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MR1-dependent antigen presentation

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Abstract

MR1 is a non-classical class I molecule that is highly conserved among mammals. Though discovered in 1995, only recently have MR1 ligands and antigens for MR1-restricted T cells been described. Unlike the traditional class I molecules HLA-A, -B, and -C, little MR1 is on the cell surface. Rather, MR1 resides in discrete intracellular vesicles and the endoplasmic reticulum, and can present non-peptidic small molecules such as those found in the riboflavin biosynthesis pathway. Since mammals do not synthesize riboflavin, MR1 can serve as a sensor of the microbial metabolome and could be key to the early detection of intracellular infection. This review will summarize the current understanding of MR1-dependent antigen presentation.

Keywords

MR1; Antigen presentation; Mucosal associated invariant T cells

1. Introduction

MHC class I molecules with an antigen presenting function include the classical class I molecules (HLA-A, -B, -C) and the non-classical class I molecules (HLA-E and CD1). In 1995, a new non-classical class I molecule was discovered: MHC-Related Protein 1 (MR1) [1]. Although it shares a high degree of homology with classical class I molecules that reside on chromosome 6, MR1 like CD1, is on chromosome 1. Furthermore, unlike classical class I molecules, human MR1 is monomorphic with a high degree of homology between species [2,3]. It has been identified in multiple tissues, including liver, lung and gut [4], but for many years its function was unknown. There was early speculation that given the monomorphic nature of MR1, it might bind a conserved ligand [5].

Early studies found that MR1 associated with β -2 microglobulin (B2M), suggesting an immunological function [6,7]. In 2003, Treiner et al. characterized a subset of T cells, Mucosal-Associated Invariant T (MAIT) cells, that were restricted by MR1 [8]. These MR1-

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restricted T cells (MR1Ts) were defined by a semi-invariant T-cell receptor (TCR), specifically V α 7.2 (TRAV1–2)-J α 33 in humans and V α 19-J α 33 in mice, and were not found in germ-free mice. In humans, there is a high percentage of MAIT cells at mucosal sites, in the liver, and in the blood where they represent 1–10% of CD3+ T cells [9]. Surprisingly, MAIT cells were found to react to a variety of bacteria and fungi, including *Mycobacterium tuberculosis* (Mtb), even in individuals who had never been exposed to Mtb [10,11]. Unlike traditional CD8+ T cells, MAIT cells can be found in the thymus with existent effector function, meaning they are capable of releasing pro-inflammatory cytokines like TNF- α and IFN- β , and can lyse infected cells via granzyme and perforin [12–15]. Napier et al. have provided a concise summary of the development and functions of MAIT cells [16].

Despite the rapid growth in our understanding of MAIT cells, the study of MR1 antigen presentation was hampered by the fact the MR1 antigen was unknown. Studies have consistently shown that unlike classical class I, very little MR1 was detectable on the cell surface in the absence of a ligand [7,17]. The discovery that MR1 ligands can be derived from the riboflavin biosynthesis pathway has led to significant advances in our understanding of MR1 trafficking and antigen presentation [18,19]. Since mammals cannot synthesize riboflavin and only certain bacteria and fungi possess the enzymes necessary for riboflavin synthesis [18], MR1 is poised to be a detector of intracellular infection by sampling the microbial metabolome; the low level of surface MR1 indicates there are tight regulatory mechanisms in place to prevent improper translocation to the cell surface in the absence of ligand. In this review, we will discuss MR1 ligands, MR1 structure, and MR1 regulation with a specific focus on MR1 distribution and translocation.

2. MR1 ligands and structure

2.1. MR1 ligands

Even before ligands for MR1 were identified, it was clear MR1 was capable of presenting antigens from a variety of microorganisms to MR1Ts. The monomorphic nature of MR1 and the observation that MR1 from different species activated mouse MAIT cell hybridomas [20] suggested that MR1 would display a limited repertoire of ligands, but their discovery proved challenging. Some data suggested it was unlikely to be a peptide. For example, MR1 did not require TAP [8,10,11,21]. However, there were also data indicating the ligand was sensitive to proteases and that MR1 coimmunoprecipitated with the peptide loading complex, which implied a possible peptidic ligand [7,11]. However, these observations might also be explained by as yet unknown carriers or chaperones.

The identification of ligands for MR1 has been seminal in the field. Using an assay that relied on the efficiency of MR1 refolding, Kjer-Nielsen et al. found that MR1 could fold properly in the presence of B2M and RPMI-1640, which indicated the presence of an MR1 ligand in RPMI-1640 [18]. Since RPMI-1640 contains vitamins, some of which are synthesized by bacteria and fungi, investigators examined the B vitamins and found that folic acid increased folding of MR1. Using mass spectrometry, 6-formyl pterin (6-FP), a product of the photodegradation of folic acid, was found to be a dominant ligand. However, 6-FP failed to activate Jurkats that had been transduced with a MAIT TCR (Jurkat.MAIT

cells). Since *Salmonella* Typhimurium supernatants can activate Jurkat.MAIT cells, MR1 was refolded with the supernatant and analyzed by mass spectrometry. The predicted antigens were collectively called ribityllumazines and were thought to be derived from the riboflavin biosynthesis pathway [18]. Chemical synthesis of these antigens activated Jurkat.MAIT cells, while 6-FP was found to be a competitive antagonist [22]. A new inhibitory ligand, acetyl-6-FP, has also been identified and is 100 times more potent than 6-FP [23].

At that time, all of the microbes known to activate MAIT cells possessed the enzymes necessary for riboflavin biosynthesis. However, one of the predicted antigens, 6-hydroxymethyl-8-D-ribityllumazine (rRL-6-CH₂OH), was not a known intermediate in the riboflavin biosynthesis pathway. Subsequent work found that the riboflavin precursor 5-amino-6-D-ribitylaminouracil (5-A-RU) can combine with glyoxal or methylglyoxal to form the antigen 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU) and 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), respectively [19]. Glyoxal and methylglyoxal are byproducts of glycolysis, and therefore could originate from the microbe or the host cell. These pyrimidine based ligands are the most potent MR1 antigens identified to date but they are unstable unless they are stabilized by MR1. 5-OP-RU can breakdown to rRL-6-CH₂OH, thus explaining how this antigen is biochemically derived. The discovery of MR1 ligands has allowed the development of MR1 tetramers, which now facilitates more specific studies of MR1Ts and their characterization [24].

An important question that arose from the discovery of riboflavin based antigens was whether it was possible for other small molecules that are outside the riboflavin biosynthesis pathway to act as MR1 antigens. It is known that MAIT cells with different TCRs can discriminate between MR1 antigens, but these antigens were all based on riboflavin intermediates [25]. New studies support the hypothesis that there are MR1 antigens outside of the riboflavin biosynthesis pathway. First, a non-canonical MR1T cell characterized by TRAV12–2 TCR usage was found to detect infection with *Streptococcus pyogenes*, a microbe incapable of producing riboflavin [26]. The antigen presented remains unknown but this suggested that MR1 is able to present antigens derived from pathways other than riboflavin biosynthesis. Second, an *in silico* screen of small molecules identified dozens of MR1 ligands. Sixteen compounds upregulated MR1 surface expression and eight were antigenic [27]. Diclofenac, a cyclooxygenase inhibitor, activated Jurkat.MAIT cells to a similar level as measured by IL-2 production when compared with 5-OP-RU, though the concentration of 5-OP-RU was 100 fold less than that of diclofenac. While the clinical significance of these findings is unknown, they raise important questions about whether common medications can result in MR1T cell activation or inhibition.

2.2. MR1 structure

MR1, like MHC-I, is characterized by three alpha domains, a transmembrane domain, and cytoplasmic tail. An analysis of human MR1 versus mouse MR1 found a high degree of homology: the $\alpha 1$ and $\alpha 2$ domains are 90 and 89% similar, respectively; the $\alpha 3$ domains share 73% homology [28]. Prior to the discovery of any MR1 ligands, it was clear that MR1 associated with B2M [6,7]. The crystal structure of MR1 confirmed that the $\alpha 3$ domain

binds to B2M. The MR1 binding groove is characterized by an A' pocket and an F' pocket and compared to MHC-I, the MR1 binding groove contains more aromatic amino acids [18,29]. Mutational analysis pointed to the A' pocket as the antigen binding pocket because alternations in amino acids in the A' pocket attenuated the ability of MR1 to activate the mouse MAIT T-T hybridomas 8D12 and 6C2 [30].

The crystal structure of MR1 revealed features required for ligand binding and T cell activation. First, all the ligands known to bind MR1 do so in the A' pocket. The A' pocket is characterized by the aromatic amino acids tyrosine 7, tyrosine 62, tryptophan 69 and tyrosine 156, and the basic amino acids arginine 9, lysine 43 (K43), and arginine 94. 6-FP and the pyrimidine based ligands form a covalent bond with K43 [18,19], but this is not an absolute requirement for an antigen since multiple antigens, such as the ribityllumazines and diclofenac, do not form this bond. The aromatic amino acids in the A' pocket argue that pi-pi stacking is a prerequisite for binding to MR1, implying that ligands must contain aromatic rings. Mutagenesis studies of the TCR showed that the α chain plays more of a role in MR1 recognition than the beta chain [31]. Crystallography revealed that the ribityl tail of antigens formed a hydrogen bond with tyrosine 95 of the CD3 α loop. 6-FP does not make contact with the CD3 α loop, explaining why it cannot activate T cells [23,32].

3. MR1 regulation

3.1. MR1 and disease

Given ubiquitous MR1 expression and the high prevalence of MAIT cells, especially at mucosal sites, it seems that MR1 is an important part of the host immune response to infection. Studies on individuals with active tuberculosis found fewer circulating MAIT cells in the peripheral blood compared to healthy controls and those with latent tuberculosis [10,11]. This finding has also been observed in patients with cystic fibrosis, where the number of peripheral blood MAIT cells is lower compared to healthy controls [33]. Similarly, Grimaldi et al. showed that MAIT cells were decreased in the blood of patients with severe sepsis and septic shock. Furthermore, they observed that a failure to reconstitute the MAIT cell population was associated with a risk of developing a new infection while in the intensive care unit [34]. Studies using an MR1 knockout (MR1-KO) mouse point to the importance of MR1 for survival in the early stages of infection. First, MR1-KO mice had decreased survival following intraperitoneal injection with *Klebsiella pneumoniae* compared to wild type mice [35]. The MR1-KO mice had decreased production of IL- β , TNF- α , and IL-17. However, the increase in mortality of MR1-KO mice was not observed when mice were challenged with *Escherichia coli* (*E. coli*), *Shigella dysenteriae*, or *Yersinia enterocolitica*. Second, MR1-KO mice depleted of CD4⁺ and CD8⁺ cells died rapidly following the administration of a live vaccine strain of *Francisella tularensis* [36]. In contrast, mice depleted of CD4⁺ and CD8⁺ T cells, but retaining an intact MR1 and MAIT cell system, were chronically infected but lived approximately 2 months. Other studies have found that MAIT cells were important for the early control of infection, though they did not necessarily confer a mortality benefit [10,37,38]. In addition to direct antimicrobial effects, another mechanism behind the early protection afforded by MAIT cells is that they promoted differentiation of monocytes to dendritic cells via production of GM-CSF [39].

It is not surprising that the ubiquitous expression of MR1 could lead to improper immune activation. In this regard, MAIT cells have been associated with autoimmune diseases, such as inflammatory bowel disease and multiple sclerosis. For instance, there are fewer MAIT cells in the blood of patients with ulcerative colitis and Crohn's disease [40–42]. Two of these studies also demonstrated an increase in IL-17 production and accumulation of MAIT cells at the site of inflamed gut mucosa. However, different results were reported by Hiejima et al. where the number of MAIT cells in inflamed tissue was lower compared to tissue from healthy controls [42]. With respect to the role of MAIT cells in multiple sclerosis, Willing et al. found MAIT cells in the brain lesions of patients with multiple sclerosis [43]. Whether dysregulation of these T cells is MR1 dependent is not known. Nonetheless, the role of MR1 in human disease is an important question and a better understanding of the key regulatory mechanisms will allow us to better understand the pathophysiology of these diseases.

From these observations, it would appear that MR1 Ts play a role in the host response to microbial infection but have the potential to cause tissue damage. Given the possibility that commensal bacteria can produce MR1T antigens, we hypothesize that the loading of MR1 is tightly regulated. *In vitro* studies show that exogenously added antigen can reach MR1 and activate MR1Ts. However, *in vivo*, the mucosal barrier may limit how much antigen reaches antigen presenting cells. Therefore, we postulate that the intracellular location of MR1 would suggest a role in sampling intracellular microbial metabolites and that molecular mechanisms enable appropriate loading of MR1.

3.2. MR1 distribution and translocation

Although little endogenous MR1 is present at the cell surface, it is clear from antibody blocking studies that loaded MR1 must be on the cell surface in order to make contact with the TCR [10,11] and that MR1 bound to an antigen activates MR1Ts via the TCR [26]. The amount of cell surface MR1 can be increased by overexpressing MR1. In addition, MR1 surface expression increased with culturing at 26° Celsius and with TLR2 stimulation [44,45]. Coimmunoprecipitation in mouse fibroblasts expressing Ii and DM showed that both proteins associated with MR1, but MHC-II did not associate with MR1 [21]. Overexpression of Ii and DM enhanced the activation of mouse MAIT T–T hybridomas 8D12 and 6C2 and inhibition of Ii with shRNA caused a substantial reduction in their activation. Furthermore, overexpression of Ii caused MR1 to go from the endoplasmic reticulum (ER) to endocytic vesicles characterized by being LAMP1 positive. However, Ii and DM are not absolute requirements for MR1-dependent antigen presentation because multiple cell lines that lack the MHC-II machinery including Ii, can present antigens via MR1 to MAIT cells [10,11,13,20]. Earlier studies also showed that MAIT cells are increased in Ii knockout mice, which is consistent with the observation that MR1-dependent antigen presentation is not dependent on Ii [8,10].

In the last year, there have been two papers that specifically examined MR1 distribution and ligand-dependent cell surface translocation. First, using the airway epithelial cells BEAS–2B expressing MR1-GFP, it was shown that MR1 was in discrete intracellular vesicles as well as the ER [46]. This was consistent with earlier work using the mouse B cell line CH 27 [21]. The MR1 vesicles in BEAS-2Bs shared features of the late endosome as approximately 50%

of the vesicles were LAMP1 positive and 40% were Rab7 positive [46]. In addition, approximately 70% of MR1 vesicles stained with B2M; almost none stained with the early endosome marker Rab5. While these airway epithelial cells were inefficiently infected with Mtb relative to monocyte derived dendritic cells, they were very efficient on a per-cell basis at the activation of MR1Ts [47].

Given the presence of MR1 vesicles, the role of vesicular trafficking proteins was examined to define those proteins required for MR1-dependent antigen presentation. To that end, shRNA was used to knockdown vesicular trafficking proteins in epithelial cells, followed by infection with Mtb. The activation of Mtb-reactive MR1, HLA-E, and HLA-B45 restricted T cell clones was assessed using IFN- γ release. The simultaneous use of these disparate T cell clones allowed for identification of vesicular trafficking proteins that are specifically required for MR1-dependent antigen presentation. Several candidates were identified and validated with siRNA knockdown. While the mechanisms regulating MR1 translocation to the cell surface are not fully known, the vesicular trafficking proteins Syntaxin 18, VAMP4 and Rab6 were identified as key proteins for MR1-dependent antigen presentation of Mtb [46]. Syntaxin 18 is primarily localized to the ER [48]. VAMP4 and Rab 6, on the other hand, are mostly localized to the *trans*-Golgi network (TGN) and both have been implicated in endosome-TGN trafficking [49–51].

MR1 translocation to the cell surface following the addition of a ligand can be measured empirically with flow cytometry. Following 6-FP treatment, the number of MR1 vesicles decreased and more MR1 was found on the cell surface. Brefeldin A (BFA), an inhibitor of ER to Golgi transport, substantially blocked 6-FP mediated MR1 translocation. While intracellular infection produced a subtle increase in surface MR1 by flow cytometry [11], exogenously added ligands caused a substantial translocation of MR1 from intracellular pools to the plasma membrane. Interestingly, while Syntaxin 18 knockdown decreased MR1-dependent antigen presentation of Mtb and reduced MR1 translocation to the cell surface following 6-FP treatment, VAMP4 and Rab6 knockdown decreased MR1-dependent antigen presentation of Mtb but had no effect on 6-FP mediated MR1 translocation [46]. These data suggest different trafficking pathways for exogenous antigens versus those derived from an intracellular infection.

Additional data support this hypothesis. First, while 6-FP pretreatment eliminated the MR1-dependent response to *Mycobacterium smegmatis* supernatant, the response to Mtb infection was diminished but still intact [46]. Second, a different study in THP-1 cells showed that inhibition of endosomal acidification had no effect on MR1-dependent antigen presentation using *E. coli* supernatant but significantly reduced presentation when intact *E. coli* were used [45]. Taken together, there are at least two possibilities: 1) either there are different pools of MR1 for sampling of an intracellular infection versus an exogenously added antigen or 2) antigens from an intracellular infection access MR1 at a different location in the MR1 trafficking pathway than do exogenously added ligands like 6-FP. The exact location of antigen loading in the setting of Mtb infection is not known, but the fact that VAMP4 colocalized with MR1 vesicles, in conjunction with its functional role in endosomal trafficking, suggests that VAMP4 plays a role in sampling MR1 ligands from the Mtb phagosome.

Using C1R cells transfected with MR1-GFP and confocal microscopy, McWilliam et al. found that in the absence of ligand, the majority of MR1 is retained in the endoplasmic reticulum [52]. No cytoplasmic MR1 vesicles were identified in this report. The results were confirmed biochemically using endoglycosidase-H sensitivity, which showed that most MR1 was Endo H sensitive in the absence of an MR1 ligand; the results were confirmed in human PBMCs as well. These data were consistent with earlier work using P388 cells, which also showed that MR1 was Endo H sensitive [6]. The kinetics of MR1 translocation were characterized using the inhibitory ligand acetyl-6-FP and the antigen 5-OP-RU. With 5-OP-RU, MR1 surface expression peaked at 4 h while with acetyl-6-FP, it peaked at 8–16 h. While BFA inhibited MR1 translocation to the cell surface, the protein synthesis inhibitor, cycloheximide, had no effect with 5-OP-RU. Unlike 5-OP-RU, cycloheximide had a sustained effect after 2 h with acetyl-6-FP [52]. Surface expression of MR1 was not upregulated with intracellular infection with *Salmonella enterica* (*S. enterica*), though this was thought to be due to limited antigen availability. In order to quantify the effect of cycloheximide and BFA in the setting of infection, PBMCs were infected with *S. enterica* with or without cycloheximide and BFA. The infected cells were incubated with autologous PBMCs and the percentage of TNF positive cells was quantified. Cycloheximide treatment led to a ~40% reduction in the number of TNF positive cells while BFA led to a ~70% reduction [52].

After addition of either acetyl-6-FP or 5-OP-RU, MR1 becomes Endo H resistant, signifying movement through the Golgi [52]. To determine whether MR1 was associated with B2M, immunoprecipitation of MR1 was done with and without exogenously added MR1 ligands. The results showed that MR1 does not associate with B2M when no ligand is present. Treatment with BFA and acetyl-6-FP demonstrated B2M in association with MR1 in the ER, but MR1 was unable to translocate to the cell surface. The authors noted that this indicated that acetyl-6-FP was able to reach MR1 in the ER. Whether other ligands are able to access MR1 in the ER is unclear. To determine whether MR1 undergoes recycling, a fluorescently labeled antibody to MR1 was used to label surface MR1 and track its internalization. After 2–4 h, 50% of surface MR1 was internalized and was associated with both early and late endosomal markers [52]. Finally, some of these internalized molecules are able to be loaded with antigen and recycle back to the cell surface.

McWilliam et al. further explored the role of MR1 residue K43 [52]. Most of the MR1 antigens identified thus far form a covalent bond with K43, thus eliminating the positive charge on the lysine residue. It was hypothesized that this loss of positive charge would allow MR1 to escape the ER and proceed to the cell surface. To test this hypothesis, MR1 mutants K43A and K43R were expressed in C1R cells. K43A, which lacked a positive charge, was able to associate with B2M and was found on the cell surface despite the lack of exogenous antigen. In contrast, the K43R mutant, which is less efficient at forming a covalent bond with MR1 antigens, could not reach the cell surface despite the addition of acetyl-6-FP. The authors propose that K43 acts as a “molecular switch” for MR1; once the charge is lost via a covalent bond, MR1 is able to leave the ER and reach the plasma membrane. However, MR1 antigens like the ribityllumazines and diclofenac do not form a covalent bond with K43 and yet are able to activate MR1Ts [18,27]. In addition, while the amount of endogenous MR1 at the plasma membrane in the absence of a ligand is small, the

fact that MR1 reaches the plasma membrane without an antigen indicates there are additional mechanisms governing MR1 trafficking, which might include an endogenous ligand.

A key difference in these two reports is the intracellular distribution of MR1. While MR1 is in the ER in BEAS-2B cells, there is also a vesicle pool that appears important for antigen presentation in the setting of Mtb infection. The differences observed between BEAS-2B and C1R cells raises the possibility that different cell lines may have different distributions of MR1, or may serve different functions with regard to MR1T activation. In BEAS-2Bs, it is not clear whether MR1 antigens from a microbe are captured only by MR1 vesicles or if there is also some loading taking place in the ER. Another confounder is whether folic acid is in the media and whether any of it undergoes photodegradation resulting in 6-FP.

McWilliam et al. used folic acid free media, but folic acid and its degradation products would likely be present *in vivo*. It remains unclear how much of a role folic acid or 6-FP in the media play in allowing for the egress of MR1 from the ER or whether they serve as an endogenous ligands for MR1 *in vivo*.

Although MR1 is capable of binding exogenously added antigens, such as bacterial supernatants, the extent to which this occurs *in vivo* is not known. Mucosal surfaces have mucus as a barrier that might prevent direct contact between exogenous antigens and the epithelium. As a result, we propose a model for MR1 trafficking in which MR1-dependent antigen presentation of antigens derived from an intracellular infection utilizes a pathway distinct from exogenously derived ligand [Fig. 1]. In this model, there is a pre-formed pool of MR1 available to rapidly sample the intracellular environment. We propose that MR1 vesicles play an important role in sampling microbial antigens contained within endosomal compartments. How these vesicles are formed is not known, but they could originate from the Golgi or they could be derived from MR1 that has recycled from the plasma membrane. Whether an endogenous ligand exists that allows MR1 to leave the ER and the nature of such a ligand (folic acid derived, protein based, etc.) are key unanswered questions. The precise mechanisms by which MR1 becomes loaded with intracellular microbial antigens remain to be elucidated. One possibility is that the pre-formed pool of MR1 could transiently traffic to the cell surface where it can then recycle back into the cell for subsequent acquisition of microbial ligands. It is also possible that vesicular MR1 directly interacts with ligands derived from intracellular microbes. A third possibility is that some antigens from an intracellular microbe reach the ER via yet undefined mechanisms. Exogenous ligands, like 6-FP, acetyl-6-FP, and 5-OP-RU, are loaded in the ER thus allowing MR1 to translocate to the cell surface. Whether there are chaperones that stabilize MR1 in the absence of antigens is also an important unanswered question. In either case, we postulate that MR1, like MHC-I/II will depend upon chaperones to facilitate the loading of the small molecule metabolites.

4. Conclusions

In the last few years, our understanding of MR1 biology has increased substantially. MR1 is tightly regulated with little cell surface expression. Although monomorphic, MR1 can bind a number of different ligands derived from the riboflavin biosynthesis pathway. While more limited, there are data supporting the hypothesis that MR1 can bind ligands outside of the

riboflavin biosynthesis pathway. Structurally, MR1 is tailored to bind cyclic compounds in the A' pocket and upon acquiring a ligand, MR1 translocates to the cell surface where it can present antigen to MR1Ts. MR1 is unusual in that there is a pre-formed intracellular pool that is poised for the rapid sampling and presentation of the microbial metabolome. The data are mixed with respect to the intracellular compartment where ligand loading occurs and it may be that the distribution of MR1 and the location of ligand loading depend on the cell type. Several lines of evidence suggest there are inherent differences between MR1 antigen presentation of an exogenous antigen versus antigen derived from an intracellular microbe, and while the biological significance of these findings is unclear, the intracellular distribution of MR1 suggests it is poised to serve as a sensor for an intracellular infection.

Several questions about MR1 remain unanswered. First, there is no evidence that MR1 ligands undergo any additional processing prior to being presented to MR1Ts, but it is unclear whether MR1 directly captures ligands or if there is active shuttling or chaperoning of microbial ligands. Given the small size of the ligands, innovative solutions are needed to accurately track the ligand and the formation of loaded MR1. Second, we do not know if there are ligands that bind the F' pocket. Recent data show that there is a more diverse array of MR1 ligands than previously thought and it is conceivable that the F' pocket could participate in binding or stabilization of ligands. Another unknown is whether MR1 itself is stabilized by a chaperone protein or proteins in its pre-loaded state. Chaperones are a key feature of MHC I/II, but thus far, no chaperones for MR1 have been described. We hypothesize that MR1 associates with a protein or biochemical chaperone to facilitate stability. Finally, with its conserved binding groove and the widespread tissue expression, MR1 could be a useful target to provide immunotherapy. Undoubtedly, the further elucidation of the mechanisms by which MR1 ligands are sampled, stabilized and presented will yield further insight into the role of MR1Ts both in the host response to intracellular infection and in autoimmunity.

Abbreviations

MR1	MHC related protein 1
B2M	β -2 microglobulin
MAIT	mucosal associated invariant T cell
MR1	TMR1-restricted T cell
TCR	T-cell receptor
Mtb	<i>Mycobacterium tuberculosis</i>
MR1-KO	MR1 knockout
ER	endoplasmic reticulum
BFA	brefeldin A

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Fig. 1.

Model of MR1 trafficking. A) MR1 is synthesized in the endoplasmic reticulum and passes through the Golgi where it either (1) goes directly to the plasma membrane or (2) remains in an intracellular vesicle. Whether an endogenous ligand facilitates MR1 egress from the ER is not known. MR1 at the plasma membrane may recycle back into the cytoplasm via recycling endosomes. MR1 vesicles may then be loaded with antigens from Mtb and then translocate to the plasma membrane. It is also possible that some MR1 ligands from Mtb are transported to the ER (3) via an unknown mechanism and are then loaded onto MR1. Syntaxin 18 is a vesicular trafficking protein involved in ER to Golgi transport. VAMP4 and Rab6 are vesicular trafficking proteins involved in endosome to *trans*-Golgi trafficking. Knockdown of all three of these vesicular trafficking proteins resulted in a decrease in MR1-dependent antigen presentation in the setting of Mtb infection. B) exogenous ligands traverse the cell membrane and are loaded onto MR1 in the endoplasmic reticulum. These ligands may be endocytosed or interact with specific transporters that shuttle them to MR1. After loading, MR1 passes from an Endo-H sensitive state to a post-Golgi, Endo-H resistant state, on its way to the plasma membrane. Syntaxin 18 knockdown affects 6-FP mediated translocation of MR1 to the cell surface.