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Molecular Insights Into MR1-Mediated T Cell Immunity: Lessons Learned and Unanswered Questions

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ABSTRACT

The major histocompatibility complex class-I related protein, MR1, is an evolutionarily conserved antigen presenting molecule that binds and displays organic metabolites to T cells, including mucosal associated invariant T (MAIT) cells and diverse MR1-restricted T cells (MR1T). Structural studies have elucidated how MR1 can accommodate a range of chemical scaffolds that arise from foreign, synthetic, and self-metabolites, although the full spectrum of metabolites that MR1 presents remains unclear. Presently, MAIT and MR1T cell recognition of MR1-antigen complexes represents a new immune recognition paradigm and is emerging as a critical player in protective immunity, aberrant immunity, tumor immunity, and tissue repair. Moreover, the limited allelic variation of MR1 makes it an attractive therapeutic target. This review will address the unique features and capability of the MR1 molecule to display several classes of small molecules for T cell surveillance. We will also address the molecular basis underlying MAIT and MR1T TCR recognition of MR1-binding ligands.

1 | Introduction

Understanding antigen-mediated immunity has progressed substantially by identifying the molecular and structural features that underlie T cell receptor (TCR) recognition of antigen (Ag) presentation molecules. The majority of our knowledge of Ag presentation stems from research on TCR recognition of peptide and lipid-based Ags presented by MHC and CD1 proteins, respectively. Another facet in T cell immunity has recently been reached with the expansion of the antigenic repertoire of T cells to include "small molecule metabolites" that are presented by the MHC class I-related molecule "MR1". Initially, a population of unconventional T cells was reported to be mucosal-associated invariant T (MAIT) cells that were restricted to MR1 molecules [1–3]. MAIT cells, in humans, are an abundant innate-like T-cell population that express a semi-invariant TRAV1-2^{+} TCRα chain (referred to as TRAV1-2^{pos} hereafter) and a limited repertoire of TCRβ chain. These cells were shown to be activated by a wide variety of bacteria and yeast [4, 5]. This suggested that these microbes shared conserved ligand(s), even though their nature was unknown. In 2012, we identified the MR1 binding Ags as small molecule metabolites derived from vitamin B (VitBAg) [6]. Since then, we and others have identified a broad range of MR1-binding mono-, bi- and poly-cyclic compounds (150–400 Da) derived from microbial and non-microbial sources [7–16]. These MR1 Ags are presented to a broader family of diverse αβ MR1-restricted T cells (MR1T) and γδ MR1-reactive T cells, some of which exhibit self-reactivity, suggesting a role in

tissue homeostasis and malignancy. MAIT and diverse MR1T cells are emerging as critical players in anti-microbial immunity, due to their unique ability to recognize and respond to conserved microbial Ags, and are also implicated in tissue repair, inflammation, modulation of graft versus host disease, and anti-tumor activities, possibly by presentation of as yet undefined classes of metabolites [17–20].

To fully understand MR1-mediated T cell immunity, we have to define the range of antigens that MAIT and MR1T cells are capable of detecting, the molecular underpinnings of their antigen specificity and TCR recognition, as well as the variables that control their functions. We hypothesize that the heterogeneity within the TCR chains of MAIT and MR1T cells can provide a mechanism of metabolite Ag discrimination. Over the last decade, several X-ray crystal structures of TCR-MR1-Ag complexes have helped to shape our understanding of the molecular features of TCR recognition of MR1-Ag molecules [6, 7, 12, 21–24]. This review highlights the uniqueness of the MR1-Ag presentation in comparison to other Ag presentation systems, then addresses the MR1 capability to bind metabolite Ags. We also provide an overview of the most recent discoveries regarding the diversity of MR1-reactive T cells (MAIT and diverse MR1T cell subsets) and the molecular basis underpinning TCR-MR1 recognition.

2 | MR1 as a Metabolite-Presenting Molecule

2.1 | MR1 Conservation Across Mammals

MR1 is ubiquitously expressed in all human cells; however, the level of MR1 at the cell surface is correlated with Ag availability and is generally low compared to the cell surface expression of HLA molecules. MR1 is highly conserved over ~170 million years of mammalian evolution. Human MR1 gene orthologs have been found in placental mammals, including mice [25, 26], sheep [27, 28], cow [27], rat [29], pig [30] and bats [31], as well as non-human primates [26, 32], and marsupials [33]. However, MR1 orthologs have not been identified in some lower jawed vertebrates ranging from teleosts to monotremes [33]. Nonetheless, about 90% of the amino acids that form the putative ligand-binding groove are conserved across humans and mice [26, 34]. This suggests that there is significant selection pressure to preserve conserved MR1-responsive T cells across mammalian species [35, 36].

2.2 | Molecular Features of the MR1 Molecule

MR1 shares the general architecture of the CD1 and MHC-I proteins. The MR1 heavy chain, which consists of three domains (α1, α2, and α3), forms a non-covalently associated heterodimer complex with the β2-microglobulin (β2m) chain. In 2012, we determined the first crystal structure of MR1 loaded with a folate-based ligand (6-FP) [6]. The three-dimensional structure of the MR1 antigen-binding groove was shown to be ideally shaped to capture and display small heterocyclic metabolites, uniquely forming a specific Schiff base with many ligands through a conserved Lysine residue in the Ag cleft. The antigen-binding cleft of MR1 (~750 $Å^3$) comprises two pockets (A′ and F′), which lie between α1 and α2 helices of the MR1 heavy chain on top of an antiparallel β-sheet (Figure 1A). All the currently known MR1 ligands reside in the A′ pocket, where the aromatic and charged amino acids form an 'aromatic cradle' lining the cleft (Figure 1B). MR1 ligands are mainly located at the base of the A′ pocket, where those Ags that can form a covalent "Schiff-base" interact with the amino group of Lys43 via their carbonyl group (Figure 1C,D). As Lys43 is situated at the base of the MR1 pocket, the Ags are buried deep within the cavity with comparatively small exposure to solvent. The F′ pocket is significantly shallower than the A′ pocket and is lined with mostly polar residues. Presently, no biological ligands have been identified to bind the F′ pocket, although crystallization components such as bis-tris propane have been observed to reside here [6, 24]. Further research is required to understand the role of the F′ pocket in MR1 biology.

2.3 | MR1 Polymorphism

Since its discovery, MR1 has been widely regarded as a monomorphic protein. Recently, this notion has been revised with the identification of at least six human MR1 alleles, each characterized by a few single-nucleotide polymorphisms (SNPs) [37]. The allele *MR1*01* has been deemed 'wild type' with an allele frequency of 71%, while *MR1*02* has an allele frequency of 25%. *MR1*02* harbors a His17Arg (α1-domain) mutation previously observed in a chronic myeloid leukemia patient following a lymphocyte transfusion [38] (Figure 1A). This His17Arg mutation is also maintained in the less frequent *MR1*04* allomorph, which carries an additional Arg9His mutation buried within the A′ binding pocket. Interestingly, we identified an immunodeficient patient who lacked circulating MAIT cells to be homozygous for this Arg9His substitution [39]. This MR1 variant reveals a conformational shift in the antigen-binding cleft that impedes the binding with MAIT microbial Ags. We hypothesize that the allelic variations may alter the MR1 ligand repertoire as well as the intracellular pathways of MR1 processing and antigen loading, warranting further analysis. In addition, three intronic SNPs have been found in human MR1, one of which impacts MR1 expression associated with susceptibility to tuberculosis [40]. None of the xenogenic MR1 variants observed in non-human primates and opossums have been found to impact antigen presentation or TCR reactivity [32, 33, 41]. So, MR1 is an attractive target for therapeutic applications due to its minimal allelic variation.

2.4 | Schiff Base "Molecular Switch" Formation and MR1 Translocation to the Cell Surface

Several investigations have been conducted to elucidate the mechanistic pathways by which MR1 is translocated to the plasma membrane [42, 43]. In 2016, McWilliam et al. proposed that MR1 does not present antigens in the steady state, but rather most MR1 molecules are retained in the endoplasmic reticulum (ER) in a "ligand–receptive conformation". Free of the β2m protein, such that only a few MR1 molecules escape the ER with the assumed assistance of an uncharacterized, presumably endogenous ligand(s) [44]. Once an MR1 antigen is captured by an APC, the immature MR1 in the ER binds. This neutralizes

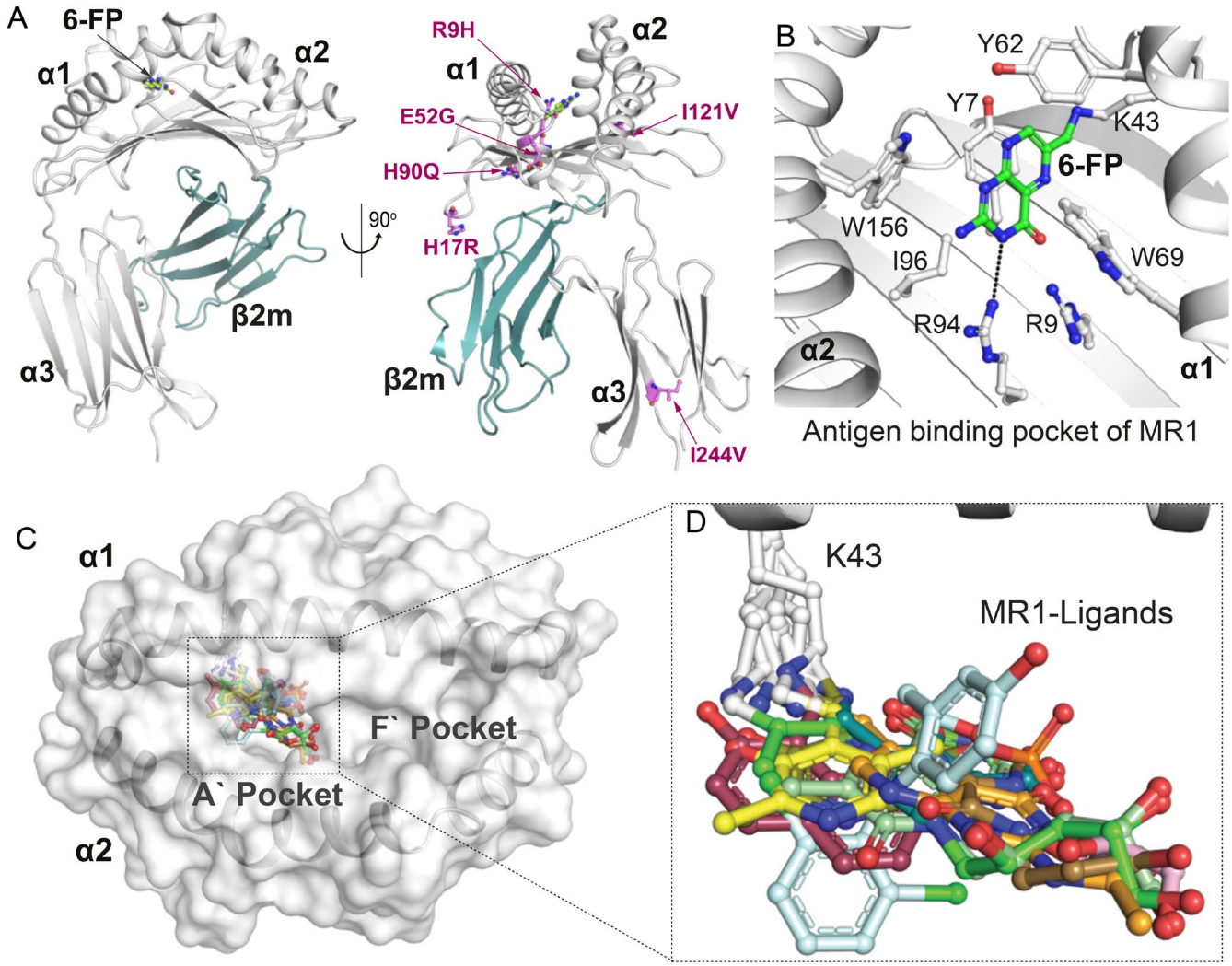

FIGURE 1 | Crystal structure of MR1-Ags. (A) Cartoon presentation of the structure of the binary MR1-6-FP complex (PDB; 6GUP). MR1 and β2M, and 6-FP ligand are colored white, gray, and green, respectively. Mutations in various MR1 alleles are shown in pink. (B) The interactions of 6-FP within the A′-pocket of the MR1 binding cleft. MR1-Ag interacting residues are shown as white sticks, and the H-bond is shown as a black dashed line. (C) Surface presentation of the MR1-Ag binding A′ and F′ pockets that are formed between MR1 α1 and α2 helices. All recognized MR1-ligands, to date, dock in the A′ pocket as shown in (D).

the basic charge of MR1-Lys43, allowing MR1 folding and association with β2m, which is thought to promote subsequent MR1-β2m-Ag complex trafficking from the ER through the golgi complex to the surface of the cell for Ag presentation. Since some ligands, like ribityllumazines, lack the reactive carbonyl group, they cannot form a Schiff base and thus weakly upregulate MR1 on the cell surface. The current model for MR1 trafficking proposes that the "Molecular Switch" function is enabled by MR1 folding through Schiff-base formation between the MR1-Lys43 and the Ligand. MR1 trafficking is affected when this lysine is mutated to a neutral alanine (Lys43Ala), which results in constitutive MR1 surface expression even in the absence of ligands [45]. Conversely, MR1 remains confined within the ER when this lysine is mutated to another positively charged arginine (Lys43Arg), as the Schiff-base formation is not permitted [44]. Indeed, we identified two MR1 ligands (DB28 and NV.18; See Below), which can be captured in the MR1-binding cleft without forming the Schiff-base adduct and are therefore retained with MR1 in the ER in an immature form [14]. It appears that the ability of MR1 ligands to upregulate MR1 cell surface expression is significantly influenced by the capacity of the ligand to form a Schiff-base adduct with MR1.

2.5 | Roles of Endoplasmic Reticulum Chaperones in MR1-Ag Presentation

Since MR1 molecules primarily reside intracellularly within the ER, it is thought that ER chaperones may assist in stabilizing unliganded MR1 and maintaining a pool of receptive MR1-β2m in the ER. Indeed, co-immunoprecipitation assays have shown that MR1 is capable of binding with various peptide loading complex (PLC) components, including tapasin, and that cell surface MR1 is significantly reduced in the absence of tapasin [46]. In tapasin-deficient environments, it is suggested that another tapasin-related protein (TAPBPR) may undertake the chaperone functions in the ER, contributing to MR1 antigen presentation. Alternatively, there may be a collaborative effort required of both tapasin and TAPBPR, presumably in loading Ag and thus aiding the translocation

of MR1 from the ER to the cell surface [46]. Unlike tapasin, TAPBPR is not restricted to the ER and has been observed on the cell surface, preserving its role as a chaperone on surface MHC-I [47]. Thus, it is also possible that TAPBPR may chaperone MR1 beyond the ER. Recent studies have demonstrated that MR1 binds TAPBPR in vitro, independent of ligand, and that TAPBPR association with MR1 appears to affect MR1 epitopes equivalent to the observed MHC-I sites affected upon the ER chaperone binding [48]. Applying an integrative approach, McShan et al. formulated a structural model for TAPBPR in complex with human MR1 and bovine β2m to replicate TAPBPR interactions with the ligand-free human MR1-β2m [48]. NMR models propose conformational changes due to contact with TAPBPR, including widening of the binding groove and slight shift of the α3 and β2m interface within MR1. While few reports have been conducted to elucidate the interactions between ER chaperones (e.g., tapasin and TAPBPR) and MR1 in vitro, there are currently no structural details on how human chaperones bind human MR1-β2m.

Despite growing knowledge of the MR1 antigen presentation pathways, the molecular mechanisms by which MR1 is maintained and processed intracellularly, as well as the roles of ER chaperones in MR1-Ag loading, are still not fully understood. We also do not yet know how MR1 ligands are transported to the ER and why not all Schiff-based ligands upregulate MR1 to a similar level.

3 | Diversity of MR1 ligands

To date, three different classes of MR1-binding vitamin-B-derived Ags (VitBAgs) have been described (Figure 2). Beyond MR1 ligands related to vitamin B, several categories of self, exogenous, or environmental chemicals were also described as MR1 ligands. In this section, we will discuss the diversity of the MR1 binding metabolites and further define the molecular interactions underpinning their capture and recognition by MR1.

3.1 | MR1-Binding VitBAgs

3.1.1 | Microbial Vitamin B_2 Derivatives That Activate MAIT Cells

The biosynthetic intermediate of riboflavin (vitamin B_2) comprises the major class of VitBAgs ligands that we described to activate MAIT cells [6]. The main source of these MAIT agonists in humans is the infection with riboflavin-synthesizing bacteria and yeast. Indeed, the capacity of certain microbial species to stimulate MAIT cells via the MAIT TCR correlates well with their ability to synthesize riboflavin [49]. Since humans are incapable of producing riboflavin, the existence of MR1 ligands derived from riboflavin in the host is considered a "molecular signature" of infection. Through systematically mutating the *rib*-operon in *Lactobacillus lactis*, Corbett et al. identified 5-amino-6-D-ribitylaminouracil (5-A-RU) as a crucial mediator for MAIT cell activity [21]. 5-A-RU is a highly labile precursor that contains a free amine, and during bacterial infection, can non-enzymatically react with aldehydes/ketones derived from microbial or mammalian metabolism, such as glyoxal and methylglyoxal, to produce the pyrimidine-based MR1 antigens (Figure 2). These include the most potent, although relatively unstable, 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) as well as the slightly less potent 5-(2-oxoethylidene amino)-6-D-ribitylaminouracil (5-OE-RU). These ribityl-uracil-based Ags are then stabilized within the MR1 ligand-binding pocket via the formation of a Schiff base interaction with the MR1-Lys43 through their α-imminocarbonyl group [21]. When the pyrimidine derivatives are free in water and not stabilized by MR1 binding, they exhibit very low chemical stability and undergo ring closure with dehydration to form the more thermodynamically stable dual-ring "ribityl-lumazine" derivatives (Figure 2). Indeed, the half-life of 5-OP-RU and 5-OE-RU in physiological conditions is ~90 and 15 min, respectively. Unlike ribityl-pyrimidines adducts, the ribityl-lumazines, such as 7-methyl-ribityllumazine (RL-7-Me) and 7-hydroxy-6-methyl-8-D-ribityllumazine (RL-6-Me-7-OH), do not form a Schiff base with MR1, and this is associated with slow and modest MR1 upregulation on the cell surface and with weak MAIT cell activation [7].

Later, Harrif et al. used mass spectrometry to investigate the repertoire of MR1 binding Ags, discovering functionally diverse Ags derived from *E. coli* and *M. smegmatis* [50]. They characterized ribityllumazines including 6-(2-carboxyethyl)-7-hydroxy-8-ribityllumazine (photolumazine I [PLI]) and 6-(1H-indol-3-yl)-7-hydroxy-8-ribityllumazine (photolumazine III [PLIII]), as well as the 7,8-didemethyl-8-hydroxy-5-deazariboflavin (FO) that can be recognized by diverse MR1T TCRs [51]. This suggests that the microbial MR1 ligandome is highly diverse, containing ligands that are not 5-A-RU-derived. Accordingly, MR1 can function as an "immunological sensor" of the repertoire of microbial ligands.

We have determined several high-resolution crystal structures of MAIT TCR-MR1-5-OP-RU and TCR-MR1-5-OE-RU complexes [7, 10, 21] (see next section on the description of the TCR-mediated interactions). These structural studies showed that the uracil rings of these Ags are positioned between Tyr7 and Tyr62 of the aromatic cradle of the MR1 pocket. Here, the ribityl-based ligands are oriented differently in the MR1 pocket compared to the folate ligands; specifically, 5-OP-RU is rotated by about 75° compared to 6-FP (Figure 3A). Notably, this tilted conformation of the ribityl-based Ags enabled the ribityl tail to form intermolecular H-bonds with the conserved MR1 residues of Arg9, Arg94, Tyr152, and Gln153, as well as the intramolecular H-bond between the ribityl tail's 3′-OH group and the ring's amino group. These interactions reduced the flexibility of the ribityl tail and anchored these Ags well in the MR1 ligand-binding cavity for TCR ligation (discussed in the next sections).

3.1.2 | Vitamin B_9 Metabolites That Competitively Inhibit MAIT Cell Activation

We also described an additional group of VitBAgs that bind MR1, but instead of activating MAIT cells, they inhibit MAIT activation. This includes the photodegradation product of folic acid (vitamin B_9), mainly 6-formylpterin (6-FP), along

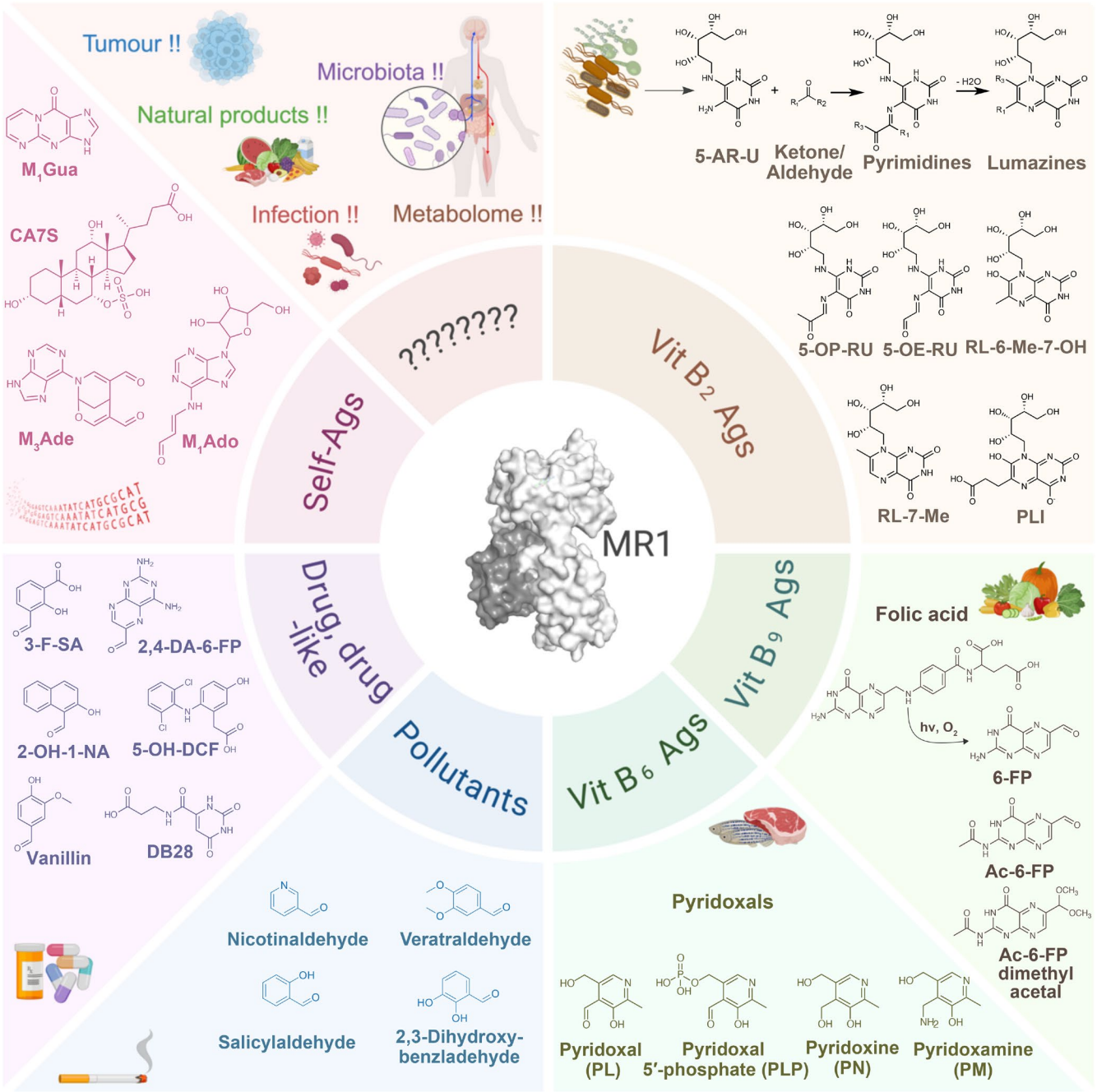

FIGURE 2 | Schematic of various categories of MR1 ligands, including microbial and non-microbial derived VitBAg, drug, drug-like, environmental, and self-antigens.

with its synthetic derivative, acetyl-6-formylpterin (Ac-6-FP) [6, 10]. These are bicyclic pterin-containing compounds, with a highly reactive carbonyl group (Figure 2), which facilitate binding to MR1, neutralize MR1-Lys43, and upregulate MR1 on the surface of APCs. Our structural studies reveal that both 6-FP and Ac-6-FP formed Schiff base interactions with MR1-Lys43, and the pterin rings were sandwiched within the aromatic cradle (MR1-Tyr7, Tyr62, Trp69, and Trp156) of the A′-pocket in a similar planar orientation with respect to each other (Figure 3A). Notably, Ac-6-FP is 100 times more potent than 6-FP as an MR1 up-regulator and MAIT inhibitor [10], making it an immunosuppressive candidate in vivo, as shown in mouse models [12, 52].

3.1.3 | Vitamin B_6 (Pyridoxal) Metabolites

The third class of VitBAgs characterized so far is vitamin B_6 metabolites [13]. Pyridoxal (PL) and its phosphorylated derivative, pyridoxal 5′-phosphate (PLP), which are two of the six structurally related known B_6 vitamers, can bind MR1 molecules (Figure 2). PL and PLP induce dose-dependent cell surface overexpression of *MR1*01* and MR1 expressing the Arg9His mutant, which is associated with the *MR1*04* allotype, in a manner that is dependent on Lys43-mediated Schiff-base synthesis (Figure 3A). The crystal structures of MR1-PL and MR1-PLP showed how these ligands were accommodated within the A′ pocket of MR1, where their aldehyde groups

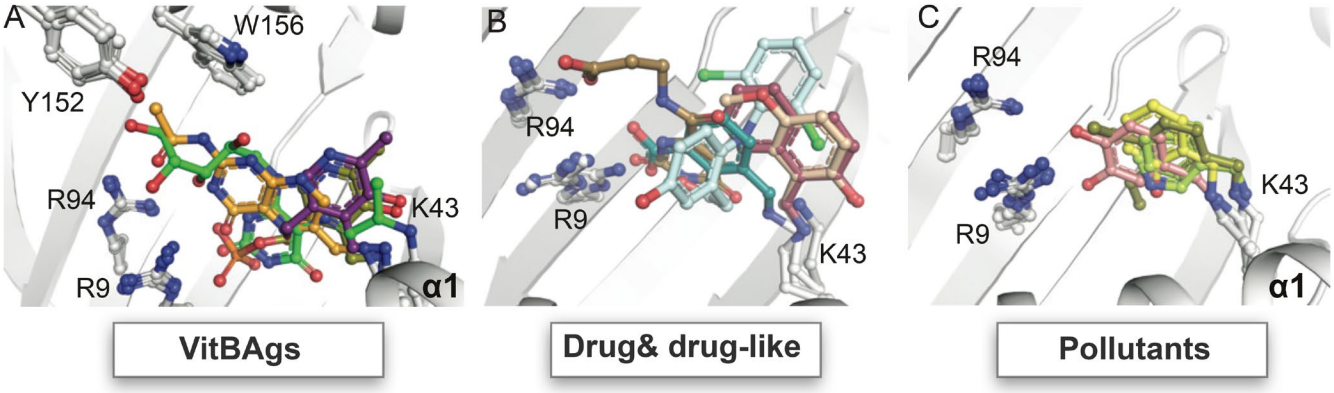

FIGURE 3 | MR1 presentation of small metabolites. Superimpositions of the Ag binding pocket of MR1 binding: (A) VitBAgs: 5-OP-RU; (PDB: 6PUC), Ac-6-FP (PDB; 4PJ5); PL (PDB; 9CGR); and PLP (PDB; 9CGS), (B) Drug and drug-like Ags: 5-OH-DCF (PDB; 5U72); HMB (PDB: 5U2V); 3-F-SA (PDB: 5U6Q); 2-OH-1-NA (PDB: 5U16) and DB28 (PDB: 6PVC), (C) Pollutants ligands: Salicylaldehyde (PDB; 9BU0); veratraldehyde (PDB; 9BTY); nicotinaldehyde (PDB; 9BTZ); and 3,4-dihydroxybenzaldehyde (PDB, 9BTX). MR1-ligands are represented as colored sticks.

formed Schiff base bonds with the ε-amino group of MR1-Lys43. The pyridine ring of PL was sequestered by a few hydrophobic and water-mediated H-bond interactions within the aromatic cradle of MR1. Further, the monophosphate ester group of PLP formed hydrogen bonds with the evolutionarily conserved residues of MR1-Arg9 and Arg94 that protrude up into the pocket. Given the preferential uptake of PL by cancer cells [53–55], PL reactive MR1T cells may detect the accumulation of PL inside cancer cells, enabling their preferential cancer detection.

3.2 | MR1-Binding Drug, Drug-Like and Diet-Derived Xenobiotic Molecules

We have reported different classes of exogenous MR1-binding ligands [12, 14, 16]. First, in 2017, we described in Keller et al. over 20 drugs and drug-like metabolites, with various chemical scaffolds to be presented by MR1 [12] (Figures 2 and 3B). These include aspirin analogues like 3-formyl-salicylic acid (3-F-SA), a photodegradation product of the chemotherapeutic amethopterin (2,4-diamino-6-formylpterdine [2,4-DA-6-FP]), 2-hydroxy-5-methoxybenzaldehyde (HMB), the sirtinol inhibitor (2-hy-droxy-1-naphthaldehyde [2-OH-1-NA]), as well as the anti-inflammatory drug diclofenac (DCF) and its metabolite 5-hydroxy-diclofenac (5-OH-DCF). These drug-related ligands displayed differing orientations and interactions within the conserved aromatic cradle of the A′-pocket of MR1 (Figure 3B). Except for the DCF metabolites, these ligands can form a Schiff base interaction with MR1-Lys43. The crystal structure of MR1-5-OH-DCF showed that MR1-Lys43 acquired a similar orientation to those in the covalently bound ligands [6, 12] (Figure 5B). Most of these exogenous compounds cannot activate MAIT TCRs transduced T cell lines, except the weak agonist DCF. Additionally, this report showed that MAIT cell stimulation or proliferation was competitively inhibited in vivo by the 3-F-SA compound. In another study, via *in silico* screening, two synthetic MR1 ligands, 3-[(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl) formamido] propanoic acid (DB28), and its ester analogue, methyl 3-[(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl) formamido] propanoate (NV18.1) were identified, which can bind MR1 while being retained in the ER [14] (Figure 2). Both DB28 and NV18.1 fail to neutralize the positively charged MR1-Lys43 or promote MR1 trafficking to the cell surface, thereby down-regulating MR1 levels on the cell surface. However, these xenobiotic ligands are not host-derived, and whether there could be host-derived ligands that mimic these effects remains unclear.

In 2022, we established a fluorescence-based polarization (FP) assay to measure the binding affinities of ligands for MR1 in vitro, enabling a rapid screening method for MR1 ligands [16]. Using the FP assay, we identified two MAIT non-stimulating ligands derived from diet: vanillin and ethylvanillin [16]. Both are found in vanilla extract, a common ingredient in food and medicine. Our structural studies demonstrate that ethylvanillin can bind within the A′-pocket of the MR1-binding cavity, establish Schiff-base bonding with MR1-K43, and potentially upregulate MR1 on the cell surface. Collectively, the diversity in the chemical identities of these metabolites reveals the malleability of the MR1 cleft to accommodate mono-, bi-, and polycyclic scaffolds.

3.3 | MR1-Binding Environmental Chemicals and Pollutants

We recently described components of the cigarette smoke that modulate MR1 biology, thereby impacting the T cell activities and functions in the lungs. Several aromatic components of cigarette smoke (CS) can bind MR1 and impact MR1 cell surface levels; some of which also competitively inhibit MAIT cell activation (Figure 2) [8]. Namely, some MR1-binding CS compounds increased MR1 translocation to the cell surface, including nicotinaldehyde, salicylaldehyde, veratraldehyde and 2,3-dihydroxybenzaldehyde. The aromatic rings of these CS ligands adopted planes like that of Ac-6-FP. They formed Schiff base bonds with MR1-Lys43 and were located within the base of the A′ pocket, mostly through van der Waals interactions (Figure 3C). These MR1-binding CS ligands result from the combustion of organic matter in cigarettes (sugars and cellulose) and flavor additives for both tobacco and e-cigarettes. Interestingly, nicotinaldehyde is considered a thirdhand CS component that is produced by the reaction of

environmental oxidants with the nicotine absorbed onto indoor surfaces. Our in vivo studies reveal that cell exposure to these CS-based ligands results in the inhibition of MAIT cell activation and reduction of MAIT cell responses to influenza A virus infection. Indeed, in MR1-deficient mice, the development of symptoms of chronic obstructive pulmonary disease (COPD) associated with CS exposure was partially avoided. These findings show that CS disrupts the MR1–MAIT axis and may increase the risk of infection and worsening of existing conditions. Nevertheless, the impact of other environmental and pollutant compounds on the MR1–MAIT cells axis requires further investigations.

3.4 | MR1 Binding Host-Derived Metabolites

One of the main questions in the field of MR1 biology for years is whether MR1 ligands can originate from the host, for example, the human metabolome, and how this may impact T cell functions. This research topic was recently explored. First, Ito et al. investigated the hepatic MR1 ligandome and discovered that MR1 can present sulfated bile acids, such as cholic acid 7-sulfate (CA7S), to MAIT cells (Figure 2) [11]. It is well known that the human liver has a significant number of MAIT cells that exhibit an activated phenotype even if they are in the non-proliferating state [56]. Here, CA7S induced modest cell surface MR1 expression and activated reporter cells expressing MAIT TCRs, but with a weaker potency than the microbial MR1 antigens [11]. This MAIT TCR recognition was blocked by both Ac-6-FP and anti-MR1 antibodies in a dose-dependent manner, reinforcing that CA7S is presented by MR1 in a conventional way. In vivo studies showed that these bile derivatives facilitate MAIT cell survival and trigger the expression of a homeostatic gene signature [11]. However, structural studies are required to further understand the molecular basis of this interaction.

MAIT cells are also enriched in blood circulation and other organs of the human body, suggesting the presence of more ubiquitous ligands. Indeed, nucleobase-containing adducts were identified as a novel class of diverse MR1T cell self-antigens, after observing the enrichment of certain nucleic acid metabolic pathways in stress-related conditions (Figure 2) [15]. These endogenous adducts are formed by the condensation of nucleobases and nucleoside compounds with carbonyl-containing species like malondialdehyde (MDA), 4-hydroxy-2-nonenal, and 4-oxo-2-nonenal (4-ONE), which are abundant during cellular oxidative stress in healthy cells and cancer cells [57]. Biochemical investigations revealed that 8-(9H-purin-6-yl)-2-oxa-8-azabicyclo [3.3.1] nona-3,6-diene-4,6-dicarbaldehyde (M_3Ade), (2E)-3-([9-[3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-9H-purin-6-yl] amino)prop-2-enal (M_1Ado) and 3H,10H-pyrimido[1,2-a] purin-10-one (M_1Gua) (Figure 2), among others, are able to activate distinct MR1T clones [15]. M_3Ade is formed by the condensation of adenine with a trimer of MDA [9, 15]. The discovery of these self-metabolites opens the doors for more functional and biochemical investigations into the repertoire of the host-derived MR1 ligandome, which would enrich our knowledge of MR1 biology. In conclusion, the repertoire of MR1-binding ligands is probably quite large (Figure 1C), and further research is required to determine the diversity and biological significance of the MR1-binding ligands in different contexts such as infections, cancers, and other diseases.

4 | Mucosal-Associated Invariant T (TRAV1-2^{pos}) Cells

MAIT cells express a semi-invariant αβ TCR, where the α-chain is composed of TRAV1-2^{+}TRAJ33/20/12^{+} in humans and of orthologous TRAV1^{+} TRAJ33^{+} in mice, paired with a limited array of TCR β-chains including TRBV6/20 in humans and TRBV13/19 in mice [4, 10, 21, 22, 45]. MR1 binds to and presents microbial Ags, such as 5-OP-RU, on the surface of the infected cells. These MR1-Ag are identified by the conserved TCRs and activate MAIT cells. Consequently, MAIT cells are considered to play a protective role in antibacterial immunity. In addition, MAIT cells have been implicated in a variety of autoimmune disease settings like inflammatory bowel disease, multiple sclerosis, and rheumatic diseases, as well as cancers [58, 59]. Once stimulated, MAIT cells produce pro-inflammatory cytokines in Th1/Th17-like responses and can also express perforin and granzyme B, killing the infected cells [60, 61].

4.1 | MAIT TCR Recognition of Microbial Antigens

In the ternary crystal structures of the human MAIT TCR in complexes with microbial Ags, the TCRs adopt orthogonal central docking modes above the A′ pocket of MR1, with α and β chains staying over the MR1 α2 and α1 helices, respectively [23, 24, 62] (Figure 4A,B). Although MAIT TCRs utilize variable TRBVs, including TRBV20, TRBV6-1, and TRBV6-4, TCR recognition is minimally impacted with differing TRBV usage [10, 22]. This notion was corroborated when mutagenesis studies revealed that no specific residue in the β chains of MAIT TCRs is essential for MAIT cell stimulation [45]. Overall, our structural data showed that the semi-invariant TRAV1-2 α-chain is crucial for MAIT activation, whereas the MAIT β-chain and its hypervariable CDR3β loop “fine-tune” reactivity to specific antigens, allowing for ligand discrimination [10, 19, 22, 50, 63, 64].

5-OP-RU and 5-OE-RU contribute less than 1% of the surface area of the MR1-5-OE-RU and 5-OP-RU complexes available for TCR binding [21]. The 2′-OH group of ribityl moieties formed a single hydrogen bond with the conserved Tyr95α ‘lynch pin’ from the CDR3α loop of the TRAV1-2α chain of MAIT TCRs (Figure 4C). Mutagenesis of this Tyr95α affected MAIT recognition and activation [24]. In the crystal structure of MAIT TCR-MR1-RL-6-Me-7-OH, the ligand's ribityl chain was positioned similarly within the MR1 ligand binding domain as 5-OP-RU and 5-OE-RU, forming the conserved H-bond with TCR-Tyr95α. However, the lumazine derivative was a far weaker MAIT agonist compared to the pyrimidine Ags. These raised some questions: (1) whether the interaction between Tyr95α and the ribityl moiety of the Ags is the key factor for MAIT stimulation? (2) Why, then, are the ribityl-pyrimidines significantly more effective in activating MAIT cells than ribityl-lumazines that still retain the ribityl chain?

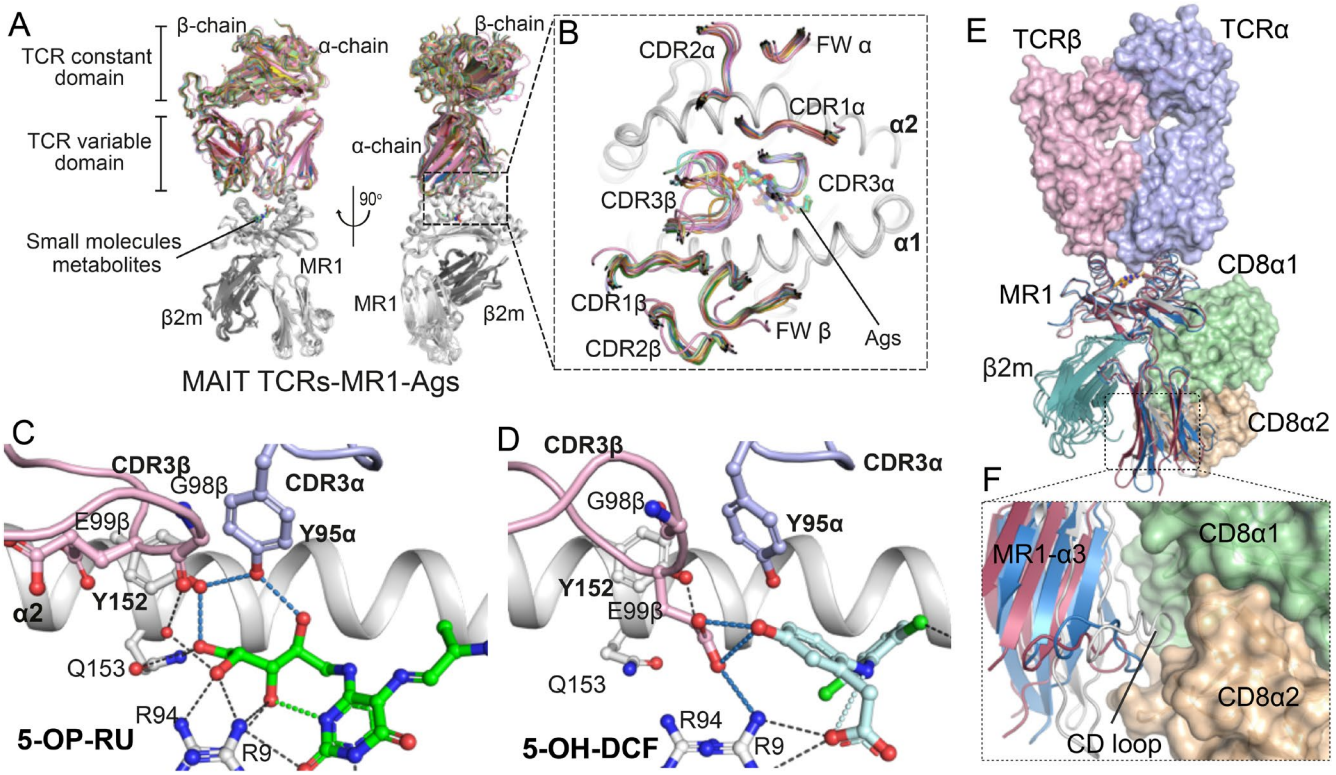

FIGURE 4 | TCR-MR1-Ag "Interaction Triad" formation with MAIT agonists. (A) Superimposition of the MAIT TRAV1-2^{pos} TCR-MR1-Ags structures with zooming into the top view of the Ag-binding cleft of the respective MR1 molecule, displaying similar docking of the TCR CDR loops atop the MR1 molecule (B). (C) MAIT TCR-MR1-Ag interaction triad (encompassing the 5-OP-RU, TCR-Tyr95α, and MR1-Tyr152). (D) Recognition of 5-OH-DC by classical MAIT AF-7 TCR. CDR3α and CDR3β are colored light blue and light pink, respectively. (E) Superimposition of the binary MR1-6FP, CD8αα-MR1-Ac-6-FP, and MAIT-TCR-MR1- Ac-6-FP complexes. (F) Zoomed view of the interaction between CD8aa and the CD loops of the a3 domains of MR1. MR1 molecules were shown as colored ribbons, and TCR and CD8αα were shown as surface representations.

(3) In addition to this interaction, how do the ribityl and non-ribityl components of riboflavin Ags influence MR1-TCR interactions and promote MAIT activation? (4) Why is 5-OP-RU the most potent MAIT agonist identified to date? (5) Finally, whether the ligand's capacity to form a Schiff base with MR1-Lys43 is necessary for the robust activation of MAIT cells and the upregulation of MR1? To address these questions, we recently explored the effect of several synthetic analogues of 5-OP-RU and RL-6-Me-7-OH, referred to as "Altered Metabolite Ligands" (AMLs) on the MAIT-MR1 axis [7].

4.2 | AMLs, TCR-MR1-AML "Interaction Triad" and MAIT Stimulation

This group of AMLs, alongside biochemical and functional assays, allowed us to make some general observations on the MAIT TCR-MR1 axis: (1) Any subtle modifications in the Ag scaffolds exhibit a significant effect on the stability of AMLs (half-life time ranging from 15 min to > 100 h), but without a significant impact on the stability of the refolded MR1-AMLs complexes in solution. (2) This inherent stability of the ligand, together with its capability to form a Schiff base adduct with MR1, as well as the precise chemical composition of the AMLs ribityl and non-ribityl components, modulates the Ag's capability to upregulate MR1 on the cell surface. (3) The efficiency of MR1 cell surface upregulation is inversely correlated to ligand polarity and the associated hydrophilicity of the MR1-AMLs interface. (4) Although the ribityl tail of riboflavin ligands is a prerequisite for MAIT stimulation, it is not essential, but probably even detrimental for MR1 upregulation. (5) MR1 upregulation and MAIT stimulation are not interdependent processes, where there is no apparent correlation between the ligand's capacity to upregulate MR1 on the surface of the cells and its capability to activate MAIT cells. (6) Our findings revealed that modifications at the 2′-OH and 3′-OH positions of the ribityl moiety significantly impact MAIT TCR recognition; however, the ribityl 4′-and 5′-hydroxyl moieties are not vital for MAIT cell activation.

Based on surface plasmon resonance (SPR) affinity results of MAIT TCRs with MR1-AMLs, MR1-AMLs tetramer staining, and the potency of AMLs to stimulate MAIT cells, we categorized the explored AMLs as strong agonists, moderate agonists, and non-activating ligands. The crystal structures of 11 MAIT TCR-MR1-AMLs ternary complexes provided molecular insights into the activation potency of these Ags. Namely, an intricate network of H-bond interactions—termed the "interaction triad"—that is formed between the evolutionarily conserved Tyr95α from the CDR3α loop of the MAIT TCRs, the ribityl-moiety, and Tyr152 of MR1 (Figure 4C). Indeed, this structural motif of "interaction triad" was found to be conserved among the published TCR-MR1-5-OP-RU structures of various MAIT TCRs of TRAV1-2 with TRBV6-1, TRBV6-4, and TRBV20, which explained similar affinities to MR1-5-OP-RU [10, 24]. So, this triad is the structural limiting

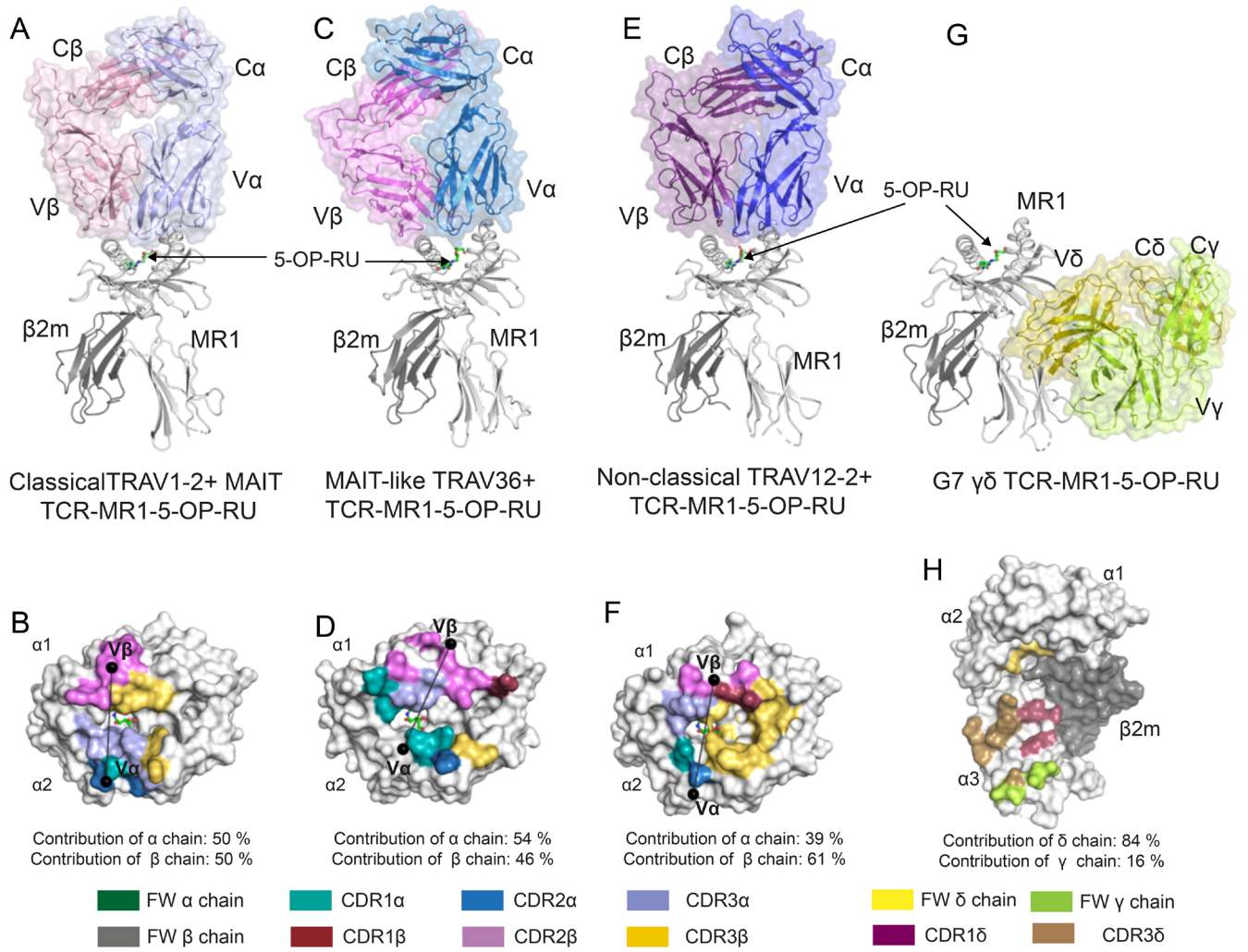

FIGURE 5 | Crystal structures of the ternary complexes of (A, B) A-F7 TCR-MR1-5-OP-RU (PDB; 4NQC), (C, D) TRAV36^{+} TCR-MR1-5-OP-RU (PDB; 5D7L), (E, F) non-classical TRAV12-2^{+} TCR-MR1-5-OP-RU (PDB; 6XQP), and (G, H) G7 γδ TCR-MR1-5-OP-RU (PDB; 6MWR). Top panels (A, C, E, G) show ribbon diagrams of the ternary complexes. The MR1 and β2-microglobulin molecules are colored white and gray, respectively, and 5-OP-RU is presented as green sticks. A-F7 TCRα, light blue; A-F7 TCRβ, pink; MAV36 TCRα, sky blue; MAV36 TCRβ, violet; TRAV12-2 TCRα, slate; TRAV12-2 TCRβ, violet-purple; TCRγ, lemon; G7 TCRδ, yellow orange. The lower panels (B, D, F, H) illustrate the TCR footprints on the molecular surface of MR1-5-OP-RU. The centers of mass of the TCR variable domains are shown as black spheres. The atomic footprints of CDR loops are colored as follows: CDR1α, teal; CDR2α, sky blue; CDR3α, light blue; Frameworks of α-chain, dark green; CDR1β, maroon; CD2β, violet; CDR3β, yellow orange; Frameworks of β-chain, dark gray; Frameworks of δ chain, yellow orange; Frameworks of γ chain, Lemon; CDR1δ, raspberry; CDR3δ, deep olive.

feature for MAIT stimulation, and if it is partially or entirely disrupted, the resulting Ags are either moderately or non-activating. Indeed, the structure of MAIT TCR-MR1-Ac-6-FP demonstrates that Ac-6-FP does not activate MAIT cells because it forms an H-bond with TCR-Tyr95α, but does not interact with MR1-Tyr152, hence distorting the interaction triad. In addition, we recently determined the crystal structures of two mouse MAIT TRBV13-2^{+} TCRs (M2A and M2B) in complex with mouse MR1-5-OP-RU [34]. These mouse MAIT TCRs are located on top of the mouse MR1-5-OP-RU and exhibit a conserved interaction triad and a molecular footprint that is comparable to that of human MAIT TCR-MR1-5-OP-RU complexes. Collectively, these studies revealed the conserved nature of the MAIT TCR–TCR-MR1-5-OP-RU interaction triad, with preserved selectivity for the microbial antigens.

4.3 | Diclofenac and Breaking the Rules

In addition to the ribityl-based MAIT agonists, diclofenac metabolites also activated Jurkat.MAIT-A-F7 cells, albeit less strongly. In the crystal structure of MAIT AF7-MR1-5-OH-DCF, the Tyr7 and Trp69 residues are molecularly shifted to make space for 5-OH-DCF to be accommodated within the MR1 pocket (Figure 4D). Getting this orientation in the MR1 pocket allowed the establishment of H-bond interactions with the CDR3β loop but also hindered the formation of the interaction triad between 5-OH-DCF, MR1-Tyr152, and TCR-Tyr95α. This deformed interaction triad significantly decreased the MAIT A-F7 activation efficacy toward 5-OH-DCF when compared to the riboflavin derivatives [12]. These results also support the critical roles that ribityl tail acquisition

and Schiff base formation play in the efficacy of MR1 ligand MAIT activation.

4.4 | Functional Co-Receptors for MAIT Cells

In humans, the majority of MAIT cells express CD8, which aids in modulating the activation of MR1T cells [17, 22, 65]. Recently, Souter et al. demonstrated that CD8 seems to co-stimulate MR1T cells via direct interactions with MR1 [65]. Nevertheless, CD8 engagement is not strictly necessary for MAIT (TRAV1-2^{pos}) TCR recognition of microbial Ags, but CD8 deficiency reduces or even eliminates immune responses to the low-affinity MR1T TCR recognition of non-microbial ligands, such as Ac-6-FP. We determined the crystal structure of the human CD8αα-MR1-Ac-6-FP complex, which revealed a largely conserved binding mode of CD8αα dimer with MR1, similar to its binding sites on the MHC-I molecules (Figure 4E,F). Specifically, the CD loop of the MR1 α3-domain projected into the area between the two CD8αα subunits, forming an extensive pattern of H-bonds and van der Waals interactions. Additionally, the MR1-α3 domain developed a broad network of interactions with the CDR1-like and CDR2-like loops of CD8α1 and CD8α2 subunits. However, a small interface and few interactions were observed between CD8αα subunits and the MR1-α2-domain and β2m (Figure 4F). Overall, the CD8αα dimer extensively interacts with the CD loop of the MR1 α3-domain and, to a lower extent, the α2- and β2m, revealing several aspects of CD8αα-MR1 complex formation and stability. We also showed that these CD8-MR1 interactions are critically important for: (1) boosting the specific recognition MAIT TRAV1-2^{pos} TCRs of the potent 5-OP-RU Ags and, consequently, enhancing cytokine production by MAIT cells, and (2) promoting the reactivity of the other MR1T subsets (see next section) to weaker activating MR1-Ags (non-microbial) or MR1 auto-reactivity [65]. In conclusion, CD8 acts as a coreceptor for MR1T cells and fine-tunes MR1T cell responsiveness.

5 | Diverse Populations of Non-MAIT MR1T Cells

We and other researchers described more diverse populations of MR1T cells that do not use the TRAV1-2 gene (referred to as TRAV1-2^{neg} MR1T cells), and therefore lack the Tyr95α residue previously found to be conserved in TRAV1-2^{pos} MAIT cells [17, 19, 22, 66–68]. These atypical T cells made up less than 0.1% of αβ-T cells in human blood. Some of these TRAV1-2^{neg} MR1T cells recognize microbial [19, 22] or non-microbial antigens presented by MR1 [9, 15, 17, 22, 66–69].

5.1 | TRAV1-2^{neg} MR1T Cells Specific for Microbial Antigens

Microbially reactive TRAV1-2^{neg} MR1T cells use a broad range of TRAV and TRBV genes, including TRAV36^{pos}, TRAV19^{pos}, and TRAV12-2^{pos} subsets and differ phenotypically from TRAV1-2^{pos} MAIT cells [19, 22, 70]. The crystal structure of the atypical MAV36 (TRAV36-TRBV28), in complex with MR1-5-OP-RU, revealed that, unlike the TRAV1-2^{pos} TCRs, the α-chain of MAV36 TCR had distinct contacts and docking footprint with MR1-5-OP-RU; however, the 2′-OH of the ribityl moiety was still recognized convergently via the CDR1α loop [22] (Figure 5). Here, the Asn29α of the CDR1α of TRAV36 chain formed H-bond and salt-bridge interactions with the ribityl chain and MR1-Tyr152, respectively, thereby partially compensating for the absence of Tyr95α. However, there were no longer any direct or indirect contacts between MR1-Tyr152 and the ribityl moiety (Figure 6A). The deformed MAV36 TCR-MR1-ligand interaction triad has a significant impact on MAV36 affinity for MR1-5-OP-RU, with > 5-fold lower affinity than classical MAIT TCRs. Collectively, we hypothesized that the dynamic compensatory interactions surrounding the TCR-MR1-ligand "interaction triad" are responsible for the 5-OP-RU activation potency of both TRAV1-2^{pos} and TRAV1-2^{neg} MR1 T cells.

Thus, the MR1 reactive TRAV1-2^{neg} cells may exhibit altered specificity toward the microbial ligands compared to the classical MAIT TRAV1-2^{pos} cells [19, 22, 50, 64, 68]. Here, Meermeier et al. identified a TRAV1-2^{neg} MR1T clone, referred to as "D462-E4" (TRAV12-2/TRBV29-1), that revealed a different pattern of anti-microbial activity [19]. Indeed, the D462-E4 T cell clone sensed the infection of both riboflavin-producing and auxotroph microbes *Streptococcus pyogenes* (group A strep), in an MR1-dependent manner. We solved the crystal structure of

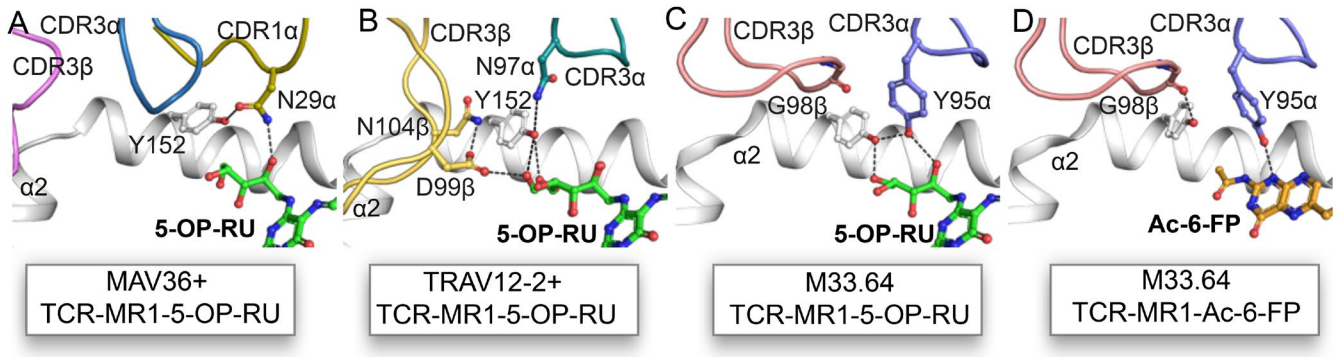

FIGURE 6 | Recognition of 5-OP-RU by TRAV36+, TRAV12-2+, and author-reactive M33.64T cell receptors recognition of MR1-Ags. (A) Interactions of the CDR1α-loop (olive) of the MAV36+ TCR with 5-OP-RU, including the locations of CDR3α (sky blue) and CDR3β (violet). (B) The interface between the CDR3β (yellow) of the TRAV12-2+ TCR, depicting the direct and indirect polar contacts with the 5-OP-RU ligand, whereby the CDR3α-loop (teal) did not directly interact with the ligand. (C, D) The interactions of the CDR3α- (light blue) and CDR3β (salmon) loops of the M33.64 TCR with MR1-5-OP-RU (C) and MR1-Ac-6-FP (D). The MR1 residues-ligands H-bonds are colored black.

the D462-E4 TCR-MR1-5-OP-RU complex that showed a different molecular footprint of the TCR on MR1, in comparison to TRAV1-2^{pos} TCR-MR1-Ag and MAV36^{pos} TCR-MR1-Ag complexes [70] (Figure 5). Surprisingly, the TRBV29-1 β-chain and its CDR3β loop dock over the F′-pocket of MR1, surrounding and projecting into it. Nevertheless, the CDR3β loop was still anchored close to the A′-cleft, and its Asp99β formed an H-bond with 5-OP-RU (Figure 6B). In summary, the heterogeneity in the MR1-restricted T cell repertoire leads to different TCR docking on MR1, thereby providing a wide scope for differing ligand recognition and specificities. Moreover, the microbial "MR1-ligandome" can include a wide range of metabolites not dependent on the riboflavin pathway; however, the identity of these ligands is still unknown.

5.2 | MR1 T Cells Specific for Self-Antigens

Several studies revealed that a subset of MR1T cells respond clonally to tumor cells and/or healthy cells, while not responding to microbial antigens, most likely via identifying self/endogenous MR1 ligands [17, 22, 66–68]. Some of these MR1T cells exhibit T-helper-like functional capabilities, express polyclonal TCRs, and can recognize diverse cancer types [68], with one clone, "MC.7.G5", that expresses TRAV38-TRAJ31/TRBV25-1 TCR, described to exhibit pan-cancer reactivity [17]. Nevertheless, Jurkat cells overexpressing the TCR from the MC.7.G5 clone preferentially recognized APC cells expressing the *MR1*04* allele, and this was implied not to be cancer-specific [71]. Thus, it became important to identify the ligands that MC.7.G5 TCR-T cells recognize to better understand the observation that cancer cells are preferentially recognized by these T cells. Recent evidence indicates that Pyridoxal (PL) bound to MR1 activates MC.7.G5.TCR-T cells, yet the molecular basis of this recognition is unknown [13]. Of note, MC.7.G5.TCR-T cells are activated to a much greater level by APCs expressing *MR1*04*, to which PL binds with greater affinity than *MR1*01*. There may be other ligands recognized by MC.7.G5 cells beyond PL, considering the promiscuous character of some discovered MR1T clones.

In another report, another class of MR1 endogenous-Ags associated with tumor metabolic dysregulation was discovered to be the nucleobase-containing adducts [15]. While some of these self-adducts stimulated at least one MR1T clone, this highlighted the promiscuous Ag recognition of MR1T cells [15]. Interestingly, MR1 tetramers loaded with M_1Ado adduct were able to stain some tumor-infiltrating T cells isolated from lung cancer biopsies [15]. Given the recent finding about the oligomorphic nature of MR1 and that many tumors stimulate MR1T cells, such TCRs could be potentially used in cancer immunotherapy. However, the efficient translation of cancer-targeting MR1 T cells from bench to clinic requires a better understanding of the ligands they recognize and determining the molecular underpinning of the MR1T TCRs' recognition of these tumor Ags.

5.3 | MR1 Auto-Reactive αβ and γδ MR1T Cells

Different populations of αβ and γδMR1T cells were described to exhibit MR1-centric reactivity, rather than antigen-centric reactivity [22, 72, 73]. Specifically, Gherardin et al. described a human typical MAIT T cell clone (M33-64) exhibiting MR1 auto-reactivity when it was stained with MR1-WT tetramer loaded with various antigens, including Ac-6-FP lacking the ribityl moiety, in addition to the MR1-Lys43A tetramers (unloaded with Ags). The biochemical studies confirm the binding of the M33-64 TCR to MR1-5-OP-RU and MR1-Ac-FP, but with different binding affinities. The M33.64 TCR ternary complex structures with MR1-Ac-6-FP and MR1-5-OP-RU were very similar (Figure 6C,D), albeit with subtle minor variation around the ligands. In the M33.64 TCR-MR1-5-OP-RU ternary structure, the TCR-MR1-ligand interaction triad was observed, explaining the TCR recognition of 5-OP-RU (Figure 6C). In comparison, the M33.64 TCR-MR1-Ac-6-FP structure showed an H-bond between TCR Tyr95α and the pterin ring. In addition, the MR1-Tyr152 was displaced to form another H-bond with Gly98β of the CDR3β, thus the 'interaction triad' was observed (Figure 6D). Collectively, this suggests that the autoreactivity of M33.64 is attributable to the CDR3β loop of the TCR.

In addition to autoreactive αβ AMR1T cells, distinct subsets of human Vδ2^{-} γδT cells have been isolated from healthy donor PBMCs and tissues exhibiting inherent autoreactivity to MR1 [72, 74]. Namely, the γδTCRs of the G7 (Vδ1–Vγ9), G19 (Vδ1–Vγ8), G21 (Vδ1–Vγ8), and G83.C4 (Vδ3–Vγ8) T cell clones were stained with both MR1-5-OP-RU and MR1-6-FP tetramers [72, 74]. This auto-reactivity was validated using functional and biochemical assays. The crystal structure of the G7 γδTCR-MR1-5-OP-RU ternary complex showed a significantly distinct mode of binding for the G7 γδTCR in comparison to the previously identified αβTCR in ternary complexes, where it binds the underside of MR1, interacting with the MR1 α3 domain [72]. The G7 γδTCR-MR1-5-OP-RU interface interactions were primarily mediated by the δ chain of the TCR to dictate the docking and stability of G7 TCR binding with MR1 (Figure 5G,H). We also determined the ternary crystal structure of the G83.C4 (Vδ3–Vγ8) γδTCR with MR1-5-OP-RU, uncovering a third mode of binding, where the G83.C4 TCR docked on the side of the MR1 α2-domain toward the A′ pocket of the ligand-binding groove. Here, the Vγ8 chain docked centrally over the α1- and α2-helices, whereas Vδ3 chain was positioned skewed to the MR1 α2-domain, close to the side of the MR1 ligand-binding groove (Figure 5H). The δ chain also dominated the interface between the G83.C4 TCR and MR1, where the CDR3δ loop was positioned near the aromatic roof of the MR1 antigen-binding pocket. Collectively, we have characterized a population of human γδ MR1T cells with a variety of phenotypes that adopt diverse binding modes with MR1, including beneath the MR1 antigen-binding cavity. These γδ MR1T cells were discovered in both normal and pathological tissues, indicating a potential function in humans.

6 | Conclusions and Future Directions

Since 2012, our structural data have revealed the versatile antigen-binding capacity and adequate plasticity of MR1 to accommodate structurally diverse chemical scaffolds. The MR1 ligands discovered thus far are captured within the A′ pocket of MR1, and no ligands have been described to reside in the shallower F′ pocket. Further, the MR1 cleft is larger than the known ligands, which suggests that significantly bigger and architecturally diverse scaffolds bind MR1. Indeed, in the

context of steady-state [17, 68], bacterial infections [19, 50] and cancers, there is evidence for the existence of unknown MR1 ligands. Thus, the repertoire of the MR1 ligandome will likely continue to grow, and some of these may serve as antigens for distinct populations of MR1 T cells. For example, the determination of the identity of the tumor-associated MR1 antigens will enable the production of MR1 tetramers that will aid in the assessment of the physiological roles and prevalence of the cancer-reactive MR1T cells. Overall, further research is required to assess the significance of the MR1 binding ligands in different contexts such as infections, cancers, and other diseases.

MR1 reactive T cells exhibit selective reactivity toward metabolic Ags associated with MR1, suggesting key roles in host immunity. We showed that different TCR usage among the MR1T cells facilitates distinct TCR docking modalities on MR1, which in turn led to divergent mechanisms of MR1-antigen reactivity. It is well established that the classical MAIT-MR1 axis is crucial for anti-microbial immunity; nevertheless, the functions of other subsets of MR1T cells are still being uncovered, even if some clones exhibit anti-cancer capabilities. Therefore, further investigation is required to determine the molecular basis underpinning MR1T TCRs recognition of various self and non-self Ags presented by MR1 and to ascertain the functional capability and physiological relevance that various MR1T cell subsets play in health and diseases.

Acknowledgments

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Conflicts of Interest

J.M. and J.R. are inventors on patents describing MR1 ligands and MR1-tetramer reagents.

Data Availability Statement

The authors have nothing to report.

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