Published in final edited form as:

Immunol Rev. 2015 March ; 264(1): 154–166. doi:10.1111/imr.12271.

MR1-restricted mucosal associated invariant T (MAIT) cells in the immune response to *Mycobacterium tuberculosis*

Marielle C. Gold^{1,2,3}, Ruth J. Napier¹, and David M. Lewinsohn^{1,2,3}

¹Pulmonary & Critical Care Medicine, Oregon Health & Science University, Portland, OR

²VA Portland Health Care System (VAPORHCS), Portland, OR

³Molecular Microbiology & Immunology, Oregon Health & Science University, Portland, OR

Summary

The intracellular pathogen *Mycobacterium tuberculosis* (Mtb) and its human host have long co-evolved. Although the host cellular immune response is critical to the control of the bacterium information on the specific contribution of different immune cell subsets in humans is incomplete. Mucosal associated invariant T (MAIT) cells are a prevalent and unique T-cell population in humans with the capacity to detect intracellular infection with bacteria including Mtb. MAIT cells detect bacterially derived metabolites presented by the evolutionarily conserved major histocompatibility complex-like molecule MR1. Here we review recent advances in our understanding of this T-cell subset and address the potential roles for MR1-restricted T cells in the control, diagnosis, and therapy of tuberculosis.

Keywords

MR1; mucosal associated invariant T cells (MAIT); tuberculosis

The natural history of tuberculosis suggests humans have evolved resistance mechanisms to control infection with Mtb

Mycobacterium tuberculosis (Mtb) remains a leading cause of infectious mortality worldwide (1). Although the Mtb bacillus was identified as the causative agent of tuberculosis (TB) in 1882, substantial gaps exist in our understanding of the pathogenesis of the disease and the mechanisms by which the human immune system contains the bacterium. It has long been argued that humans have evolved innate resistance to control Mtb; however, the lack of natural infection animal models has made it difficult to define these mechanisms (2–5). Nonetheless, exposure to Mtb, which is transmitted via the aerosol route, could result in a number of outcomes. First, effective mucociliary clearance as well as other airway-associated mechanisms could result in the clearance of the bacterium prior to infection. Second, Mtb exposure could result in intracellular infection, but effective innate

Correspondence to: Marielle C. Gold, Oregon Health & Science University, Pulmonary & Critical Care Medicine, 3181 SW Sam Jackson Park Road, Mail code VA R&D 11, Portland, Oregon 97239, Tel.: +1 503 220 3428, goldm@ohsu.edu.

The authors have no conflicts of interest to declare.

and/or adaptive mechanisms would clear the bacterium. Third, Mtb exposure could result in an infection that persists, disseminates, or progresses. Although no conclusive test exists to determine if an individual has cleared Mtb, the measurement of Mtb-specific T-cell immunity has provided circumstantial evidence. Here, the measurement of Mtb-specific T cells, using either γ -interferon release assays (IGRA) (6) or less specifically using the tuberculin skin test (TST) (7) has been used to define those who are productively infected. However, household contact studies show that 30–50% of individuals repeatedly exposed to Mtb remain TST-negative (8). In this context, it is impossible to distinguish the absence of infection from clearance. Indeed, transient conversion may be a better indicator and has been documented in a number of cases (6, 9, 10), suggesting that T-cell responses to Mtb antigens were generated and waned due to antigen clearance.

The cellular immune response is critical to the control of tuberculosis

Another indication that humans have evolved mechanisms to control Mtb is apparent in those individuals with a positive IGRA or TST, in which more than 90% will remain healthy. Specifically, containment of infection is attributable to an effective cellular immune response. Both human and animal models have shown that different T-lymphocyte subsets and their effector molecules are required for preventing the uncontrolled growth of the bacterium. Nonetheless, the distinct contribution of each cell subset is difficult to ascertain primarily because in experimental knockout animal models the remaining T lymphocytes may provide compensatory mechanisms. This finding highlights how in humans defining the specific role of each cell is challenging, particularly in the context of rational vaccine design (11). Nevertheless, a clear protective role exists for T-cell subsets and their associated effector molecules, particularly the cytokines IFN- γ and TNF. Specifically, Th1 CD4⁺ T cells have been shown to be essential to control bacterial growth. Mtb-infected mice and humans lacking CD4⁺ T cells such as those co-infected with human immunodeficiency virus (HIV) (12, 13) rapidly progress to TB. Consistent with this, Th1-type cytokines and IL-12 and their receptors are associated with protection against mycobacterial infections (14). Humans who harbor the bacterium and are treated with TNF blockers are likely to rapidly progress to TB (15). While CD4⁺ T cells are a primary source of IFN- γ , conventional CD8⁺ T cells and unconventional TCR $\alpha\beta$ bearing lymphocytes such as MHC-related protein 1 (MR1)-restricted mucosal associated invariant T (MAIT) cells (the subject of this review), CD1 family-restricted T cells such as iNKT cells and GEM cells (reviewed in this volume by Moody and Van Rhijn), $\gamma\delta$ TCR T cells, as well as NK cells, and potentially innate lymphoid cells, also play a role in either the early control of infection with Mtb or in the acquisition of a successful adaptive response.

While CD4⁺ and CD8⁺ T cells share a number of functions CD8⁺ T cells have attributes that allow for their unique contributions to bacterial control. Specifically and germane to infection with Mtb which is an intracellular pathogen, CD8⁺ T cells can detect those cells that are directly infected with intracellular bacteria. CD8⁺ T cells perform this function via their T-cell receptor (TCR) or antigen receptor. Classical, or HLA-Ia-restricted T cells detect antigens in the context of cell surface HLA-Ia molecules that sample and present pathogen-derived peptide antigens. HLA-Ia is present on all nucleated cells, resulting in the ability of CD8⁺ T cells to target virtually any cell in the body. This is in contrast to CD4⁺ T cells

whose TCRs recognize peptide ligands presented by HLA-II molecules that are largely limited to professional antigen presenting cells such as those of myeloid lineage, and B cells. Furthermore, CD8⁺ T cells preferentially lyse Mtb-infected cells with high bacterial burden (16). In addition to these conventional CD8⁺ T cells, a number of additional CD8⁺ T cells subsets can be distinguished by their TCR usage, HLA restriction, and their affinity for particular ligands.

In this review, we focus exclusively on a subset of CD8⁺ T cells known as MAIT cells. Human MAIT cells were originally identified in 1993 by virtue of their expression of the semi-invariant TCR *TRAV1-2/TRAJ33* (17). Subsequently, MAIT cells were shown to be restricted by the evolutionarily conserved MHC-like molecule MR1 (18). However, major advances in their characterization have occurred in the last several years. Specifically, a physiological role for MAIT cells was identified in their ability to detect infection with a variety of bacteria and fungi including Mtb (19, 20). Subsequently and critical to the study of MAIT cells, vitamin B metabolites were identified as ligands for MR1 with the capacity to activate MAIT cells (21). A number of properties suggest MAIT cells are ideally suited to contribute to the containment and control of infection with Mtb. MAIT cells are universally prevalent in humans, including Mtb naive individuals. MAIT cells are endowed with the capacity to function rapidly following detection of an Mtb-infected cell (22). MAIT cells are enriched in the human respiratory tract, where Mtb first interacts with its human host (19). In response to Mtb-infected cells, MAIT cells can produce IFN- γ , TNF, and lyse infected cells (19, 20, 23). These aforementioned functions all point to the possibility that MAIT cells contain and control Mtb upon initial exposure. However, MAIT cells can also serve as an early source of the cytokines that are essential to the acquisition of effective conventional T-cell immunity, suggesting they have the capacity to shape the downstream adaptive immune response. Finally, as described herein, MAIT cells have recently been shown to be more heterogeneous than previously thought. This highlights the likelihood that MAIT cells may have yet unknown functions and leaves open the possibility that MAIT cells adapt and potentially contribute to immunological memory in a manner analogous to conventional T cells. Accordingly, we postulate a number of practical uses for MAIT cells including their use as a diagnostic marker for exposure to Mtb and in vaccination and therapeutic strategies.

MAIT cells: an unconventional CD8⁺ T-cell subset

Conventional T cells recognize antigen presented in the context of an HLA-Ia molecule through the TCR, a heterodimer comprised of a TCR α and TCR β chain. TCRs are the result of somatic recombination from a set of TCR genes in combination with random nucleotide additions. The recombination event and TCR α and TCR β pairing results in an array of highly diverse TCRs that allow for the recognition of a broad range of antigens displayed by HLA-I molecules. Subsequent to TCR rearrangement, T cells undergo selection by thymic epithelial cells, and those that survive the process of positive and negative thymic selection exit to the periphery. Conventional T cells that enter the periphery exist as naive T cells that circulate between the blood and the lymph. Once naive T cells encounter dendritic cells bearing cognate peptide/HLA in lymph nodes, T cells are primed and undergo clonal expansion. Stimulation of the TCR in conjunction with costimulatory molecules results in T cells that divide, differentiate, and gain effector function over the course of several days.

MAIT cells are similar to conventional T cells in that they also undergo TCR rearrangement and selection in the thymus. However, in contrast to conventional T cells, MAIT cells have a unique developmental pathway that results in their ability to perform as functional T cells prior to leaving the thymus, and by inference prior to exposure to peripheral antigens. This inherent functional capacity has resulted in MAIT cells being termed innate or innate-like T cells. Similarly, the more fully characterized T-cell subset known as iNKT cells (24) shares this capacity. MAIT cells and iNKT cells are both characterized by the expression a semi-invariant TCR. In humans, MAIT cells were initially defined by the expression of a TCR α chain encoded by the *TRAV1-2-TRAJ33* gene and iNKT cells by *TRAV11-TRAJ18* (17). Both populations are distinguished by their restriction by non-polymorphic MHC-like molecules. MAIT cells are restricted by MR1 and iNKT cells by CD1d, each on chromosome 1 in humans. MR1 has been shown to bind microbial derived vitamin B metabolites, while CD1d binds lipid-containing ligands. Thus, both of these T-cell subsets use a semi-invariant TCR, are restricted by an oligomorphic MHC-like molecule, and recognize non-peptide ligands. While MAIT cells are prevalent in humans and less frequent in laboratory mice, the opposite is the case for iNKT cells.

Development of MAIT cells

In the recombination process that generates the TCR chains, the semi-invariant TCR α chains used for MAIT and iNKT cells can be preferentially generated compared to those generally used conventional TCRs (25, 26). For example, MAIT TCRs shared among individuals can be generated solely from germ-line encoded sequences or with only 1 or 2 nucleotide additions (18, 26, 27). Therefore even prior to the selection of MAIT and iNKT cells, the TCR α chains for these subsets are more efficiently generated. Subsequently, MAIT and iNKT cells are selected in the thymus by their respective MHC-like molecules, namely MR1 for MAIT cells and CD1d for NKT cells. While a number of self-lipid antigens have been identified as candidates for iNKT cell selection (24), a self-ligand for MR1 remains to be identified.

In contrast to conventional T cells, innate-like T cells undergo a developmental pathway distinct from conventional T cells that results in the thymic acquisition of functional capacity. While the details of the developmental pathway for MAIT cells are incomplete, the developmental pathway for innate cells is distinct from conventional T cells. Conventional T cells are selected on thymic epithelial cells, while innate-like T cells are selected on hematopoietic cells. In mice, CD1d (28), MR1 (29), and H2-M3-restricted innate T cells (30) are selected on hematopoietic cells. Hematopoietic cell selection appears sufficient for the acquisition of thymocyte effector capacity (31), possibly due to influences on the strength of TCR signaling as well as interactions with SLAM family members (32). Specifically, CD4⁺CD8⁺ double positive (DP) thymocytes have been shown to select iNKT cells and MAIT cells in mice (29). In human cells, MR1 has generally been difficult to detect on the cell surface. However, high levels of MR1 are expressed by a distinct subset of human DP thymocytes (22) that could have the capacity to select MAIT cells.

In contrast to iNKT cells, the cell surface receptors and transcription factors required for MAIT cell development remain to be identified. Studies of murine iNKT cell development

have shown that the accessory SLAP/SAP family members are important but not required for the development of iNKT cells. In human MAIT cells, reliance on SAP was suggested to be unnecessary, because humans lacking SAP still have MAIT cells (33). However, as with iNKT cells accessory molecules may play important but nonessential roles in MAIT cell development.

The master regulator for iNKT cell effector programming is the transcription factor PLZF. Intrathymic expression of PLZF directs the iNKT cell effector capacity and also instructs iNKT cells to home to non-lymphoid sites after thymic egress. PLZF alone can confer the iNKT cells its innate effector phenotype (34, 35). Although PLZF mRNA has been detected in MAIT cells isolated from adult human blood (33, 35), the requirement for PLZF in MAIT cell programming remains controversial. For iNKT cells, a TCR agonistic signal has been shown to be required for PLZF induction (36). Because MAIT cells are presumably selected in a TCR-dependent fashion, it is difficult to reconcile why MAIT thymocytes express low levels of PLZF (37). However, MAIT cells from the mucosal tissues of second trimester human fetuses were Ki67 positive, had the capacity to proliferate, expressed high levels of PLZF, and displayed a mature phenotype (37). These findings suggest that in humans, in the absence of *bona fide* microbial colonization, MAIT cells in mucosal tissues are being stimulated in a TCR/ligand dependent fashion by either endogenous or exogenous ligands that remain to be identified. In humans, PLZF is expressed by a number of different lymphocyte subsets suggesting that, in contrast to mice, PLZF may play a more general role in lymphocyte development (38). Ultimately, whether PLZF is sufficient for imparting a specific phenotype to human MAIT cells remains to be determined.

Post-thymic development and maintenance of MAIT cells

Once innate-like T cells exit the thymus, they can be found as naive cells in the cord blood and as expanded activated effectors in adult peripheral blood (22). This further supports the idea that MAIT cell programming in the thymus endows them with innate effector function prior to encounter by exogenous microbial antigen that results in an activated phenotype. Innate T cells are thought to preferentially localize to non-lymphoid tissue sites. For example, iNKT cells preferentially localize to the liver and the spleen. For MAIT cells, knowledge regarding the signals that mediate MAIT thymus egress or localization to specific tissue sites is incomplete. For liver-resident iNKT, localization and retention is dependent on PLZF (39). MAIT cells were termed mucosal due to the finding they were present in the intestinal lamina propria (18). MR1 tetramer positive cells have been identified in the small intestine (40); however, the extent to which MAIT cells are enriched in the intestine is unclear. As is the case for iNKT cells, MAIT cells are present in the human liver where they can represent over 20% of T cells (41, 42). Furthermore, MAIT cells, as defined by the expression of CD3, TRAV1-2, and CD161^{hi} expression have been identified in a variety of sites including peripheral blood, lymph nodes, lung, liver, rectum, small intestine, and skin. Moreover, MAIT cells are present in the human airway where they represent about 20% of CD8⁺ T cells (Gold and Lewinsohn, unpublished data). At present, analyses of MAIT cells at different sites have been performed on a limited number of individuals and therefore the extent to which MAIT cells localize to specific tissue sites remains to be determined.

Evidence for MAIT cell adaptation in the periphery

While MAIT cells are present as effectors in the thymus and cord blood, a number of findings suggest MAIT cells can adapt to their environment in response to microbial exposures. In mice, expansion of MAIT cells in the periphery is dependent on microbial colonization. MAIT cells are undetectable in the periphery of germ-free mice and administration of MAIT-activating bacteria results in peripheral expansions of MAIT cells (18, 20). In humans, the frequency of MAIT cells, as defined by their TCR rearrangement, is increased in the peripheral blood compared to the thymus (25). When defined by their ability to produce TNF in response to Mtb, pathogen-reactive MAIT cells increase in frequency in peripheral blood compared to cord blood (22). These findings support the hypothesis that exogenous antigens are responsible for MAIT cell expansion and maintenance.

It was initially postulated that the usage of a semi-invariant TCR would be associated with limited ligand discrimination (43). However, recent studies point to greater MAIT TCR heterogeneity than initially defined based on the usage of the canonical *TRAV1-2/TRAJ33* TCR α chain (17, 18, 27). Specifically, the *TRAV1-2* chain can rearrange with additional different *TRAJ* genes including *TRAJ12*, *TRAJ20*, and *TRAJ33* (19, 40, 44, 45). Furthermore, random amino acid additions in the CDR3 region contribute to further diversity in the CDR3 region used to detect the MR1/ligand complex. Moreover, the number of TCR β chains associated with the MAIT cell TCR is more diverse than originally reported.

At present, the degree of MAIT cell TCR heterogeneity in the thymus is unknown, and hence the TCR repertoire of MAIT cells is not known. Analysis of the TCR repertoire of functional pathogen-reactive MAIT cells from 4 individuals in response to 3 different classes of microorganisms demonstrated the selective usage of MAIT cells TCRs in the response to each pathogen for a given subject. These data suggest that MAIT cell TCR usage is a reflection of the host's microbial exposure history (44). Although no unique TCR could be found to be uniquely associated with a specific pathogen, the TCRs used to detect each pathogen within one individual were distinct. This study would suggest that different classes of antigens or microorganisms elicit MAIT TCRs that have the capacity to selectively recognize discrete ligands. Furthermore, the oligoclonal expansions that were observed for each individual would support this hypothesis. This finding suggests that MAIT cells, along with conventional T cells, could have the capacity to exhibit immunologic memory. Longitudinal studies of individuals with well-defined microbial exposures will be needed to further explore this hypothesis.

MAIT cells detect intracellular infection via MR1/ligand

MR1 is a highly conserved MHC-like molecule with antigen presentation function. The MHC-like molecule MR1 is the restricting molecule for MAIT cells. MR1 shares a number of structural features with MHC-Ia molecules. Both encode an immunoglobulin superfamily heavy chain (HC) that combines with a light chain known as β 2-microglobulin (β 2m). The HC and β 2m of MHC-Ia molecules combine to form a groove that is receptive to 8–11 amino acid peptides while the MR1 HC/ β 2m complex binds small organic compounds. In sharp contrast to MHC-Ia molecules that are highly polymorphic, a single gene encodes MR1. MR1 is the most evolutionarily conserved MHC-like molecule among mammals (43,

46–48) and shares homology with *Xenopus* and chicken nonclassical MHC molecules (49). Like CD1d, MR1 is encoded on chromosome 1 and is distinct from the MHC locus on chromosome 6.

Studies by Hansen and colleagues (43, 50–53) provided initial evidence that MR1 had antigen presentation function. Specifically, MR1 mutations of residues previously shown to disrupt the ability of MHC-Ia molecules to bind peptide and interact with the TCR showed these to be similarly critical to MAIT hybridoma activation (51). Recent work from the laboratories of McCluskey/Rossjohn and Adams (54–58) has elucidated the molecular basis for MAIT TCR recognition of ligands presented in the context of MR1. The crystal structure for human MR1 demonstrated similar folds in the heavy chains between MR1 and HLA-A2 that allows for antigen loading (21). However, as will be described below, ligands for MR1 were found to be ribityllumazines derived from vitamin B synthesis (21, 59). As predicted by Huang et al. (51), both groups have demonstrated that the MAIT cell TCR is oriented in a perpendicular manner with regard to the MR1/ligand complex, an orientation also described for classical T cell recognition of HLA-I. In contrast, the iNKT TCR is oriented in a parallel manner in conjunction with CD1d/ligand. These crystallographic studies suggest that the TCR α and TCR β chains both contribute to the recognition of MR1/ligand. However, studies in which mutagenesis of both the TCR and MR1 were performed to define residues critical to MAIT cell activation suggest that the TCR α chain contributes to the majority of the interaction between the MAIT TCR and MR1 itself consistent with a requirement for conserved residues in the TRAV1-2 chain. Notably, TRAV1-2/TRAJ rearrangements containing a tyrosine at position 95 of the TCR α have been shown to be essential for antigen recognition by the MAIT TCR (54–56, 58). The role of the TCR β chain appears to predominantly accommodate recognition of different MR1 ligands that themselves can alter the conformation of the MR1 molecule (55, 58). Thus MAIT cells have the ability through different TCRs to bind MR1 in complex with a number of different ligands.

MR1 ligands

Prior to the identification of MR1 ligands, Young *et al.* (60) provided evidence that MR1 bound an unexpected class microbial-derived ligands that were proteinase K resistant and not common lipids. Recently, vitamin metabolites derived from the riboflavin biosynthesis pathway were described as a new class of T-cell antigens capable of activating MAIT cells. Riboflavin, also known as vitamin B2, is an essential micronutrient of the human diet. Humans cannot synthesize riboflavin *de novo* and are therefore dependent on food goods and commensal organisms as a primary source. Riboflavin deficiencies have been correlated with stunted growth, failure to thrive, and increased mortality (61). While most microbes and all plants produce riboflavin, the biosynthetic pathways are markedly different between fungi and archaea. Biosynthesis of riboflavin begins with one molecule of GTP, which undergoes several enzymatic reactions that yield potentially several pyrimidine precursors, including, 6,7-dimethyl-8-ribityllumazine, and finally riboflavin (62).

Kjer-Nielson *et al.* (21) showed that riboflavin derivatives secreted by *Salmonella* including, 6-hydroxymethyl-8-d-ribityllumazine (rRL-6-CH₂OH), 7-hydroxy-6-methyl-8-d-ribityllumazine (RL-6-Me-7-OH) and 6,7-dimethyl-8-d-ribityllumazine (RL-6,7-diMe) bind

MR1 and activate T cells expressing the canonical MAIT cell TCR. Although all three molecules bound MR1 and activated MAIT cells, rRL-6-CH₂OH was found to be the most potent suggesting the potential for multiple MAIT cell ligands. The same study showed that 6-formyl pterin (6-FP), derived from vitamin B9 or folic acid, also bound to MR1, but was unable to activate MAIT cells. The diversity of TCR usage described above in conjunction with the ability of MR1 to bind small molecule ligands suggests the possibility that additional ligands and antigens remain to be found.

In support of the hypothesis that MAIT cells recognized riboflavin metabolites, microbes proven to activate MAIT cells, including *Mtb*, have the genes required for riboflavin biosynthesis. In contrast, bacteria that are non-stimulatory to MAIT cells, such as *Listeria monocytogenes* and *Enterococcus faecalis*, lack this pathway (19, 20). More recently, Corbett *et al.* (59) showed that molecules in the riboflavin biosynthesis pathway directly upstream of RL-6,7-diME, namely 5-amino-6-D-ribitylaminouracil (5-A-RU), are necessary for the generation of MAIT cell antigens. Their data suggest that 5-A-RU can react spontaneously with other organic small molecules to produce new and distinct compounds that can bind MR1 and activate MAIT cells. These molecules, termed ‘neo-antigens’, include 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU) and 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU). These data suggest that in addition to previously defined riboflavin intermediates, molecules that are byproducts of the pathway may represent new antigens for MAIT cells. This is intriguing, as the riboflavin pathway and physiological byproducts hypothetically differ between microbes suggesting there may be microbe-specific antigens. This would support new evidence of MAIT TCR heterogeneity (40, 45, 58) and particularly the potential for unique TCR repertoires of MAIT cells that are specific to different microbial classes in human peripheral blood (44).

MR1-dependent antigen processing and presentation

The processing and presentation of protein antigens in the context of HLA-Ia, which has been extensively characterized, allows for the presentation of peptide antigens derived from the cytosol or phagocytic compartments (63, 64). Cytosolic proteins are degraded into peptides via the proteasome, and peptides translocated via the transporter associated with antigen processing and presentation (TAP) to the endoplasmic reticulum (ER), where they are loaded into HLA-Ia molecules. This carefully choreographed process is termed antigen processing and is facilitated by HLA-Ia chaperones at each stage. In brief, classical HLA-Ia molecules are stabilized by calnexin prior to association with β 2m and with calreticulin prior to loading with peptide. After the peptide-loading complex (TAP, tapasin, calreticulin, and ERp57) facilitates the loading of HLA-Ia binding peptides, these loaded molecules then translocate to the cell surface.

While it is believed that all cytosolic proteins can gain access to the HLA-Ia processing pathway, those present in phagocytic and endosomal compartments can also be loaded onto HLA-Ia. Given the relatively low abundance of these proteins, it has been postulated that specialized mechanisms have been developed to sample particulate-associated proteins, such as those derived from *Mtb* that resides in a phagosome. Here, there is evidence to support

direct sampling of the phagosomal environment, as well as sampling of proteins that are secreted into the cytosolic environment (63, 64).

Peptides derived from pathogens such as viruses and intracellular bacteria are not inherently distinct from those derived from the host. As a result, specificity in CD8⁺ T-cell recognition of pathogen-derived peptides rests in a TCR repertoire that has the capacity to respond to diverse peptidic ligands but selected to minimize self-reactivity. In contrast, the observation that MR1 can present non-peptidic, bacterially derived small molecules such as the ribityllumazines provides inherent selectivity in the presentation of bacterially derived compounds, but raises a number of questions regarding the processing and presentation of these small molecules. Given the potential abundance of these compounds in environments such as the intestine, it would seem that the loading of MR1 must be carefully regulated to avoid indiscriminant MAIT cell activation.

MR1 is not normally expressed on the cell surface

While MR1 is thought to be widely expressed based on transcriptional profiling (46, 52), little is known regarding the abundance and intracellular location of the native protein. In some regards, MR1 shares features of HLA-Ia in that it is β 2m associated and must be correctly folded prior to expression on the cell surface (50–52). In contrast to HLA-Ia, MR1 is not expressed on the cell surface unless it is overexpressed (51). However, increasing evidence suggests that outside of the thymus, cell surface expression of MR1 is dependent on the presence of an exogenous ligand. In the presence of intracellular infection with Mtb, modest cell surface expression of MR1 could be seen (19), an observation concordant with the finding that intracellular infection could result in the activation of MR1-restricted MAIT cells (19, 20). This hypothesis is further supported by the recent observation that addition of the MR1 ligand 6-FP resulted in cell surface expression of MR1 (58). These findings leave open the question as to whether or not intracellular MR1 contains a native ligand, and if so the mechanism by which it becomes loaded with bacterially derived ligands. The strongest evidence for the presence of an endogenous ligand comes from the work of Hansen and colleagues (53). In the context of exogenous expression of both mouse and human MR1, the group finds that properly folded MR1 is required for MR1 cell surface expression, and that cell surface expression is associated with the activation of self-reactive MAIT cell hybridomas. We note that overexpression of endogenous MR1 does not result in the activation of Mtb-reactive MAIT cell clones (Gold and Lewinsohn, unpublished observations). Further support of bacterially derived ligands being discrete from endogenous ligand comes from Young *et al.* (60). Here, the interaction of the MAIT TCR was tested for its ability to recognize MR1 in the presence or absence of bacteria. Following mutational analysis of the MAIT TCR along with the MR1 molecule, differential amino acid usage was found to mediate TCR interaction in the presence of the bacterial antigen. At present, the structure of the endogenous ligand is not known.

Although the precise mechanisms by which MR1 antigens are processed and presented are not known, current evidence suggests that the protein resides in an endosomal compartment. Early work done using epitope-tagged hMR1 expressed in the HeLa cell line demonstrated dependence on β 2m, and interestingly demonstrated association of the hMR1 with proteins

associated with the peptide loading complex (50). However, mice deficient in TAP (18, 27) have enhanced MAIT cell frequencies. Furthermore, work using self-reactive MAIT cell hybridomas demonstrated that neither proteasome function, TAP, calreticulin nor MHC-Ia proteins were required for MAIT cell activation (52), a finding confirmed by the TAP independent recognition of Mtb-infected cells (19). As a result, it would appear that MR1 antigen processing occurs in a compartment distinct from that of HLA-Ia. In support of this hypothesis, Huang *et al.* (52) found that following the introduction of intact MR1 into fibroblast (LTK-) and B-cell lines (CH27), the MHC-II associated chaperones invariant chain (Ii) and DM were associated with MR1. Furthermore, overexpression of each protein was associated with enhanced activation of self-reactive MAIT cell hybridomas, and inhibition of vacuolar acidification resulted in diminished MAIT cell activation. These findings suggested that MR1 could reside in a late endosomal compartment, and that processing of MR1 self-antigen could depend on the presence of members of the MHC-II loading complex and an acidic environment. Confocal microscopy confirmed the co-localization of mouse MR1 with LAMP and calreticulin, both markers of the late endosome.

Little is known at present about the mechanisms underlying MR1 processing and presentation of Mtb or other intracellularly derived ligands. As mentioned above, TAP is not required. Furthermore, the requirements for MHC-II-associated chaperones or an acidic environment have not been elucidated. Given the presence of Mtb in an endosome, the ready access of Mtb-derived proteins to the MHC-II pathway, and the dynamic shuttling of vesicles that has been observed (65, 66), it is tempting to speculate that vesicular trafficking will play a role in the processing and presentation of MR1-derived ligands. However, current evidence does not indicate whether MR1 is shuttled to the Mtb phagosome for further loading, or whether Mtb-derived ligands are shuttled to an MR1-containing compartment for loading. In either case, it would appear that successful loading of ligand is a prerequisite for the translocation to the cell surface.

Nonetheless, the available information would suggest the loading of MR1-derived ligands is tightly regulated, presumably to avoid MAIT cell activation when it might be detrimental. One possibility is that the loading of MR1 depends upon the presence intracellular MR1 ligand, a circumstance that might either reflect the presence of the pathogen in an endosomal environment, or might require that the host cell participate in the generation of the ligand. The latter possibility, recently described by Corbett *et al.* (59), suggested that the 'neo-transitory' antigen 5-A-RU in conjunction with glyoxal/methylglyoxal intermediates could result in the MR1 ligands 5-OP-RU and 5-OE-RU. It also seems plausible that MR1, like both MHC-I and MHC-II, requires chaperones that direct MR1 to the appropriate environment to facilitate either the exchange of endogenous ligand for those that are pathogen-derived or for the loading of 'empty' MR1.

MAIT cells in infection with Mtb

The interaction of MAIT cells with the Mtb-exposed airway

Surprisingly little is known regarding the early events that occur in the respiratory tract immediately after exposure to Mtb (3), especially in humans. Generally, research has been focused on bacterial uptake by myeloid derived antigen presenting cells (APC) such as

macrophages and DC. Mtb is an intracellular pathogen that needs to infect and propagate in host cells yet our understanding of which cells harbor the bacterium is limited. The cell type and the different cell functions and location are likely to influence both the establishment of the infection and/or influence the outcome or severity of the disease. Pathogens and particles are transmitted via the aerosol delivery of 2–5 micrometer particulates (67). Traditionally, the significance of this observation has focused on delivery of particles to the alveolus. However, particles of these sizes also have ample opportunity to interact with the epithelium of the airways (68). A wide variety of mycobacteria including Mtb have the capacity to infect bronchial epithelial cells (69–77). Moreover, lung epithelial cell lines as well as primary bronchial epithelial cells infected with Mtb efficiently process and present bacterially derived antigens to both classically and non-classically restricted T cells (19, 22, 44, 78).

Potential contributions by MAIT cells to the innate control of Mtb

Although the contribution of innate and/or HLA-Ib restricted T cells to anti-TB immunity at human mucosal sites is incomplete (11), a role for such cells has been shown in mice. iNKT cells activated through the superantigen α -galactosylceramide stimulated innate and adaptive immune mechanisms to enhance anti-TB immunity (79). We postulate that due to their anatomical localization and innate-like properties, humans MAIT cells are poised to act as early sentinels in response to respiratory pathogens such as Mtb. *In vivo* studies demonstrate a role for MR1 and MAIT cells in murine models. In aerosol infection models with *M. bovis* BCG and the live vaccine strain of *Francisella tularensis*, lung bacterial control was decreased in mice lacking MR1 or MAIT cells (80, 81). Furthermore, in mice lacking MR1, infection with *Klebsiella pneumoniae* resulted in increased bacterial burden and significantly higher mortality within the first 4 days of infection. In humans, MAIT cells are enriched in the respiratory tract. In the airway of healthy adults, TRAV1-2⁺ T cells represent about 20% of all CD8⁺ T cells (MCG/DML, unpublished data). In humans, the majority of TRAV1-2⁺ T cells directly isolated from lungs are pathogen reactive T cells. In individuals with active TB, MAIT cells are further enriched in the BAL (Wong *et al.*, manuscript submitted) while nearly absent from the blood (19, 20). Although a comprehensive analysis of the effector functions of MAIT cells remains to be performed, MR1-restricted MAIT cells have been shown to produce IFN- γ and TNF in response to Mtb-infected airway epithelial cells. Additionally, MAIT cells can induce target cell lysis of epithelial cells and contain granzyme (23, Sharma *et al.*, manuscript submitted) previously shown to have anti-microbial properties (82). Given this Th1-like cytotoxic effector phenotype, MAIT cells in the airway have the potential to provide pro-inflammatory cytokines and potentially kill Mtb-infected cells in the Mtb-infected lung.

MAIT cells have the ability to detect Mtb infection of both hematopoietic and non-hematopoietic cells that all express MR1. Furthermore, as innate effectors, MAIT cells can perform their effector functions in a rapid fashion without the need for prior exposure to Mtb. In this regard, MAIT cells may have pleiotropic effects in both the innate and the adaptive response to Mtb. One illustrative mechanism by which MAIT cells could mediate innate control of Mtb is through the production of IFN- γ . Long-standing evidence supports an important role for IFN- γ in the control of Mtb both in mice and humans (83–86). As

recently highlighted, the contribution of IFN- γ by conventional human CD4⁺ T cells has been shown to be insufficient to control infection with Mtb and the coordinated response by a number of different cell types will be essential to control the bacterium (11). IFN- γ elicits a number of innate resistance mechanisms including the antimicrobial enzyme nitric oxide synthase (NOS2), autophagy, and vitamin D-dependent antimicrobial peptide production, all shown to mediate control of Mtb in macrophages (87–94). In humans, polymorphisms in the NOS2A gene suggest a contribution by NO in the susceptibility and control of Mtb (95, 96). In humans with TB, iNOS has been detected in human granuloma and alveolar macrophages, which in some instances can be induced by Th1 cytokines (97–100). Similarly, in the human airway, the continuous production of NO by human airway epithelial cells is dependent on cytokines (101, 104). Specifically, stimulation with cytokines including IFN- γ induces NOS2-dependent NO production by primary and transformed epithelial cells (104–108). In mice, IFN- γ contributed to an indoleamine-2,3-dioxygenase dependent protective response to infection with Mtb that was mediated by non-hematopoietic cells. Therefore, IFN- γ can exert its properties on both hematopoietic and non-hematopoietic IFN- γ responsive cells including epithelial cells (109). Could MAIT cells, which are in close juxtaposition to the airway epithelium, provide IFN- γ and/or TNF locally and contribute to innate epithelial cell dependent control of Mtb? Could such control occur prior to the acquisition of adaptive immunity? Does the interaction of epithelial cells and innate T cells have important consequences for the subsequent development of adaptive immunity? In this regard, we postulate that the recognition of infected epithelial cells by T cells provides necessary recruitment and/or maturation signals to DCs.

Potential MAIT cell contributions to the acquisition of Mtb-specific T-cell responses In addition to their proximity to airway epithelial cells, MAIT cells are in close juxtaposition to lung-resident DCs that form a mesh-like network in the airways (110, 111). Ample studies provide conclusive evidence that DCs are the cells responsible for priming adaptive T cells *in vivo* (112). In mice infected with Mtb via the aerosol route, priming of Mtb-specific T cells occurred in the mediastinal draining lymph nodes in a DC-dependent manner (113, 114). In contrast to the rapid kinetics of the acquisition of T-cell responses to most pathogens, in aerosol infection of mice, Mtb-specific T cells were not detectable in the lung for 2–3 weeks after infection. The delay in the priming of antigen-specific T cells was due to a delay in trafficking to the lymph node by the DC presenting Mtb antigens (113, 114). Nevertheless, early dissemination of the bacteria may result in accelerated T cell priming and better control of Mtb (115). For effective priming of Th1 immunity to occur, immature DC must take up foreign antigens and traffic to the lymphoid tissues (116). Paradoxically, optimal Th1 priming requires an early source of IFN- γ to induce DC to express a mature phenotype and increased Th1-promoting cytokine production (117). Here we postulate that the rapid production of IFN- γ by MAIT cells may facilitate the acquisition of Th1 immunity (118–120).

MR1-independent MAIT cell functions

Increasing evidence points to the ability of MAIT cells to perform TCR independent functions. Specifically, the combination of IL-12 and IL-18 induced MAIT cells to produce IFN- γ in an MR1-independent fashion (121). Furthermore, TLR8 induction of IL-12/IL-18

by liver monocytes resulted in IFN- γ production by MAIT cells from human livers where MAIT cells appear enriched (41, 42). Such indirect responses to TLR agonists suggest that MAIT cells could contribute to immune regulation with both protective and pathological outcomes especially under states of co-infection.

Potential MAIT cell contributions to immunopathology

MAIT cells have been linked to immunopathology in a number of reports. A number of studies have identified a relationship between MAIT cells and multiple sclerosis (122–127). Specifically, MAIT cells are decreased in the blood of patients with active multiple sclerosis and return to the blood in remitting disease. Similarly, patients with irritable bowel disease (IBD) have decreased peripheral blood MAIT cells (128). The functional significance of MAIT cells under these conditions remains to be determined. TCR-independent stimulation showed MAIT cells can produce IL-17 and IL-22 in addition to IFN- γ and TNF. This is in contrast to results obtained from MR1/ligand stimulation that consistently show MAIT cells produce IFN- γ and TNF. Nevertheless, the potential exists for MAIT cells, through both TCR dependent and independent stimulation, to provide protective or pathological outcomes. In the context of infection with Mtb, MAIT cells could tip the balance from control (clearance/LTBI) to lack of control (inflammation). For example, could unregulated TNF production by MAIT cells contribute to the dualistic role of TNF in TB (129)? Theoretically, targeted suppression of MR1-dependent MAIT cells could be performed using MAIT inhibitory molecules such as 6-FP or Acetyl-6-FP (21, 58).

MAIT cells as a diagnostic or as a target of vaccination

The observation that MAIT cells are nearly absent from the peripheral blood in those with TB, and concomitantly enriched in the lung suggests a dynamic relationship between the presence of Mtb and its associated metabolites and the localization of MAIT cells. Furthermore, MAIT cells are restored to the blood of active TB patients after anti-TB therapy (Sharma *et al.*, manuscript submitted). As a result, it is possible that the measurement of peripheral blood MAIT cells could provide clinically useful information regarding disease status. While the greatest risk of development of TB occurs in the first two years following exposure, only a minority of those with evidence of exposure progress to clinical disease. The identification and treatment of those at risk to progress has the potential to hasten the eradication of TB (130). We postulate that those with ‘sub-clinical’ TB may have diminished MAIT cell frequencies in the peripheral blood, and therefore MAIT frequencies could serve as an adjunct in the identification of this group.

Because of the prevalence, location, and effector functions of MAIT cells in conjunction with their ability to detect nearly all intracellularly infected cells, MAIT cells could be targeted to aid in the clearance or control of Mtb. To some extent, the potential use for MAIT cell targeting vaccination or therapy will depend on whether or not vaccination can elicit long-lived memory. Alternately, the presence of MAIT cells in the lung and other tissues, and their inherent effector function could suggest a role for these cells as a target of host-directed therapy. It is also possible that MAIT cell ligands could be used as adjuvants in the delivery of traditional antigens. In this regard, we note that some ligands appear to have the capacity to activate a diverse array of MAIT cells, and as such could be used as a

MAIT cell adjuvants. Specifically, the rRL-6-CH₂OH MR1 ligand has been shown to stimulate all TRAV1-2⁺ CD161^{hi} MAIT cells (40).

Human MR1-restricted T cells are a unique T-cell subset. While they can share the effector functions of conventional CD8⁺ cytolytic T lymphocytes (CTL), MAIT cells are distinct in their thymic programming that endows them with the capacity to act rapidly in response to infection. Furthermore, while many functions between conventional CTLs and MAITs may appear redundant, the mechanisms by which MR1-restricted T cells detect infection are distinct. Recognition of microbial metabolites in the context of the evolutionarily conserved MR1 antigen presentation molecule allows MAIT cells to potentially play a role in a wide variety of infections and in immune modulation. At this point, the lack of knowledge of the breadth of MR1 ligands and the processing and presentation pathway by MR1 is a major limitation in our potential to use these cells in a therapeutic setting. Currently, correlates of protective immunity to tuberculosis remain to be defined. Specifically, the contribution of innate and/or lung resident T cells is unexplored. Recent advances in our understanding of MR1-restricted T cells suggest a co-evolutionary relationship between MAIT cells and Mtb. It is tempting to speculate that strong selection pressures between Mtb and the human host have contributed to the evolution and maintenance of MR1-restricted T cells.

Acknowledgments

This work was supported by the Department of Veterans Affairs with resources and facility access provided by the VA Portland Health Care System and the National Institutes of Health (NIH) grants AI078965 and AI48090 and the Mucosal Immunology Studies Team (MIST) U01 AI095776-01 funded by the National Institute of Allergy and Infectious Diseases.

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