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MAIT cells and microbial immunity

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Abstract

Mucosal-associated invariant T (MAIT) cells, the most abundant T cell subset in humans, are increasingly being recognized for their importance in microbial immunity. MAIT cells accumulate in almost every mucosal tissue examined, including the lung, liver, and intestinal tract, where they can be activated through T cell receptor (TCR) triggering as well as cytokine stimulation in response to a host of microbial products. In this review, we specifically discuss MAIT cell responses to bacterial and fungal infections, with a focus on responses that are both MR1-dependent and -independent, the evidence for diversity in MAIT TCR usage in response to discrete microbial products, protective immunity induced by MAIT cells, and MAIT cell antimicrobial functions in the context of these infections.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Mucosal-associated invariant T (MAIT) cells, the most abundant T cell subset in humans, are increasingly being recognized for their importance in microbial immunity. Features such as their abundance at the mucosal interface, selective recognition of antigens broadly expressed by many bacterial and fungi, and expression of proinflammatory cytokines and cytolytic products, uniquely position them to influence early events in defense against pathogens. We review collective data that supports a model of MAIT cell responsiveness which unites critical aspects of innate and adaptive immunity to infection.

Keywords

Antimicrobial responses; Infection; Mucosal immunology; MAIT cells; MR1; Unconventional T cells

INTRODUCTION

Human mucosal-associated invariant T (MAIT) cells are an abundant population of $\alpha\beta$ -TCR T cells that display anti-microbial Th1-like cytotoxic capacity in their response to a range of microbial infections. MAIT cells were originally defined by their usage of semi-invariant T-cell antigen receptor (TCR) that engages antigenic ligands presented by the HLA-Ib major histocompatibility complex (MHC)-related protein I (MR1). MAIT cells were first identified by Porcelli *et al.* in 1993 as a T cell population using a semi-invariant *TRAV1-2/TRAJ33* pairing of the TCR α ¹. These T cells were further defined in mice and humans as requiring an intact immune system and the MHC class I light chain (β -2-microglobulin (β 2M)) but not TAP, CD1, MHC class I, or class II; indicating a requirement for a novel class of antigen presentation². A pivotal study by Treiner *et al.* in 2003 demonstrated that MAIT cells have a

developmental and functional dependence on MR1 and the host microbiota³. That MAIT cells could play a role in the control of microbial infection was suggested their cytolytic capacity in response to a variety of microbial infections (Table 1)^{4,5}. Phenotypic MAITs were described as those that co-expressed the TRAV1–2 TCR, CD161, and CD26^{6,7}. From this definition, MAIT cells were largely found within the CD8⁺ or CD8[−]CD4[−] T cell compartments. The first MR1 ligands were of microbial origin, and included derivatives of vitamin B9 (folate) as well as pyrimidine intermediates derived from the vitamin B2 (riboflavin)–synthesis pathway^{8,9}. This discovery and synthesis of these ligands allowed for the development of the first MR1-tetramer in 2013 and facilitated a more broad view of the TCR usage and phenotype of MAIT cells¹⁰. For example, this allowed for the observation of a small subset of CD4⁺ MR1-tetramer⁺ MAIT cells in humans and mice^{10,11}. Additionally, recent studies have demonstrated the existence of TRAV1–2 negative MR1-restricted T cells such that MAIT cells are a subset of MR1-restricted T cells (MR1Ts)^{12–14}. Together, these studies provided the background for our current understanding of the role of MR1T cells in bacterial and fungal immunity.

MR1-restricted T cell responses to bacteria and fungi

MR1-dependent T cell responses

The first studies to definitively show that MAIT cells were reactive to bacterial and fungal products presented by MR1 were published by Gold *et al.* and Le Bourhis *et al.* in 2010^{4,5}. Le Bourhis *et al.* purified human TRAV1–2⁺ CD161⁺ T cells and showed that they could be activated by monocytes infected with *Escherichia coli* (*E. coli*) or *Mycobacterium abscessus* in an MR1-dependent fashion. Due to the low frequency of MAIT cells in mice, transgenic mice expressing TRAV1/TRBV19 (V α 19/V β 6) TCRs were engineered to show that MAIT cells were activated by a wide array of bacterial and fungal species. In these experiments, *Candida albicans* (*C. albicans*), *Candida glabrata*, *E. coli*, *Klebsiella pneumoniae* (*K. pneumoniae*), *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Staphylococcus aureus* (*S. aureus*), and *Staphylococcus epidermidis* were all capable of activating MAIT cells. However, *Enterococcus faecalis* (*E. faecalis*) and *Streptococcus pyogenes* (*S. pyogenes*), as well as five unrelated viruses, were unable to elicit a response by MAIT cells⁵. In parallel, Gold *et al.*⁴ were studying the human immune response to *Mycobacterium tuberculosis* (Mtb), and specifically seeking to explain the observation that individuals who had never been exposed to Mtb had a substantial population of T cells that could nonetheless recognize infected cells *ex vivo*. These cells were cytolytic and made pro-inflammatory cytokines like TNF- α and IFN- γ . In an effort to describe these T cells in more detail, T cell clones were isolated from the blood of healthy individuals and patients with TB. Surprisingly, all of these clones were dependent on MR1 for their recognition of Mtb-infected cells. In addition to Mtb, these clones were broadly reactive to pathogenic microbes such as *C. albicans*, *E. coli*, Mtb, *Mycobacterium smegmatis* (*M. smegmatis*), *Mycobacterium bovis* BCG, *Salmonella enterica* serovar typhimurium (*S. enterica* typhimurium), and *S. aureus*, but not viruses or *Listeria monocytogenes*⁴. Importantly, the data from these two studies suggested that while viruses did not activate MAIT cells, a diverse array of bacteria and fungi could (Table 1). Based on the use of a

semi-invariant TCR, and highly conserved presentation molecule, these studies suggested that MAIT cells might detect a conserved microbial ligand.

The ensuing discovery that MAIT cells could recognize a class of small molecules generated during microbial riboflavin biosynthesis^{8,9} suggested that this pathway was necessary for the generation of activating MR1 ligands (Table 1). In these and other subsequent studies, it was demonstrated that for some microbes including *S. enterica* typhimurium^{9,15}, *L. lactis*⁹, *E. coli*¹⁶, and *Streptococcus pneumoniae* (*S. pneumoniae*)¹⁷, an ability to synthesize riboflavin through functional Rib enzymes was critical to MR1-dependent recognition of these microbes (Table 1). Specifically, for these bacterial species, the RibD enzyme was required for production of 5-amino-6-D-ribitylaminouracil (5-A-RU), a precursor to riboflavin, and a critical component of these ligands. The 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) ligand, formed when 5-A-RU combines with host or bacterial derived methylglyoxal, is the most potent ligand identified to date⁹. Interestingly, there is now evidence that some bacteria produce non-riboflavin pathway-derived MR1 ligands as well. For example, MR1T clones characterized by *TRAV1*-2-negative TCR usage recognized *S. pyogenes* in an MR1-dependent manner even though *S. pyogenes* lacks the enzymatic pathway for riboflavin biosynthesis¹⁴. Additionally, the 6-formyl pterin (6-FP) ligand, that is antagonistic for MAIT cells⁸, can activate a population of *TRAV1*-2 negative MR1Ts¹³. As 6-FP is derived from folic acid, another vitamin synthesized by some bacteria and fungi, this is another possible source of microbial MR1 ligands. Clearly, diversity in MR1T TCRs is associated with differential ligand recognition. How diverse MR1 ligands contribute to protective immunity in the context of infection is not yet known.

MR1-independent cytokine driven MAIT cell responses

While MR1-dependent recognition of bacterial and fungal antigens by MAIT cells is well established, there is growing evidence for MR1-independent cytokine-driven responses. It is well-known that virus-specific mouse CD8⁺ T cells can be induced to produce IFN- γ in an antigen independent manner by sensing cytokines including type I IFNs, IL-12 and IL-18. However, in humans the responses to IL-12 and IL-18 are more distinctly linked to cell populations expressing killer cell lectin-like receptor subfamily B member 1 (KLRB1) or CD161, such as MAIT cells^{18,19}. The role of these cytokines in mediating anti-viral MAIT cell responses is covered by the Klenerman group in this issue. The relative contribution of inflammatory cytokines as compared to MR1-dependent MAIT cell activation in the context of bacterial infection is discussed below. Ussher *et al.* evaluated the role of IL-12 and IL-18 using a co-culture assay¹⁸. To distinguish the role of TCR-dependent and -independent signaling, THP-1 cells were cultured with either fixed *E. coli*, which contains MR1 ligands, or with fixed *E. faecalis*, which does not synthesize riboflavin. In this context, *E. faecalis* stimulated MAIT cells in a TCR-independent and IL-12 and IL-18-dependent manner. In contrast, while *E. coli* stimulated TCR-dependent MAIT cell responses early in the co-culture, the responses shifted to TCR-independent responses later¹⁸. More recently, Jo *et al.*²⁰ found that production of IL-12 and IL-18 by cells infected with fixed *E. coli* was sufficient to induce cytokine production by purified human liver MAIT cells after a longer co-culture. These data contrast with other studies demonstrating that human MAIT cell responses to live bacterial stimuli are MR1-dependent, even after longer co-incubations e.g.

^{4,5,14}. To explain these discordant results, we speculate that the availability of antigen could be limiting in fixed bacteria allowing for cytokine-driven activation to play a more dominant role. In the context of microbial infection in the mucosa, the relative role of cytokine-driven versus TCR-mediated activation of MAIT cells remains to be elucidated.

Other groups have demonstrated MR1-independent activation of MAIT cells in the context of microbial infection *in vivo*. In an initial study by Chua *et al.*, murine MAIT cells lost their *in vivo* anti-mycobacterial activity when the IL-12 p40 subunit was deleted or blocked²¹. Subsequently, Meierovics *et al.*²² showed that MAIT cell control of *Francisella tularensis* LVS in macrophages was dependent on IL-12, using blocking with an anti-IL-12p40 antibody. Although addition of the IL-12p40 blocking antibody in both of these cases reversed the ability of MAIT cells to control growth of bacteria, the role of IL-12 itself is unclear, as IL-12p40 is also a subunit of IL-23. Furthermore, in the case of Meierovics *et al.*, *ex vivo* blocking assays with an anti-MR1 antibody also blocked bacterial growth inhibition and cytokine production to the same extent as the IL-12p40 antibody suggesting possibly redundant or complementary roles in this infection model. In a subsequent *in vivo* study, the same group demonstrated that MAIT cell stimulation of monocyte differentiation in the context of LVS infection was MR1-independent²³. The differentiation required MAIT cell-dependent production of GM-CSF, and the role of IL-12 or IL-18 in MAIT cell activation was not investigated. Shaler *et al.* have demonstrated another MR1-independent pathway for MAIT cell activation through bacterial superantigens²⁴. Overall, given the localization of MAIT cells at common sites of infection, both MR1-independent and MR1-dependent MAIT cell activity is likely to play a role in defense against certain infections *in vivo*.

Recognition of microbially-derived ligands on MR1

The ability of MR1 to sample bacterial and fungal metabolites would suggest that MR1-dependent MAIT cell responses have a unique and complementary role in the immune response to microbes. MR1 is a monomorphic and highly conserved molecule^{25,26} that is widely expressed across a variety of tissues and cell types²⁷, including both professional antigen presenting cells and MHC class II-negative cells, such as epithelial cells. Unlike classical class I molecules, MR1 is sequestered intracellularly, with very little found constitutively on the cell surface (Figure 1)^{28–31}. As discussed above, MR1 ligands can be derived directly from riboflavin biosynthesis, or are neo-antigens formed through interactions between a riboflavin precursor and host- or pathogen-derived glyoxal or methylglyoxal^{8,9}. Recent reports^{12–14,32} and our unpublished data also suggest the presence of MR1 ligands that are not derived from riboflavin biosynthesis. Additionally, an *in silico* screen of multiple small molecules showed that MR1 can bind synthetic drugs like diclofenac, leading to activation of MAIT cells³². Whether or not these and other MR1 ligands can influence the MR1-dependent MAIT cell recognition and response to *in vivo* infection remains to be determined. However, as many of these ligands require metabolically active microbes, MR1 appears to serve as a sensor of the microbial metabolome, poised to detect infections from wide array of microbes.

The presentation of MR1 ligands to MR1Ts presents a paradox for the immune system in its ability to sense microbial metabolites in a context that would allow for appropriate sensing

of infection, while avoiding MR1T activation to ligands that might be found in healthy mucosal environments. In this context, the mechanisms by which microbially-derived ligands are generated and presented on MR1 represent a critical outstanding question in the field (Figure 1). MR1 resides in the ER, and is thought to traffic from the ER only in the presence of a ligand. However, the source of this ligand, i.e. self, exogenous, or microbial, in a physiological setting is not known. Others have demonstrated that intracellular MR1 can also be observed in an endosomal vesicle pool, depending on cell type and experimental conditions^{29–31}. Here, the mechanisms by which ligands derived from intracellular bacteria are sampled is incompletely understood. Indeed, sampling of intracellular bacteria for peptide antigens has often required specific mechanisms to sample the phagosome. Increasing evidence would suggest that the pathway by which MR1 ligands derived from intracellular microbes may require distinct pathways than those for extracellular microbes or exogenously added ligands^{28,29,33}. Specifically, these data would suggest that vesicular trafficking, possibly via recycling of MR1, may play a key role in presentation of antigens from intracellular microbes. Evidence here comes from studies that have evaluated the requirements for the presentation of MR1 antigens in the setting of infection with Mtb, and by the comparison of the requirements for presentation of intracellular antigens versus cell surface stabilization of MR1 following the addition of exogenous ligands. First, knockdown of the vesicular trafficking protein Syntaxin-18 perturbed both MR1 dependent antigen presentation of Mtb as well as 6-FP mediated surface stabilization. In contrast, the knockdown of VAMP4 only affected Mtb-dependent antigen presentation, leaving 6-FP-dependent surface stabilization of MR1 intact²⁹. Second, THP-1 cells treated with the endosomal acidification inhibitor bafilomycin A1 demonstrated a significant reduction in MR1 dependent antigen presentation of intact intracellular *E. coli* but there was no such reduction when ligands were added exogenously from *E. coli* culture supernatants³³. In this same study, the level of surface MR1 correlated with MAIT cell activation in the context of bacterial culture supernatants but not intracellular infection. Taken together, it is likely that MR1 mediated sampling of an intracellular microbe follows a different pathway than MR1 sampling of exogenously added antigens. Fundamental questions about MR1 dependent recognition of intracellular microbes versus exogenous ligands remain. These include how and where antigens derived from intracellular or extracellular bacteria are loaded onto MR1 and the role of chaperones in this loading. For example, Huang *et al.* suggested that CLIP might serve as an MR1 chaperone³⁰. As better reagents are developed, these and many other questions will be addressed.

MAIT cell TCR heterogeneity is associated with differential responses to bacterial and fungal microbes

MAIT cells are defined by their expression of a TCR including TRAV1–2 and a limited TRBV repertoire². This was confirmed through genotypic characterization of several Mtb-reactive MAIT cell clones⁴ and validated by Reantragoon *et al.*¹⁰ using the first MR1 tetramer loaded with the rRL-6-CH₂OH antigen, and antibodies to TRAV1–2 and CD161, to define the MAIT TCR repertoire. Recent studies show accumulating evidence of TCR heterogeneity reflected by diverse TRAJ gene usage including nucleotide additions in the CDR3 regions, as well as more diverse TRBV gene usage^{4,10,13,34–37}. For example,

Gherardin *et al.* found that MR1Ts expressing non-canonical TCR α chains were autoreactive to MR1 or responded selectively to folate derivative antigens. In 2014, Gold *et al.* evaluated the functional relevance of MAIT TCRs in the context of three microbial infections. Analysis of the MAIT TCR repertoire of individuals based on the reactivity to infection by *M. smegmatis*, *C. albicans*, and *S. enterica* typhimurium revealed pathogen selectivity through oligoclonal MAIT TCR usage³⁴. Although no ‘microbe-specific’ MAIT TCRs were found, each individual preferentially used discrete CDR3-TCRs to respond to each microbe. When comparing the MAIT cell cytokine response to infection by *E. coli* or *C. albicans*, Dias *et al.* observed that different combinations of cytokines were produced by MAIT cells expressing different TCR β -chains³⁸. These studies show the ability of individual MAIT TCRs to contribute to selective recognition of microbial infections. This also raises the question of whether differential MR1T TCR signaling could lead to unique MR1T cell effector function.

In humans, numerous groups have observed either decreases in MAIT cell frequencies in the blood in the context of bacterial infection^{4,5,39–41}, or increased MAIT cell frequencies in mucosal tissues^{15,42–44}. Longitudinal monitoring of blood MAIT cell frequency and activation status during human *Vibrio cholerae* O1 infection⁴⁰, in human volunteers challenged with *Shigella dysenteriae*⁴⁵, *Salmonella typhi*⁴⁶, or *Salmonella paratyphi* A⁴⁷, or NHP infection with *M. tuberculosis*⁴⁸ demonstrated changes in frequency and activation of MAIT cells during bacterial infection. Sequential sampling of circulating MAIT cells during enteric fever, compared to infected but not diseased volunteers, revealed dynamic changes in MAIT TCR β clonotypes⁴⁷. Specifically, these clonally expanded MAIT cells were more activated following stimulation with *E. coli* derived-, *S. paratyphi* derived- or purified MR1-ligands⁴⁷. These data suggest that the MAIT TCR repertoire can be shaped by bacterial infection.

A protective role for MAIT cells in immunity to bacterial and fungal infections

The hypothesis that MAIT cells could play a protective role in the antimicrobial immune response has been supported by mouse models where the deletion of MR1, and hence MAIT cells, rendered mice more susceptible to bacterial infections^{5,21,22,49}. The first indication of a protective role for MAIT cells *in vivo* came from Le Bourhis *et al.* who used mice expressing a transgenic MAIT TCR to demonstrate that MR1^{-/-} mice were significantly less able to control growth of *E. coli* after intraperitoneal injection⁵. Three other groups have subsequently challenged WT and MR1^{-/-} mice with a range of microbial infections and found MAIT cells to influence control of bacterial infection^{21,22,49}. For example, Georgel *et al.* found that MR1^{-/-} mice infected intraperitoneally with the gram-negative *K. pneumoniae*, but not *E. coli*, *Yersinia enterocolitica*, or *Shigella dysenteriae*, sustained higher bacteria loads, lower temperatures, and survived less well in comparison to WT mice⁴⁹. In this regard, it is important to note that these results contradict the finding from Le Bourhis *et al.* that MAIT cells play a role in protection from *E. coli*. However, Le Bourhis *et al.* used transgenic mice where up to 80% of the T cell repertoire is comprised of a single MAIT TCR. Therefore, protection in this model might depend on high frequencies of MAIT cells.

In another study, Chua *et al.* investigated the role for MAIT cells in control of mycobacteria using an established respiratory infection model of *M. bovis* BCG in WT or MR1^{-/-} mice²¹. Here, mice deficient in MR1 sustained a 10-fold higher bacterial load in the lungs at 10 days post infection, but an equivalent bacterial load at 30 days post infection. Lastly, MAIT cells have also been implicated in the induction of immunity to *F. tularensis* LVS^{22,23}. Meierovics *et al.* first observed that MAIT cells expanded in the lungs of WT mice following intranasal challenge with *F. tularensis* LVS. They also showed that MR1 and IL-12p40 were required for MR1T cell expansion and bacterial growth control. MR1 deficiency was associated with defects in early mucosal cytokine production and recruitment of classically-restricted CD4⁺ and CD8⁺ IFN- γ producing T cells²². In a follow up study, the same group identified the mechanism for these MAIT cell-dependent functions. Here, the MR1T cells that were recruited to the lungs were required for the production of GM-CSF. In turn, GM-CSF was required for the maturation of inflammatory monocytes into dendritic cells enabling the subsequent recruitment of activated CD4⁺ T cells²³. In sum, these murine studies implicate a role for MR1T cells in the early stages of bacterial containment during infection. While all bacteria tested were strains capable of generating MR1T cell antigens, there was not a protective role for MR1T cells in all infection models (e.g. *E. coli*, *Yersinia enterocolitica*, or *Shigella dysenteriae*). This may be because the infection models did not reflect routes of natural infection. A role for MAIT cells in fungal immunity has not yet been studied *in vivo*.

Mechanisms by which MAIT cells influence immunity to bacterial and fungal infections

While MAIT cells are often termed “innate”, MAIT cells have features of both innate and adaptive immune cells that preclude simple categorization. MAIT cells undergo TCR rearrangement and positive selection in the thymus. While conventional T cells acquire effector function in the periphery following antigenic stimulation, MAIT cells acquire effector function in the thymus, prior to exogenous antigenic exposure. Functional maturation is further shaped in the periphery by B cells and microflora^{3,50}. Specifically, they have the capacity to secrete proinflammatory cytokines such as IFN- γ and TNF, as well as lyse target cells^{51,52}. MR1T cells are poised to play a distinct role in antimicrobial immunity. These attributes include their relative abundance in mucosal tissue sites, expansion in response to bacterial infection, regulated cytokine profiles, regulated cytolytic capacity, and instigation of multicellular immune responses.

MAIT cell localization in tissues and expansion in response to infection

MAIT cells may be developmentally programmed to reside in mucosal sites early in life. In support of this, Gold *et al.* observed that MAIT cells in the thymus, cord blood and peripheral blood express significantly less CD62L, a selectin associated with homing to lymphoid tissues⁵¹. Moreover, Leeansyah *et al.* observed that human 2nd trimester fetal MAIT cells already express CD45RO and are functionally mature in mucosal tissues. Fetal MAIT cells were enriched in lung, small intestine and liver compared to spleen and thymus^{6,53}. Furthermore, at these sites, they proliferated and produced cytokines in response to *E. coli* infection, suggesting the development of an early arm of antibacterial mucosal immunity. Numerous studies in mice, NHP, and humans have extensively mapped MAIT

frequencies in tissues. Circulating MAIT cells in mice are less numerous than in humans (<0.1% versus 1–10%, respectively)^{10,11}. However, the use of an MR1 tetramer identifies enrichments in the murine lung, liver, intestines, and female genital tract at up to 2–4% of T cells^{11,15}. A pioneering study of NHP observed MR1 tetramer⁺ cells enriched in the liver and bronchial alveolar lavage as compared to the blood⁴⁸. Within humans, studies demonstrate a higher frequency of MAIT cells are present in the intestine and jejunal mucosa (5–60% of CD4-T cells)^{6,10}, liver parenchyma and vasculature (20–50% of T cells)^{6,24,36,54,55}, placental intervillous blood (2–4% of T cells)⁴⁴ and the airway and lungs (2–4% of T cells)⁴. Interestingly, MAIT cells are present at a lower frequency in the human female genital tract⁵⁶ and lymph nodes³⁶ as compared to the blood. That different tissues may contain clonotypic enrichments of MAIT cells with different TCRs is suggested by the observation that the Va7.2/Ja12 transcript is enriched relative to the Va7.2/Ja33 transcript in the human intestine, kidney, and ovary samples³⁶.

In the context of infection, numerous studies have demonstrated that circulating MAIT cell frequencies decrease^{4,5,39–43,46,47,57–60}. The prevailing hypothesis is that MAIT cells have been recruited to infected tissues. For example, intranasal infection of mice with live attenuated *F. tularensis*, mycobacteria, or *S. enterica* typhimurium caused the recruitment of proliferating MAIT cells to the lungs^{21,22,61}. In a unique model of human bacterial infection, volunteers challenged with oral wild-type *Salmonella typhi* were evaluated for development of typhoid fever⁴⁶. In those who developed disease, MAIT cells became activated, decreased in frequency from the blood, and increased expression of gut homing chemokine receptors. Additionally, Greene *et al.* evaluated the *in vivo* response by MAIT cells to mycobacteria in NHP. Here, Greene *et al.* used an MR1 tetramer to track MAIT cells in NHP that were vaccinated intradermally with BCG and then challenged with pulmonary Mtb⁴⁸. They observed an increase in Ki-67 expression, a cellular marker of proliferation and activation, on MAIT cells at the site of vaccination and systemically during Mtb infection. Together, these studies support the hypothesis that MAIT cells are recruited to and can expand at mucosal sites in response to bacterial infections.

To further explore MAIT cell expansion in response to infection, Chen *et al.* administered attenuated *S. enterica* typhimurium intranasally to mice. Here, MAIT cells accumulated locally (up to 50% of T cells) within one-week following challenge in an MR1-dependent manner and these expansions were sustained for at least 50 days¹⁵. Additionally, these investigators and one other found that intranasal delivery of purified antigen (5-OP-RU) was not sufficient to induce MAIT accumulation^{15,32}. Instead, costimulation with Rib-pathway-deficient *S. typhimurium* or TLR 2, 3, 6, 9 agonists led to MAIT cell enrichment in the lungs that was sustained for at least 10 weeks and MR1 dependent¹⁵. While TLR-agonists alone are sufficient to activate many cells within the innate arm of the immune system, they were not sufficient for MAIT cells^{4,5}. These data would suggest that mucosal MR1T cell expansion requires both TCR-dependent signals in conjunction with co-stimulation, such as TLR signaling. This illustrates how MAIT cells cooperate with innate immune stimuli as part of the early local response to infection.

Tissue Resident MAIT Cells Display Unique Functionality

Upon stimulation, MAIT cells can make a variety of cytokines that have the potential to promote an antibacterial or anti-fungal response. It has been widely observed that they produce the pro-inflammatory cytokines IFN- γ and TNF- α ^{4-8,10,24,38,62,63}. MAIT cell effector function in response to TCR signaling is triggered more rapidly than conventional T cells. For example, a comparison of human T cell responses to Staphylococcal superantigen found that MAIT cells were activated to secrete IFN- γ more rapidly than conventional T cells²⁴. In this way, the authors concluded that MAIT cells provide an early source of proinflammatory mediators that could be pathogenic or protective depending on the nature of the bacterial stimulus. This may be due to upregulation of cytokine transcripts that poise MAIT cells for robust responsiveness⁶⁴. In the context of bacterial infection, murine MAIT cells produce IL-17, while less than 1–2% of circulating NHP and human MAIT cells produce this cytokine^{6,7,24,38,48}. However, human MAIT cells from discrete tissue sites, disease states, and inflammatory environments have distinct cytokine profiles. MAIT cells in the spleen, thymus, liver, lungs, placental intervillous blood, and colon, share a similar cytokine profile with those in the circulation^{7,44,50,51,64,65}. In contrast, MAIT cells in the female genital tract⁵⁶, intrasinusoidal liver⁵⁵, spinal fluid of patients with ankylosing spondylitis^{62,66}, salivary glands of patients with primary Sjogren's Syndrome⁶⁷, pleural fluid of patients with tuberculosis pleurisy⁵⁷, and the blood of patients with ulcerative colitis⁶⁸, make more IL-17 and less TNF- α and IFN- γ . Specifically, up to 22% of MAIT cells in the female genital tract produced IL-17 in response to *E. coli* infection *ex vivo* in contrast to those from the blood⁵⁶. Notably, Tang *et al.* observed that the pre-treatment of MAIT cells *in vitro* with IL-7 and IL-23 increased the IL-17 polarization⁵⁵. Like IL-17, IL-22 is another cytokine preferentially expressed by MAIT cells at different tissue sites. IL-22 is a cytokine associated with host defense and repair of mucosal tissue and is generally not produced by circulating MAIT cells⁷. However, MAIT cells from the spleen and small intestine from 2nd trimester fetuses could make IL-22 in response to fixed-*E.coli*, while IFN- γ producing cells were found in the small intestine, lung, and spleen⁵³. Collectively, these data suggest that tissue-resident MAIT cells will have a phenotype and function tailored to their anatomic location, proximity to microbes, and inflammatory environment. In this regard, MAIT cells might contribute directly to microbial control, could enable the subsequent development of adaptive immunity, or could contribute to autoimmunity.

MAIT cell cytolytic capacity

Cytolytic properties of immune cells, including MAIT cells, are crucial for eliminating intracellular bacterial infection, either through the induction of apoptosis of the target cells, or via the introduction of anti-bacterial proteins. Cytotoxic T cells, in humans both CD8⁺ and CD4⁺, contain granzyme B, perforin, and granulysin in preformed granules, allowing for rapid delivery to the target cell. MAIT cells also express cytotoxic molecules and are capable of lysing bacterially infected cells ranging from macrophages to epithelial cells^{4,6,7,36,45,55,69}. However, data suggest that resting MAIT cells do not express high levels of granzyme B like conventional T cells, but instead, can be licensed to produce granzyme B by bacterial infection, TCR stimulation, or IL-12/15/18^{57,64,69,70}. Importantly, this cytolytic 'licensing' could be maintained at least 6 days in an *in vitro* culture⁶⁹. Furthermore, MAIT

cells were found to be specifically activated in the context of volunteers challenged with an attenuated strain of *Shigella dysenteriae*⁴⁵. MAIT cell lysis of epithelial cells infected with *S. dysenteriae* was dependent upon MR1. Collectively, MAIT cells are cytolytic effectors that have may have licensing mechanisms to prevent inflammatory cytolytic damage that would be more likely given their abundance and proximity at the mucosal interface.

Conclusion

While conventional T cells respond sequentially and require activating signals by specialized DCs, MAIT cells respond more quickly to infectious attacks. Features such as their abundance at the mucosal interface, selective recognition of antigens broadly expressed by many bacterial and fungi, and expression of proinflammatory cytokines and cytolytic products, uniquely position them to influence early events in defense against pathogens. Collectively, these data support a model of MAIT cell responsiveness to the bacterial and fungal metabolomes which unites critical aspects of innate and adaptive immunity.

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Figure 1. The Intracellular Pathway of MHC Class I (MHC I)-Related Protein 1 (MR1) Ligand Presentation

MR1 is synthesized in the endoplasmic reticulum (ER) and passes through the Golgi where it either goes directly to the plasma membrane or remains in an intracellular vesicle.

Whether an endogenous ligand (yellow) facilitates MR1 egress from the ER is not known.

Exogenous MR1-ligands, such as 6-formyl pterin (FP) (purple) or those from an extracellular infection (orange), traverse the cell membrane and are loaded onto MR1 in the ER. MR1 at the plasma membrane may recycle back into the cytoplasm via recycling endosomes. MR1 vesicles may then be loaded with antigens from intracellular infection (pink) or from sampling extracellular infection and then translocate to the plasma

membrane. It is also possible that some MR1 ligands from intracellular infections are transported to the ER via an unknown mechanism and are then loaded onto MR1.

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Table 1

Bacteria and fungi recognized by MR1-restricted T cells

Name	References supporting MALT cell recognition	Predicted to synthesize riboflavin [†]	Requirement for Rib enzymes	References
<i>Candida albicans</i>	4,5,14,34,38	Yes		
<i>Candida glabrata</i>	5	Yes		
<i>Escherichia coli</i>	4,5,14,16,18,20,38,48,54,69,70	Yes	Yes	16
<i>Francisella tularensis</i>	22,23	Yes		
<i>Klebsiella pneumoniae</i>	5,49	Yes		
<i>Lactobacillus acidophilus</i>	5	Yes		
<i>Lactobacillus lactis</i>	9	Yes	Yes	9
<i>Mycobacterium abscessus</i>	5	Yes		
<i>Mycobacterium avium</i>	14	Yes		
<i>Mycobacterium bovis</i> BCG	4,21	Yes		
<i>Mycobacterium smegmatis</i>	4,14,29,34	Yes		
<i>Mycobacterium tuberculosis</i>	4,14,29,51	Yes		
<i>Neisseria gonorrhoeae</i>	14	Yes		
<i>Nocardia asteroides</i>	14	Yes		
<i>Pseudomonas aeruginosa</i>	5,14	Yes		
<i>Saccharomyces cerevisiae</i>	5	Yes		
<i>Salmonella enterica</i> serovar Paratyphi A	47	Yes		
<i>Salmonella enterica</i> serovar Typhimurium	4,9,14,15,31,34,45	Yes	Yes	9,15
<i>Shigella flexneri</i>	45	Yes		
<i>Staphylococcus aureus</i>	4,5	Yes		
<i>Staphylococcus epidermidis</i>	5	Yes		
<i>Streptococcus pneumoniae</i>	17,71	Yes	Yes	17
<i>Streptococcus pyogenes</i>	14	No	No	14,71
<i>Vibrio cholerae</i>	40	Yes		
<i>Vibrio parahaemolyticus</i>	14	Yes		
<i>Yersinia enterocolitica</i>	14	Yes		

[†] <http://www.genome.jp/kegg/>