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Mucosal associated invariant T cells and the immune response to infection

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Abstract

Mucosal associated invariant T cells are unique T cells localized at high frequencies at the portals of entry for many pathogens. Mucosal associated invariant T cells display a variety of characteristics that suggest their function is to act as effectors in the initial control of microbial infection at mucosal sites.

Keywords

Mucosal associated invariant T (MAIT) cells; iNKT cells; MR1; MHC-I; innate T cell; mucosal immunity; bacteria; fungi; *Mycobacterium tuberculosis*; *Klebsiella pneumoniae*

1. Mucosal associated invariant T cells remains largely unexplored

1.1 Identification of Mucosal associated invariant T cells

In 1993, Porcelli and colleagues performed the initial identification of two subsets of unconventional T cells [1]. In characterizing T cells lacking both the CD4 and CD8 co-receptors (also known as double negative (DN) T cells) from human blood, two populations were identified that appeared monoclonal in terms of the expression of their antigen recognition receptor or T cell receptor (TCR). Each of these T cell subsets was found to express a singular TCRα chain with limited sequence diversity specifically, Vα24 and Vα7.2. One subset (Vα24) would eventually be identified as invariant Natural Killer T cells (iNKT) cells and the second (Vα7.2) would be named Mucosal associated invariant T (MAIT) cells. As predicted by Porcelli “The expression of particular TCRs by DN αβT cells from multiple donors indicates that these cells, or at least a subpopulation of cells with this phenotype, recognize a limited spectrum of antigens and suggests that they may use nonpolymorphic antigen-presenting molecules”. In both cases, iNKT cells and MAIT cells

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were found to be restricted by the non-polymorphic antigen presenting molecules, CD1d [2] and MHC class I-related molecule (MR1) [3] respectively. iNKT cells, [4] have been extensively studied over the last 15 years due in large part to the relatively early discovery of a CD1d ligand known as α-galactosyl ceramide that has allowed for extensive analyses of iNKT cells [5]. In contrast, much remains to be discovered about Vα7.2^{+} MAIT cells [6] including the ligand(s) that bind MR1.

1.2 MR1 is a highly conserved non-classical MHC-Ib molecule

Mice lacking MR1 do not have T cells expressing the canonical mouse MAIT TCR, Vα19/J α33, establishing a direct link between MR1 and MAIT cells [3]. MR1 is a non-classical MHC-like molecule within the CD1 locus on chromosome 1 [7]. While the MHC locus is on chromosome 6, MR1 and classical MHC-Ia molecules share important similarities. MHC-Ia molecules are comprised of an immunoglobulin heavy chain (HC) that forms a β-pleated sheet resulting in a groove receptive to antigenic ligands. MHC-Ia molecules associate with the light chain known as β2-microglobulin (β2m) and the combination of this HC/β2m/ antigenic peptide trimeric complex is detected by antigen-specific CD8^{+} T cells via the TCR. MR1 and MHC-Ia HC molecules share high sequence homology, and hence would be expected to have functional commonality. For example, MR1 associates with the β2m light chain [8] [7] [9] and mice lacking β2m do not have MAIT cells [3, 10, 11]. Furthermore, structure function studies of MR1 performed by Ted Hansen and colleagues suggest that MR1 binds an antigen given that alterations in the putative ligand-binding groove abrogate responses by MAIT cell hybridomas [12].

In contrast to MHC-Ia molecules, the number of putative antigenic ligands that can bind MR1 will inherently be limited. Extensive polymorphism among MHC-Ia molecules allows for the presentation of a vast number of peptidic antigens. The three human MHC-Ia genes are phenomenally polymorphic with close to 1000 alleles for each locus (http://www.ebi.ac.uk/imgt/hla/stats.html). In sharp contrast, MR1 has limited polymorphism [13] with seventeen currently identified single nucleotide polymorphisms (SNP), 10 of which are synonymous (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=3140).

Even though the antigen processing and presentation pathway(s) used for MR1 remains to be defined, obvious differences are apparent between the pathways used by MHC-Ia and MR1. Specifically, in contrast to MHC-Ia-restricted T cells, selection of MR1- restricted MAIT cells is independent of the transporter associated with antigen processing and presentation (TAP). Self and foreign antigens presented by MHC-Ia molecules are principally derived from the cytosol, transported into the endoplasmic reticulum (ER) by TAP, and then loaded onto ER-resident MHC-Ia molecules. However, MAIT cell selection is not dependent on TAP as both mice and humans lacking TAP have MAIT cells [6]. This suggests that a major difference exists between the antigen processing and presentation pathways required for the MR1 and MHC-Ia selecting ligand(s).

A striking feature of MR1 is the extraordinarily high sequence homology it shares between mammals. For example, the putative ligand-binding site between mouse and human MR1 share 90% sequence homology and 80% homology with ruminants [9, 14, 15]. This supports the view that MR1 was highly conserved throughout mammalian evolution. As such, it is not surprising that MR1-restricted T cells have been identified in a variety of mammals including humans, ruminants, and mice. Interestingly, in laboratory mice, MR1- restricted T cells are present at surprisingly low frequencies perhaps owing to breeding and maintenance conditions [6, 15].

In addition, among mammals, MAIT cells also share considerable sequence homology in their TCRs further suggesting strong evolutionary pressure for conservation of this TCR [15]. Suggestive evidence that the MR1/MAIT cell interaction was conserved through evolution comes from studies showing orthologous mammalian MR1 molecules can stimulate mouse MAIT hybridomas [16].

1.3 MAIT cells have features of innate T cells

MR1-restricted MAIT cells share features of "innate" T cells such as iNKT cells [4] and T cells restricted by the non classical MHC-like molecule H2-M3 [17]. The ability of innate T cells to act without prior exposure to exogenous antigen and the rapidity of functional responses by these subsets distinguishes them from conventional T cells. Additionally, though not with H2-M3 restricted T cells, the TCR repertoire of innate T cells lacks TCR diversity as exemplified by the relatively fixed TCRα chain expressed on both MAIT and iNKT cells. Furthermore, these populations are expanded thereby facilitating a rapid functional outcome. This, in combination with the recognition of a non-polymorphic MHC molecule, which can theoretically be expressed on almost any cell type, allows for a large and "monoclonal" population to respond in a concerted fashion.

Selection of T cells, which occurs in the thymus for almost all T cell subsets, appears to differ in specific ways between innate and conventional T cells. For classically restricted T cells, thymic selection results a diverse population of T cells with broad antigenic reactivity, and limited autoreactivity. Conversely, thymic selection for innate T cells results in relatively large populations of T cells with potentially limited reactivity. For iNKT cells extensive cell division occurs in the thymus prior to entering the periphery [4]. Although MR1-restricted cells undergo selection in the thymus [11] their replicative history is unknown. Due to the paucity of MAIT cells in mice studies of mouse MAIT cells have primarily been performed using the MAIT TCRα Vα19 transgenic (Tg) mouse. Using MR1 sufficient versus MR1-deficient backgrounds two groups of investigators have independently demonstrated that selection of Vα19 TCR Tg MAIT cells requires thymic selection that is MR1 and β2m-dependent [10, 11]. In contrast to conventional T cells that are positively selected on thymic epithelial cells, innate subsets such as H2-M3-restricted T cells [17] and iNKT cells [18] are selected on hematopoietic cells. MAIT cells are similarly selected on hematopoietic although the selecting cell remains to be defined [3, 11].

In contrast to conventional T cells, where effector function is acquired in the periphery following exposure to cognate antigen, both iNKT and H2-M3 restricted cells are present in the thymus with short-term effector capabilities. It remains to be shown if human MR1-restricted thymocytes, which have a naïve phenotype ([11] and our unpublished studies), display direct ex vivo effector function. In the case of thymic iNKT cells, effector cell programming was dependent on the expression of the transcription factor PLZF [19]. Given the similarities between iNKT cells and MR1-restricted T cells it has been postulated that PLZF may play similar role. Indeed, PLZF is expressed by human peripheral blood $V\alpha7.2^{+}$ T effector cells [19]. Nonetheless, little is known about the expression of PLZF throughout early development of human MR1-restricted T cells. Interestingly, PLZF is not expressed in mouse MAIT cells, which retain a naïve phenotype even in the periphery [11].

A hallmark of MAIT cells, like iNKT cells, is their restricted TCR usage. Regardless of the source or nature of the ligand, the combination of a conserved MR1 molecule with limited allelic variation, in conjunction with the limited TCR sequences associated with MAIT cells cloned so far, suggests MAIT cells recognize a limited set of ligands. Goldfinch et al. compared the TCRα CDR3 sequences, those highly variable regions that allow for antigen discrimination, of MAIT cells in humans, mice and ruminants. In contrast to the highly variable CDR3 TCR region normally associated with MHC-Ia-restricted T cells, the TCRα

CDR3 region of MAIT cells among these mammalian species differed by at most 2 amino acids.

Recent evidence, however, suggests that MAIT cells isolated based on functional reactivity may have a more diverse TCR repertoire. In our limited assessment of TCR sequences from *Mycobacterium tuberculosis* -reactive MAIT cell clones, we have observed additional Jα region as well as Vβ chain diversity compared to previously reported MAIT cells [20]. It is uncertain if discrete subsets of pathogen-reactive MAIT cells will be associated with different TCR clonotypes. Ligand identification and functional analyses of MAIT cell clones with differing Jα regions and alternative Vβ pairing will be required to determine if Vα7.2 T cells detect different ligands. Nevertheless, it is tempting to speculate that different MAIT cell clonotypes recognize specific ligand families.

1.4 MAIT Cell Phenotypes

As mentioned above, MAIT cells have been defined through the use of their semi-invariant TCR expression. Although originally identified in the DN (CD4−CD8−) T cell compartment, MAIT cells have now been shown to be present in $CD8^+$ subsets, including the CD8αα and CD8αβ subsets, and potentially in the $CD4^+$ subset. Human MAIT cells detected through PCR-based quantification of $V\alpha7.2/J\alpha33^+$ expressing T cells were initially identified and primarily present in the $CD4^-CD8^-$ subset [1, 3, 6]. More recent studies have been performed with an antibody that labels all $V\alpha7.2^+$ T cells [11]. Because this antibody detects MAIT cells as well as conventional $V\alpha7.2^+$ T cells cautious interpretation of results using this antibody is warranted. Using this approach, $V\alpha7.2^+$ T cells were identified in all T cell compartments including DN as well as CD8 as well as CD4 subsets [19].

Functional delineation of MAIT cells allows for a precise look at pathogen-reactive MAIT cells. Using direct ex vivo analysis of *M. tuberculosis*-reactive TNF-α-producing $V\alpha7.2^+$ cells from blood, lung and thymus ([20], and our unpublished data) demonstrates the presence of these cells in the $CD8^+$, but not in the DN subset [20]. Furthermore, *M. tuberculosis*-reactive MR1-restricted T cell clones express CD8αβ. In the assessment of human FACS-sorted $V\alpha7.2^+$ T cells, Le Bourhis et al. did not distinguish pathogen reactive responses by DN versus $CD8^+$ MAIT cells [21]. Nonetheless, these authors note they have no evidence to suggest that DN or CD8 subsets are functionally distinct [22].

Growing evidence suggests functional heterogeneity among MAIT cell subsets. For example, $V\alpha7.2^+$ T cells expressing the NK receptor CD161 were capable of producing IL-17 in response to stimulation by phorbol myristate acetate with ionomycin, but not to stimulation via the TCR using α-CD3/CD28. Additional evidence in the mouse supports the possibility that discrete subsets of MAIT cells can be distinguished based on their cytokine expression profile [10]. Using MAIT TCR Tg T cells, Kawachi et al. showed that Vα19i TCR Tg T cells stimulated with α-CD3/ α-CD28 produced IFN-γ, TNF-α, IL-2, IL-4, IL-5 and IL-10. Further analysis demonstrated that the DN T cells were responsible for the IL-4, IL-5 and IL-10 production. Therefore, phenotypic differences might reflect differentiation of subsets based on the local environment or prior microbial exposure. This possibility could influence MAIT cell responses in the context of different pathogens.

As more studies define MR1-restricted T cells based on functional analyses these will allow us to determine if alternative TCRα sequences, Vβ pairing, and co-receptors are associated with functional differences. In combination, these recent findings suggest that as MR1 ligand(s) are discovered, a more complete picture of MAIT cell diversity is likely to emerge.

2. MAIT cells have a complex relationship with microbes

2.1 MR1-restricted T cells are dependent on microbes for their expansion

The first indication that MAIT cells might detect a microbial antigen was based on the observation that MAIT cells were undetectable in the gut lamina propria (LP) of germ-free mice [3]. Similarly, neonatal ruminants have modest frequencies of MAIT cells in the thymus and periphery that expand significantly and selectively in peripheral tissues over the first few weeks of life [15]. Nonetheless, while bacteria may provide ligands to induce peripheral MAIT cell expansion, it is also possible that MAIT cells expand as a result of maturation of the immune system that results from microbial colonization [23].

Studies performed over the last several years have suggested a complex bidirectional relationship between microbial colonization and optimal development of the T cell compartment in the gastrointestinal tract. Germ-free mice lack a mature and developed immune compartment that has been shown to be required for the development of both systemic and mucosal T cells [23]. In one example, *Bacteroides fragilis* colonization was sufficient for the expansion and maintenance of a regulatory $CD4^+$ T cell subset. This effect could be attributed to a single component, polysaccharide A, from *B. fragilis* [24]. Similarly, reconstitution of germ-free mice with a single microbial species known to activate MAIT cells is sufficient for the expansion of these cells in the lamina propria [21]. Therefore, the requirement for microbial colonization for MAIT cell expansion and/or accumulation in the lamina propria could result from either direct microbial antigen presentation to MAIT cells or the development of an immune niche suitable for MAIT cell expansion.

Although B cells were shown to be dispensable for thymic selection of MAIT cells [11] conventional B cells are required for the expansion and/or accumulation of MAIT cells in the lamina propria [3]. However, B1-B cells, an innate-like B cell subset that is expanded in germ-free mice, are dispensable for MAIT cell selection and survival [25] [3]. The mechanisms underlying the dependence on conventional B cells for mucosal MAIT cell expansion remain enigmatic and potentially complex.

2.2 MAIT cells detect cells infected with a variety of microbes

Recent evidence from our group and from that of Lantz and colleagues provided the first demonstration of physiologic function for MAIT cells in the detection of microbial infection [20, 21]. In our long-standing endeavor to better understand the $CD8^+$ T cell response to *M. tuberculosis* in humans we have isolated over a hundred unconventional $CD8^+$ T cell clones from the blood of *M. tuberculosis*-exposed or unexposed individuals. Although these clones were selected based on their ability to recognize *M. tuberculosis*-infected dendritic cells (DC), these T cell clones also produced IFN-γ and TNF-α in response to DC infected with *Mycobacterium smegmatis*, *Escherichia coli*, *Salmonella typhimurium*, *and Staphylococcus aureus* but not *Listeria monocytogenes*. We demonstrated these clones were MAIT cells based on their dependence on MR1 for antigen recognition, the expression of the Vα7.2 TCR chain, and lack of TAP dependence for detecting infected cells.

Similarly, Le Bourhis et al. showed that human monocytes infected with *E. coli* could stimulate a proportion of $V\alpha7.2^+$ T cells from human blood. Likewise, mouse cells infected with diverse bacteria including the Gram-negative bacilli *Pseudomonas aeroginosa* and *Klebsiella pneumoniae*, and Gram-positive *Lactobacillus acidophilus*, *Staphylococcus aureus* and *Staphylococcus epidermidis* induced cell surface expression of the activation marker CD69 on mouse MAIT TCR Tg T cells in an MR1-dependent manner. Conversely *Enterococcus faecalis* and *Streptococcus* group A did not induce mouse MAIT cell activation. In addition to bacteria, infection of cells with fungi such as *Saccharomyces cerevisiae*, *Candida galabrata* and *Candida albicans* induced CD69 upregulation in mouse

MAIT cells and *C. albicans* induced IFN-γ production by human *M. tuberculosis*-reactive MAIT clones (our unpublished work). In contrast, cells infected with viruses including vaccinia virus and adenovirus did not stimulate human *M. tuberculosis*-reactive MAIT clones [20] and, encephalomyocarditis virus, Sendai virus, Newcastle disease virus, herpes simplex virus and parainfluenza 3 virus did not stimulate mouse MAIT cells [21]. These initial observations suggest MAIT cells appear focused on detecting infection with a diverse array of bacteria and fungi but not viruses.

3. What are the MR1 ligands?

3.1 MR1 antigen processing and presentation

To date no MR1 ligand has been identified. However, strong evidence supports the view that MR1 has antigen presentation function. Consistent with its high sequence homology with MHC-Ia molecules [7, 9], initial cellular characterizations showed that MR1 can associate with components of the MHC-Ia pathway. Specifically, MR1 can associate with molecules of the MHC-Ia peptide-loading complex including β2m, calnexin, calreticulin, ERp57, TAP and tapasin [26].

Nonetheless, the presentation pathway that MR1 might utilize is unclear. Hansen and colleagues used cell lines over-expressing MR1 to analyze the endogenous MR1 processing and presentation pathway. In the absence of infection, molecules associated with the MHC-I peptide-loading complex were not required for MR1 cell surface expression and MAIT cell activation. Furthermore, using MR1 transfected cell lines, MAIT hybridoma activation did not require proteasomal processing but was enhanced if components of the HLA-II pathway were over-expressed. Moreover, inhibition of endocytic compartment acidification reduced surface MR1 expression and inhibited activation of MAIT hybridomas. However, ER-to Golgi transport was required and MR1 trafficked in endocytic compartments potentially reflecting MR1's ability to bind both endocytic and exogenous antigens [27]. As mentioned above, MAIT cell selection, as well as MAIT cell recognition of infected cells, is TAP independent. As a result, it appears that the mechanisms underlying the processing and presentation of both exogenous and endogenous MR1 ligands are divergent from those of the classical MHC-Ia pathway.

In support of an endogenous MR1 ligand, Huang et al. demonstrated that acid extracts eluted from recombinant mouse MR1 could stimulate mouse MAIT hybridomas in an MR1-dependent fashion [16]. In this case, the antigen presenting cells were B cells expressing high levels of MR1, such that it is unclear if over-expression of MR1 accurately reflects the physiological antigen processing and presentation pathway. For example, mouse MAIT hybridomas display auto reactivity to over expressed mouse MR1 in the absence of infection or added exogenous antigens. In contrast, human *M. tuberculosis*-reactive MAIT clones are not activated by cells over-expressing human MR1 (our unpublished data).

3.2 MR1 has the capacity to present microbial and self ligands

One unique aspect of MHC-Ia-restricted T cells is their ability to detect intracellular infection by sensing foreign peptide/MHC complexes. However, iNKT, which have qualities of innate as well as adaptive immunity, detect CD1d molecules that present both self and foreign ligands. Due to the similarities between iNKT and MAIT cells, it is tempting to speculate that CD1d and MR1 share a similar antigen presentation paradigm with regard to TCR-dependent activation.

If one function of MR1 is to display ligands associated with altered or stressed "self", the mechanisms underlying this process remain unclear. In this regard, unconventional MHC-I molecules such as MICA [28] and ULBP1 [29] can serve as signals for intracellular stress

that can be detected through the NK receptor NKG2D receptor. NKG2D can be expressed on NK, NKT, γδ T cells and $CD8^+$ T cells, and is present on *M. tuberculosis*-reactive MAIT clones (our unpublished data). Nonetheless, antibody blockade of ULBP1, MICA, NKG2D and its co-receptor CD94 did not abrogate responses by *M. tuberculosis*-reactive MAIT cells [20].

Pathogen-associated molecular patterns associated with induction of well-defined innate pathways such as the Toll-like receptors (TLR) and nucleotide oligomerization domain (NOD) proteins are not sufficient for MAIT cell stimulation. MR1-specific activation of mouse MAIT cells was not mediated through innate immune pathway molecules MyD88, TRIF, Nod1, Nod2, Nlrp3, Asc, and Ips 1[21]. Moreover antibody blockade of TLR2 and TLR4 did not alter the ability of human MAIT cells to detect *M. tuberculosis*-infected DC [20].

Owing to the wide variety of microbes that elicit MAIT cells, one possibility is that MAIT cells detect a common microbial antigen such as a heat shock protein that is associated with some but not all bacteria and fungi. For example, colonization of germ-free mice with a monoclonal culture of *Enterobacter cloacae*, bacteria capable of stimulating mouse MAIT cells in vitro [21], is sufficient to expand MAIT cells in the lamina propria. In contrast, infection of mice with *Enterococcus fecaelis*, a bacterium that did not stimulate mouse MAIT cells similarly did not induce MAIT cell expansion in vivo [21]. Furthermore, DC incubated with microbial products, in the absence of infection, can also stimulate MAIT cells. The *M. tuberculosis* cell wall (CW) fraction stimulated MAIT cells in an MR1-dependent manner [20]. Similarly, paraformaldehyde fixation reduced but did not abrogate the ability of *E. coli* bacteria to stimulate mouse MAIT cells [21].

Alternatively, microbial stimulation of antigen presenting cells may be sufficient to induce cell surface expression of MR1. One possible mechanism for this may be that a microbial trigger allows stabilization of MR1 at the cell surface. Cell surface MR1 protein expression has been very difficult to detect. Although we have detected MR1 on an epithelial cell line infected with *M. tuberculosis* we have been unable, as many other investigators, to visualize MR1 on DC even after infection [20]. These studies suggest that very little cell surface MR1 is present. Regardless of the source of MR1 ligands, it is possible that MR1 is regulated by its association with a chaperone that dictates its cell surface expression.

The antigen trafficking pathway that MR1 might use for self or even different microbial ligands might be different. For example, *M. tuberculosis* is an intracellular pathogen that primarily resides in the phagosome. While *M. tuberculosis* antigens presented by HLA-B molecules adhere to a traditional MHC-Ia presentation paradigm, the non-classical HLA-E antigen can be derived from the *M. tuberculosis* phagosome [30]. This example highlights the need for the immune system to detect antigens from a variety of cellular compartments. Furthermore, MAIT cells detect microbial infection of many different microbes with different cellular lifestyles. Therefore, how MR1 is loaded with either exogenous microbial antigens or endogenous self-ligands remains a key outstanding question.

3.3 Nature of the MR1 ligand

While MR1 and CD1d-restricted T cells share some functional characteristics, the non-polymorphic molecules restricting these T cell subsets diverge widely in their ligand-binding groove. CD1d binds lipids and glycolipids by virtue of its deep and hydrophobic binding groove [31]. In contrast, the MR1 molecule is more akin to MHC-Ia molecules that bind peptides. To address the nature of the putative antigen Ted Hansen and colleagues performed a comparison of the amino acid sequences that form the peptide-binding pocket of MHC-Ia and MR1. Here, the sequences of the binding pockets differed significantly

leading the authors to suggest that MR1 may use a different ligand-binding paradigm from not only MHC-Ia molecules but also other MHC-like molecules [32]. Precedence exists for MHC-Ib molecules to display modified-peptide antigens. For example H2-M3 binds N-formylated peptides [33] while ZAG binds fatty acids [34] as well as a peptide that appears glycosylated [35].

Some evidence for a lipid antigen was generated by Shimamura et al who demonstrated that mycobacterial α-mannosyl glycolipids were able to activate TCR Tg mouse MAIT cells [36]. In contrast, human Vα7.2$^+$ cells were not stimulated by the CD1d ligand α-galactosyl ceramide [37]. Human *M. tuberculosis*-reactive MR1-dependent T cell clones are stimulated by the *M. tuberculosis* cell wall (CW). However, protease treatment, as well as heat inactivation (our unpublished data), abrogated the stimulatory activity of the CW while delipidation did not suggesting that in part, the ligand contains a protein [20]. Moreover, DC loaded with heat-killed *E. coli* [21] or heat-killed *M. tuberculosis* (our own unpublished work) did not induce MAIT cell stimulation. In sum, the nature and the size of the MR1 ligand(s), and the manner in which ligand binding to MR1 might occur all remain to be defined.

4. What role do MAIT cells play in vivo?

4.1 MAIT cells have wide tissue distribution

Historically, the term MAIT cell was assigned to MR1-restricted T cells based on the finding that MAIT cells were enriched in the mucosal lamina propria of both mice and humans [3]. Recent evidence, though, suggests more broad tissue distribution. In human peripheral blood, Vα7.2$^+$ T cells are present at frequencies ranging from <1% to about 10% of T cells [1, 6, 11, 20]. Furthermore, MAIT cells were found to be present at higher frequencies in human liver tissue [22] and in the spleen of ruminants [15].

Conventional memory T cells, as well as iNKT cells, preferentially localize to non-lymphoid tissues where they display an activated effector phenotype [4, 38]. Similarly, MR1-restricted T cells, which also appear to be activated effectors, are enriched in non-lymphoid organs but present at low frequencies in lymph nodes of humans and mature ruminants [15, 20]. T cells expressing the Vα7.2 canonical TCR sequence have been seen in tumor infiltrates [39], as well as in lesions of patients with multiple sclerosis (MS) [37]. Whether or not the presence of these cells plays a pathological role remains to be determined.

The anatomical distribution of MAIT cells positions them to function in the early and rapid detection of bacterial infection. MAIT cells detect intracellular infection of a wide variety of cells including dendritic cells, macrophages, monocytes, and epithelial cells [20, 21]. Specifically, MAIT cells are capable of detecting infected epithelial cells that lack MHC-II molecules and therefore cannot be recognized by CD4$^+$ T cells. For example, *M. tuberculosis*-infected human primary large airway epithelial cells can serve as targets for MAIT cells [20]. *M. tuberculosis*-reactive MAIT clones efficiently lyse *M. tuberculosis*-infected epithelial cells (our unpublished data), and through use of the granule exocytosis pathway, could effect clearance and/or control of intracellular infection prior to the acquisition of an adaptive immune response. Furthermore, *M. tuberculosis*-reactive MAIT cells are enriched in the lung compared to the blood and the draining mediastinal lymph nodes. These data suggest that high frequencies of MAIT cells, enriched at mucosal sites and in tissues, in combination with their ability to detect intracellular infection of non-professional antigen presenting cells, places them in a unique position to provide early and rapid control of intracellular pathogens.

Accompanying this innate effector response, MAIT cells could augment a subsequent adaptive immune response as shown with other innate lymphocytes such as iNKTs, H2-M3-restricted cells, as well as NK cells. MAIT cells produce IFN-γ and TNF-α in response to *M. tuberculosis*-infected cells [20]. IFN-γ plays a key role in the maturation of dendritic cells that prime antigen specific conventional $CD4^+$ and $CD8^+$ T cells that display a Th1-type phenotype [40]. As a result, resident MAIT cells could provide an early source of IFN-γ that contributes to optimized T helper type-1 T cell responses. MAIT cells therefore have the capacity to act as innate effectors and as enhancers of adaptive immunity.

4.2 Relationship of MAIT cells to disease

Accumulating evidence supports a role for MR1-restricted MAIT cells in the host response to bacterial infection. In mice infected for 3 days with *E. coli*, bacterial control was significantly enhanced in $MR1^+$ compared to $MR1^-$ Vα19 Tg mice. This correlated with increased mRNA of the TCR transgene for mouse MAIT cells in the draining lymph nodes and increased activation of peritoneal T cells. Modest control of *Mycobacterium abscessus*, which was dependent on both MR1 and presence of TCR Tg MAIT cells, was observed 15 days after infection [21]. Furthermore, Georgel et al. have demonstrated MR1-dependent control of *Klebsiella pneumoniae* in mice [41]. Here, MR1 knock out (KO) mice challenged with *K. pneumoniae* had higher bacterial burden and a significant increase in mortality in the first four days following infection. Interestingly, MR1 KO mice surviving beyond this time had equivalent bacterial loads to wild type controls, consistent with an early role for MR1 in infection. Furthermore, pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6 and IL-17 were markedly decreased in the KO mice while IL-10 remained unaltered. At this point the MR1-dependent mechanisms underlying the inflammatory milieu are unclear. Finally, MR1 KO mice were also more susceptible to *S. typhimurium* but not to several other Gram-negative bacteria such as *E. coli, Yersinia enterocolitica and Shigella dysenteriae*.

In humans with active tuberculosis (TB), *M. tuberculosis*-reactive MR1-dependent MAIT cells were undetectable in peripheral blood in comparison to those observed in healthy individuals and those with latent TB [20]. A similar observation was made by Lantz and colleagues who showed that MAIT cell frequencies were decreased in the blood of patients with TB as well as bacterial lung infections in comparison to individuals with cancer and healthy controls [21]. A potential explanation for these findings is that MAIT cells migrate to bacterially infected tissues and are depleted from the blood. It will be of interest to determine if other infections that do not target the lung also induce depletion of MAIT cells in the periphery.

In addition to microbial detection and control, MAIT cells may also provide additional functions. In a mouse model of multiple sclerosis, increased frequencies of mouse MAIT cells were associated with diminished disease severity while the absence of MR1 was associated with exacerbation. These data suggest that MAIT cells may play a regulatory role [42]. Although the ability of MAIT cells to respond rapidly suggests they might provide protection in the initial exposure to microbes, characterizing the ontogeny of the MAIT cell responses in relationship to bacterial exposure will be necessary to define the role of this subset in vivo. At this point it is unclear if MAIT cells are capable of long-term differentiation and if they could participate in a recall response to secondary infection.

Conclusions

MR1-restricted T cells are a unique T cell subset that shares strong parallels with iNKT cells. Like CD1-restricted iNKT cells, MAIT cells are restricted by a non-polymorphic MHC-like molecule and appear to have limited ligand discrimination through their semi-invariant TCR. Like other innate T cells they are capable of rapid effector function, likely

the consequence of transcriptional profiling through PLZF. However, in contrast to CD1d restricted iNKT cells it appears MAIT cells are uniquely predisposed to detect microbial infection. Because MAIT cells are highly enriched in mucosal tissues, MAIT cells are in position to act quickly to control microbial infection. To date, MAIT cells have principally been described based on TCR usage. However, categorization based on bacterial and/or fungal reactivity will result in a more complete picture of MAIT cells. Consequently, it remains to be determined if all MR1-restricted cells are alike and share the ability to detect microbes, recognize the same antigen(s) and perform similar effector functions. In summary, MAIT cells are a unique and evolutionarily conserved T cell population that appears universally present in mammals. Due to their ability to perform rapidly, and their localization at peripheral sites, MAIT cells have the attributes necessary to act as early effectors in response to initial exposure to mucosal microbial infection.

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