

Title: Biological functions of MAIT cells in tissues

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Abstract

Mucosal associated invariant T (MAIT) cells have a recognised innate-like capacity for antibacterial host defence, consequent on the specificity of their T cell receptor (TCR) for small molecule metabolites produced by a range of prokaryotic and fungal species, their effector memory phenotype, and their expression of cytotoxic molecules. However, recent studies have identified at least two other important functions of MAIT cells in antiviral immunity and in tissue homeostasis and repair. Each are related to distinct transcriptional programmes, which are activated differentially according to the specific immune context. Here we discuss these diverse functions, we review the evidence for the newly identified role of MAIT cells in promoting tissue repair, and we discuss emerging data pointing to the future directions of MAIT cell research including roles in cancer, in antiviral immunity and recent studies in the immune response to SARS-CoV-2 infection. Overall these studies have made us aware of the potential for pleiotropic roles of MAIT cells and related cell populations in mouse and man, and have created a simple and attractive new paradigm for regulation in barrier tissues, where antigen and tissue damage are sensed, integrated and interpreted.

Highlights

- MAIT cells recognise vitamin-B2 metabolites via their semi-invariant TCR
- TCR-independent cytokine-mediated activation extends roles to anti-viral defence
- MAIT and iNKT cells share common transcriptional programmes
- Gene set enrichment analyses implicate MAIT cells in novel tissue repair roles
- MAIT cells are highly activated by SARS-CoV2 virus in Covid-19

Key words MAIT cell, iNKT cell, virus, Covid-19, SARS-CoV-2, tissue repair

1. Introduction

In this review, part of a special edition in Molecular Immunology to commemorate the work of Vincenzo (Enzo) Cerundolo, we will discuss some of the diverse functions of mucosal associated invariant T (MAIT) cells¹ in peripheral tissues. This topic was the key focus of one of the sessions in the EMBO CD1-MR1 conference in Oxford in September 2019, a few short months before Enzo passed away. Enzo was a driving force behind the conference, and despite his diagnosis he had enormous energy to devote to this. The three of us as authors of this piece owe him a great debt as new entrants into the MAIT field, and the generosity he showed in sharing his ideas, materials and time in helping establish what has been a very exciting area of immunology was remarkable.

Much of the initial work on MAIT cells that emerged from studies of CD161⁺ T cell populations, which are highly enriched in the human liver (Dusseaux et al. 2011) benefited enormously from the fundamental discoveries on human and murine invariant natural killer T (iNKT) cells from Enzo and his team (Gadola, Zaccai, et al. 2002; Gadola, Dulphy, et al. 2002; McCarthy et al. 2007). Although it is obvious now, it was less obvious initially how interconnected these two cell types were, and Enzo's guidance over time and his connections with the interested labs helped drive progress very rapidly. In particular, the development of key tools and reagents which have been generously shared by collaborators meant it has been possible to explore the functional diversity of human and mouse MAIT cells in parallel. Akin to memory CD8 T cells, MAIT cells can be triggered by cytokine dependent stimulation (Ussher et al. 2014; Jo et al. 2014). We therefore set up to investigate difference between functions triggered through the TCR and those triggered by cytokines. Interestingly, the 3 groups co-authoring here pursued a parallel track using related approaches *in vitro* and *ex vivo* and co-published their findings in 3 papers in *Cell Reports*. In this review we will

¹ *Abbreviations:* 5-OP-RU, 5-(2-oxopropylideneamino)-6-D-ribitylamouracil; 5-A-RU, 5-amino-6-D-ribitylamouracil; Covid-19, coronavirus disease-19; GSEA, gene set enrichment analysis; iNKT, invariant natural killer T; MAIT, mucosal associated invariant T; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; Tc17, type-17 CD8⁺ T cells

review the key messages of those papers – also presented at the CD1 MR1 Symposium - and how the field has subsequently progressed.

2. MAIT and viral infections

Like iNKT cells, MAIT cells can be activated via their TCR or by cytokines. The MAIT cell TCR recognises unstable pyrimidine antigens, such as 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), bound to MR1. These pyrimidine antigens are derived from 5-amino-6-D-ribitylaminouracil (5-A-RU), an intermediate in microbial riboflavin metabolism (Corbett et al. 2014). In addition, MAIT cells can also be activated independently of their TCR by proinflammatory cytokines (Ussher et al. 2014; Jo et al. 2014). The kinetics of these two mechanisms of activation differ, with interferon-gamma production occurring early (6 hours) in response to TCR-mediated activation, while much later (20-24 hours) in response to IL-12 and IL-18 (Ussher et al. 2014). In infection with riboflavin-synthesising bacteria, both pathways will be activated, although likely at different time points, while in viral infection, only cytokine-mediated activation will occur.

The first cytokines shown to activate MAIT cells in the absence of TCR triggering were IL-12 and IL-18 (Ussher et al. 2014; Jo et al. 2014). This was subsequently extended to show a major role for Type I interferons and also IL-15 (Sattler et al. 2015; van Wilgenburg et al. 2016). In other settings members of the TNF superfamily have also been shown to be impactful in cytokine mediated activation of human T cells, including TL1A (Sattler et al. 2019; Jin et al. 2013). Whereas known MAIT antigens are in bacteria and fungi, these data underpinned growing evidence for a possible role of MAIT cells in responses to viruses (Loh et al. 2016; Sattler et al. 2015; van Wilgenburg et al. 2016). Indeed, MAIT cells are activated during acute (Dengue fever, influenza) and chronic (hepatitis C) virus infections and severe influenza (van Wilgenburg et al. 2016). Activation was dependent upon IL-18 and IL-12, IL-15, or type I interferons and resulted in production of interferon- γ and upregulation of granzyme B

expression. MAIT cell activation correlated with severity in Dengue fever, and activated MAIT cells were able to control HCV replication *in vitro* via interferon-gamma production (van Wilgenburg et al. 2016). Independently, MAIT cell frequency in blood was lower in humans with a fatal outcome from influenza H7N9 infection than in those who survive, and a role for IL-18 in MAIT cell activation was found (Loh et al. 2016). To determine whether such activation contributed more to immune pathology or protection requires *in vivo* studies. A murine model of influenza A showed early activation and pulmonary accumulation of MAIT cells, with increased weight loss and mortality in MR1^{-/-} mice. Adoptive transfer of MAIT cells ameliorated severe disease, suggesting MAIT cells play a protective role (van Wilgenburg et al. 2018). Finally, a crucial role for IFN- α -dependent TNF release was recently shown *in vitro* and *in vivo* in response to adenovirus vectors (Provine et al. 2020).

3. A new role of MAIT cells in tissue repair

To define the effector response of human MAIT cells to different stimuli, the Otago group investigated the transcriptional and effector responses of MAIT cells to TCR stimulation with 5-A-RU or ligand producing *Escherichia coli* (both at 6 hours) or with IL-12 and IL-18 (at 24 hours) (Lamichhane et al. 2019). Stimulation with 5-A-RU or *E. coli* resulted in a rapid, polyfunctional, proinflammatory response. In contrast, the response to IL-12 and IL-18 was slower and more restricted. Compared with cytokine activated MAIT cells, TCR activated MAIT cells produced a broader array of proinflammatory cytokines and chemokines such as IL-1A, -1B, -2, -22, GM-CSF, CCL3, CCL4 and CCL20, and were able to recruit neutrophils via CCL2 and XCL2 in an MR1-dependent manner. In contrast, MAIT cells activated by IL-12 and IL-18 predominantly produced interferon-gamma. GSEA (gene set enrichment analysis) revealed that type I interferon signalling was upregulated in MAIT cells stimulated with *E. coli* but not 5-A-RU. In a subsequent study, the authors showed that type I interferons are an important co-stimulatory signal for enhancing TCR-mediated

MAIT cell activation (Lamichhane et al. 2020). Transcriptional analysis also revealed - upon TCR stimulation (both 5-A-RU and *E. coli*) - a tissue repair signature, previously described for unconventional, H2M3-restricted, Tc17 cells elicited in response to the commensal *S epidermidis* by Linehan et al (Linehan et al. 2018), suggesting that activated MAIT cells may also have a role in tissue homeostasis. This gene signature was similar in both human and mice, including ten genes with a range of functions which were enriched in both species: TNF, CSF2, HIF1A, FURIN, VEGFB, HMGB1, PTGES2, PDGFB, TGFB1 and MMP25. Thus, MAIT cells have antigen dependent and independent pathways that when triggered separately lead to differing outcomes.

After *in vitro* optimization of cytokine stimulation, including TL1A, IL-15, IL-12 and IL-18, the Oxford group assessed functional differences between cytokine vs TCR stimulation, or both stimuli combined, using RNAseq (Leng et al. 2019). The expectation was that the cytokine stimulation would provoke a more limited response compared to bona fide TCR stimulation of a T cell, but the response in the cytokine arm was unexpectedly broad, and a number of distinct features were noted. The expression patterns most evidently distinct in the TCR-only arm included a number of genes linked to the “tissue repair” signature described above. A subset of these genes – TNF, Furin and CCL3 – was functionally validated *in vitro* by flow cytometry. Overall this was consistent with potential homeostatic role of MAIT cells and a potential link between maintaining and repairing a barrier with more conventional antimicrobial functions at barrier surfaces.

These data integrated well with those of the Melbourne team (Hinks et al. 2019) and reinforced not only that biological message, but also the potential for dataset integration across experimental platforms, species and continents. This third, parallel study used the same RNA sequencing approach to define the basic transcriptome of tetramer-sorted MAIT cells at rest and during TCR-stimulation. In addition to human MAIT cells, this study also analysed murine MAIT cells stimulated *in vivo*, so it

was possible to compare directly across species and during different stages of infection. Murine MAIT cells were stimulated using the intracellular pathogen *Legionella longbeachae*, which induces a rapid expansion of activated pulmonary MAIT cells by 7 days, followed by long-term persistence of an expanded population after pathogen clearance, and which can then be further activated by a repeat infectious challenge (Wang et al. 2018). TCR-mediated activation induced very similar type 1 and type 17 inflammatory responses in both species, and a common array of chemokines (including XCL1, CCL3, CCL4, and CXCL16) and chemokine receptors (CCR6, CXCR6, CCR1, CCR2, and CCR5), underlining a marked evolutionary conservation of MAIT cell functions, whilst resting MAIT cells were characterised by expression of the anti-apoptotic cytokine, IL-15.

These transcription profiles were then compared using hierarchical clustering with those of a wide range of other innate and adaptive immune cells, including innate lymphoid cells, natural killer cells, $\gamma\delta$ T cells, iNKT cells, and a range of CD8⁺ conventional T cells from the Immunological Genome Project database (Heng, Painter, and Immunological Genome Project 2008). Clustering patterns showed that activated MAIT cells most closely resembled iNKT cells, but by contrast, after resolution of infection resting MAIT cells clustered most closely with unstimulated splenic $\gamma\delta$ T cells, suggesting that in different contexts MAIT cells expressed differing transcriptional programmes which were shared with other innate-like T cells. Consistent with this the Lantz group have shown in mice that, once stratified into ROR γ t⁺ (type 17) or ROR γ t⁻ (type 1) cells, MAIT cells and iNKT cells share a virtually identical transcriptome, differing only according to their TCR specificity and a handful of genes, notably increased expression of Cd8a, Ccl3, Ccl4, Itgae, Klrb1b and Klra in MAIT cells (Salou et al. 2019). Of note, a recent study of human gamma-delta populations also identified a population of CD26⁺V δ 2 T cells with a very similar transcriptome to that of MAIT cells (Wragg et al. 2020).

Furthermore, the analyses in all the three papers published in the same time frame (Lamichhane et al. 2019; Leng et al. 2019; Linehan et al. 2018) also discovered very strong enrichment of the same TCR-triggered tissue repair gene set in both humans and mice. Indeed, this similarity led to the clustering of commensal-specific H2M3-restricted Tc17 cells immediately adjacent to MAIT cells when included in a comparative analysis, providing more evidence of shared transcriptional programmes common to specific subsets of non-classical T cells.

What evidence is there that these transcriptional programmes lead to important functional consequences? Accelerated closure of intestinal epithelial cell lines in a wound healing assay *in vitro* occurs when exposed to TCR-triggered MAIT cell supernatants (Leng et al. 2019), which was demonstrated *in vivo* (Constantinides et al. 2019). Experiments were conducted in *Tcrd*^{-/-} mice, to avoid confounding by the commensal-specific tissue repair activity by $\gamma\delta$ T cells. The healing of skin punch biopsies was slower in the *Tcrd*^{-/-} *Mr1*^{-/-} mice which lack MAIT cells, and conversely could be accelerated by enhancing MAIT cell activity by topical application of the MAIT cell ligand, 5-OP-RU, prior to wounding. This series of experiments demonstrated several other interesting findings. IL-17A-producing MAIT cells are enriched in the murine skin dermis and their frequencies were influenced by the presence or absence of specific riboflavin-synthesizing gastrointestinal flora in the first few weeks of life, but not in adulthood (Constantinides et al. 2019). In adulthood their frequencies could be enhanced by topical association with the skin commensal *Staphylococcus epidermidis*, even in *Lta*^{-/-} mice, which lack secondary lymphoid organs, and not in parabiotic animals, suggesting this expansion occurred locally in the tissue, leading to a tissue-resident MAIT cell population. MAIT cells could also be expanded locally by topical 5-OP-RU, whilst commensal induced expansion was prevented by conditional MR1 knockout, suggesting that skin MAIT cell expansion was TCR-dependent.

Together, these observations suggest that MAIT cells may do much more than act as early inducers of inflammatory responses to invasive bacterial pathogens. Rather, their abundance in barrier

surfaces such as lung and skin, and capability to sense and respond to ligands produced by commensal populations, suggest an important, perhaps even dominant role could be maintenance of the integrity of surface epithelia. How important this function is at other barrier surfaces with higher (gut) or lower (lung) commensal biomass remains to be investigated.

Secondly these diverse functional responses of MAIT cells activated by different stimuli – be it TCR stimulation during homeostasis, cytokine stimulation in the context of viruses, or both in the context of bacterial infection – identify MAIT cells as multifunctional cells that express a range of activities depending on the tissue site and nature of stimulus received. This is likely to be a pattern common to other innate-like cells. In addition, in some circumstances even conventional $\alpha\beta$ T cells can respond to local stimulation. For example, we have recently reported that type-2 CD8⁺ T (Tc2) cells can be activated by leukotrienes, prostaglandins, their combination or via the TCR (Hilvering et al. 2018) (and unpublished data). The expression of common effector programmes by diverse cells, each controlled by specific master transcription factors, could be merely a relic of the manner in which the immune system has evolved, but more likely these conserved responses underscore the protective advantages provided by immunological redundancy.

4. Future directions

The distinct functions of MAIT cells have drawn much attention in infection, and increasingly attention has turned to their role in cancer, a complex area where discrepant results have been obtained. If MAIT cells can – according to the signals they receive – contribute to conventional cytotoxic and Type 1 host defence, or contribute to the induction of such defence through IFN- γ secretion, then one might predict a protective role (Sundstrom et al. 2019). In contrast, tissue repair and pro-proliferative activity such as secretion of IL-22 and growth factors, or even regulatory

activity, could promote fibrotic disorders or tumour growth. Interestingly tumour-associated bacteria such as *Fusobacterium nucleatum* could contribute to this through TCR-mediated MAIT cell activation (Li et al. 2020).

Recent papers also suggest a role for MAIT cells in the immune response to SARS-CoV-2. In Covid-19 patients, MAIT cells are profoundly depleted from the blood, even more so than other T cell subsets, and are enriched in the airways (Kuri-Cervantes et al. 2020; Parrot et al. 2020; Jouan et al. 2020; Flament et al. 2020). The residual MAIT cells in the blood are activated, with increased expression of CD69, CD38, HLA-DR, CD56, and granzyme B, and reduced expression of CXCR3 (Kuri-Cervantes et al. 2020; Parrot et al. 2020; Flament et al. 2020; Jouan et al. 2020). MAIT cell activation is associated with serum levels of IL-18 (Flament et al. 2020; Jouan et al. 2020). MAIT cells in the airways are also activated and have bias towards IL-17A and TNF production. Enrichment in the airways is associated with multiple chemokines including CXCL10 (Parrot et al. 2020; Jouan et al. 2020). While in one study, high expression of CD69 and low expression of CXCR3 on MAIT cells was associated with death (Parrot et al. 2020), another study found that CD69 expression on MAIT cells and iNKT cells on admission was associated with improved oxygenation at day 7 and increased discharge from intensive care at day 15 (Jouan et al. 2020). MAIT cells frequencies recover in the convalescent phase with phenotypic changes largely resolving. Overall, the data suggests that while MAIT cells may contribute to airway inflammation, they may also contribute to resolution of disease. Whether this reflects their tissue repair functions, which are more prominent with TCR stimulation, is yet to be determined. As, coronaviruses, like other viral pathogens, do not produce the kinds of MR1-restricted pyrimidine antigens found in bacteria and fungi, this strong evidence for virus induced activation again points to the need to understand the physiological roles of cytokine-based trans-activation.

Overall, the studies have really opened our eyes to the potential distinct roles of MAIT cells and the related cell populations found in mice and humans, and created a simple and attractive new paradigm for regulation in barrier tissues, where antigen and tissue damage (or danger) are sensed, integrated and interpreted (see figure). This two-pronged activation with distinct *in vivo* consequences could indeed be a broader feature of the innate-like or unconventional T cell family, so this issue will be of interest to many different groups in future, especially given its potential impact on a range of diseases, from bacterial and viral infection, through inflammation to cancer.

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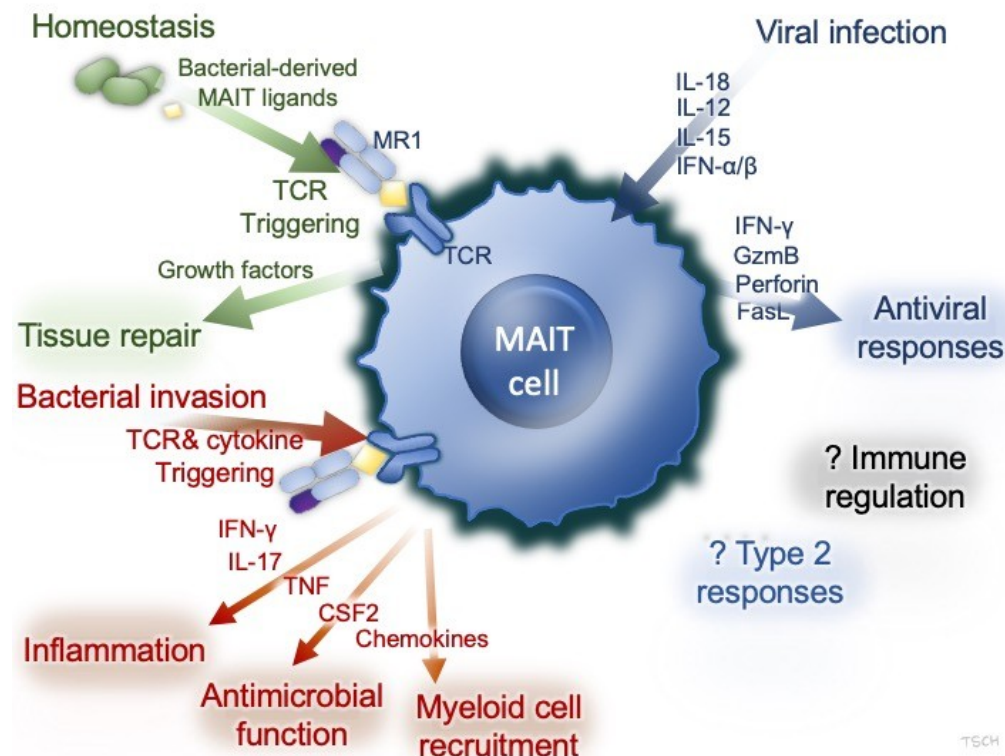
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Figure



Context-dependent MAIT cell functions. Commensal derived MAIT cell ligands, in the absence of co-stimulation trigger the MAIT cell TCR to promote secretion of growth factors leading to tissue repair and homeostasis. Where a mucosal barrier is breached and invasive bacterial infection occurs, these TCR signals are accompanied by cytokine co-stimulation inducing MAIT cell antibacterial defence functions including production of IFN- γ , IL-17, TNF, CSF2, and chemokines leading to inflammation, direct antimicrobial functions and recruitment of myeloid cells. Virally-infected cells induce TCR-independent activation via the cytokines IL-18, IL-12, IL-15, and type I interferons, which upregulates direct antiviral functions IFN- γ and cytotoxic activity. Other transcriptional programmes which may occur could include type-2 cytokine responses or immunoregulatory effects, but remain to be defined. CSF2, colony stimulating factor 2 (GM-CSF); FasL, Fas cell surface death receptor ligand;

GzmB, granzyme B; IFN, interferon; IL, interleukin; MAIT, mucosal associated invariant T; TCR, T cell receptor.