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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) cells 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 had toward helping establish what force betwen 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 (Uscher 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 review the key messages of those papers – also presented at the CD1 MR1 Symposium - and how the field discoveries on human and murine invariant natural killer T (iNKT) cells from Enzo and his team (Gadola et al., 2002a, b; 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 (Uscher 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 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-oxopropylidenamino)-6-D-ribitylaminouracil (5-OP-RU), bound to MR1. These pyrimidine antigens are derived from 5-amino-6-D-ribitylamino-purineriboside (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 h) in response to TCR-mediated activation, while much later (20–24 h) 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 TIL1A (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-12 and IL-18, 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-ARU or ligand producing Escherichia coli (both at 6 h) or with IL-12 and IL-18 (at 24 h) (Lamichhane et al., 2019). Stimulation with 5-ARU 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-1α, -1β, -2, -22, GM-CSF, CCL3, CCL4 and CXCL2, and were able to recruit neutrophils via CXCL2 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-ARU. 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-ARU and E. coli) - a tissue repair signature, previously described for unconventional, H2M3-restricted, Tc17 cells elicited in response to the commensal S. enterica 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, TGFβ1 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 IL1A, 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 transcriptional 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, γδ T cells, iNKT cells, and a range of CD8+ conventional T cells from the Immunological Genome Project database (Heng et al., 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 γδ 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 RORα- (type 1) or RORγt- (type 17) MAIT 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 Cda8a, Ccl3, Ccl4, Itgae, Kib1b and Kira in MAIT cells (Sulou et al., 2019). Of note, a recent study of human gamma-delta populations also identified a population of CD26+CD82 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...
ments were conducted in exposed to TCR-triggered MAIT cell supernatants (Leng et al., 2019), 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 Terd− mice, to avoid confounding by the commensal-specific tissue repair activity by γδ T cells. The healing of skin punch biopsies was slower in the Terd− 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 of 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 para-biotic 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 γδ 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; Flamant 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; Flamant et al., 2020; Jouan et al., 2020). MAIT cell activation is associated with serum levels of IL-18 (Flamant 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
The authors report no declarations of interest.

References

Constantinides, M.G., Link, V.M., Tamoutouis, S., Wong, A.C., Perez-Chaparro, P.J., Han, S.J., Chen, Y.E., Li, K., Farhat, S., Weckel, A., Krishnamurthy, S.R., Vujkovic-Cvijin, I., Linsen, J.L., Bouladoux, N., Merritt, E.D., Roy, S., Cua, D.J., Adamo, E.J., Bhandoola, A., Scharschmidt, T.C., Aube, J., Fischbach, M.A., Belkaid, Y., 2019. MAIT cells are imprinted by the microbiota in early life and promote tissue repair. Science 366, 3061–3065.

Dusseau, M., Martin, E., Serriari, N., Pueguet, I., Premel, V., Louis, D., Milder, M., Le Bourhis, L., Soudais, C., Treiner, E., Lantz, O., 2011. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. Blood 117, 2011. Human mucosal-associated invariant T cells contribute to antiviral immunity via IL-18-dependent activation. Proc. Natl. Acad. Sci. U.S.A. 113, 10133–10138.

McCarty, C., Shepherd, D., Fleire, S., Stronge, V.S., Koch, M., Illarionov, P.A., Bossi, G., Akther, H.D., Hackstein, C.P., Powell, K., King, T., Friedrich, M., 2020. OX40L binds ligands at 2.3 A, a maze for alkyl chains. Nat. Immunol. 3, 721–726.

Flament, H., Roulan, M., Beaudein, L., Fauchet, A., Bertrand, L., Lebourgeois, S., Pampena, M.B., Meng, W., Rosenfeld, A.M., Ittner, C.A.G., Reiter, Y., Griffiths, G.M., van der Merwe, P.A., Besra, G.S., Jones, E.Y., Batista, F.D., Trinchieri, G., Brenchley, J.M., O’Shannessy, C., 2007. The length of lipids bound to human CD1d molecules modulates the affinity of NKT cell TCR and the threshold of NKT cell activation. Eur. J. Immunol. 37, 784–796 e18.

Loh, L., Wang, Z., Sani, Z., Koutsakos, M., Timsit, J., Monteiro, R.C., Bourhis, L., Soudais, C., Treiner, E., Lantz, O., 2011. Human MAIT cells are imprinted by the microbiota in early life and promote tissue repair. Nature 490, 361–365.

Lindelöf, M., Harrison, O.J., Han, S.J., Byrd, A.L., Vujkovic-Cvijin, I., Villarino, A.V., Sheen, S.K., Shai, J., Smelkinson, M., Tamoutouis, S., Collins, N., Bouladoux, N., Dzuvez, A., Rosskopf, S.P., Arbuckle, J.L., Wang, C.R., Meyer, N.J., Betts, M.R., 2020. Comprehensive mapping of immune cell activation and in vivo evolution of the MAIT cell transcriptome in colorectal cancer. Cell Rep. Med. 1, 100039.

Linehan, J.L., Harrison, O.J., Han, S.J., Byrd, A.L., Vujkovic-Cvijin, I., Villarino, A.V., Sheen, S.K., Shai, J., Smelkinson, M., Tamoutouis, S., Collins, N., Bouladoux, N., Dzuvez, A., Rosskopf, S.P., Arbuckle, J.L., Wang, C.R., Meyer, N.J., Betts, M.R., 2020. Comprehensive mapping of immune cell activation and in vivo evolution of the MAIT cell transcriptome in colorectal cancer. Cell Rep. Med. 1, 100039.

Nalchniuk, A., Gouzou, S., Rousset, C., Song, L., Surenaud, M., Luce, S., Bailleul, K., Andreu, M., Boitard, C., Vallet-Fichard, A., Gautier, J., Arjaneen, N., Terrier, B., Pene, F., YaadanaPanab, Y., Vissou, B., Descamps, D., Timoté, J., Monteiro, R.C., Leharou, A., 2020. Outcome of SARS-CoV-2 infection linked to MAIT cell activation and cytokine toxicity: evidence for an IL-18 dependent mechanism. medRxiv. https://doi.org/10.1101/2020.08.31.20185082.

Parrot, T., Gorin, J., Ponzetta, A., Maleki, K.T., Kammann, T., Emgard, J., Potti, A.P., Linehan, J.L., Harrison, O.J., Han, S.J., Byrd, A.L., Vujkovic-Cvijin, I., Villarino, A.V., Sheen, S.K., Shai, J., Smelkinson, M., Tamoutouis, S., Collins, N., Bouladoux, N., Dzuvez, A., Rosskopf, S.P., Arbuckle, J.L., Wang, C.R., Meyer, N.J., Betts, M.R., 2020. Comprehensive mapping of immune cell activation and in vivo evolution of the MAIT cell transcriptome in colorectal cancer. Cell Rep. Med. 1, 100039.

Shea, J.J., Belkaid, Y., 2018. Non-classical T cell receptors expand the effector potential of MAIT cells. J. Immunol. 191, 191–196.

Sheeran, B., Stoker, G., Marchi, E., Jabeen, M., Olshansky, M., Kurioka, A., Pediongco, T.J., Fauchet, A., Bertrand, L., Lebourgeois, S., Pampena, M.B., Meng, W., Rosenfeld, A.M., Ittner, C.A.G., Reiter, Y., Griffiths, G.M., van der Merwe, P.A., Besra, G.S., Jones, E.Y., Batista, F.D., Trinchieri, G., Brenchley, J.M., O’Shannessy, C., 2007. The length of lipids bound to human CD1d molecules modulates the affinity of NKT cell TCR and the threshold of NKT cell activation. Eur. J. Immunol. 37, 784–796 e18.

Wherry, E.J., Meyer, N.J., Betts, M.R., 2020. Comprehensive mapping of immune perturbations associated with severe COVID-19. Sci. Immunol. 5, eabe1670.
Sattler, A., Dang-Heine, C., Reinke, P., Babel, N., 2015. IL-15 dependent induction of IL-18 secretion as a feedback mechanism controlling human MAIT-cell effector functions. Eur. J. Immunol. 45, 2286–2298.

Sattler, A., Thiel, L.G., Ruhm, A.H., Souidi, N., Seifert, M., Herberth, G., Kotsch, K., 2019. The TL1A-DR3 axis selectively drives effector functions in human MAIT cells. J. Immunol. 203, 2970–2978.

Sundstrom, P., Szeponik, L., Ahlmanner, F., Sundquist, M., Wong, J.S.B., Lindskog, E.B., Gustafsson, B., Quiding-Jarbrink, M., 2019. Tumor-infiltrating mucosal-associated invariant T (MAIT) cells retain expression of cytotoxic effector molecules. Oncotarget 10, 2810–2823.

Ussher, J.E., Bilton, M., Attwood, E., Shadwell, J., Richardson, R., de Lara, C., Mettke, E., Kurioka, A., Hansen, T.H., Klenerman, P., Willberg, C.B., 2014. CD161++ CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. Eur. J. Immunol. 44, 195–203.

van Wilgenburg, B., Schervitzl, I., Hutchinson, E.C., Leng, T., Kurioka, A., Kulicke, C., de Lara, C., Cole, S., Vasawathana, S., Limpitikul, W., Malasit, P., Young, D., Denney, L., Stop-Hcv consortium, Moore, M.D., Fabris, F., Giordani, M.T., Oo, Y.H., Laidlaw, S.M., Dustin, L.B., Ho, L.P., Thompson, F.M., Ramamurthy, N., Mongkolapaya, J., Willberg, C.B., Screaton, G.R., Klenerman, P., 2016. MAIT cells are activated during human viral infections. Nat. Commun. 7, 11653.

van Wilgenburg, B., Lob, I., Chen, Z., Pediongeo, T., Wang, H., Shi, M., Zhao, z., Koutsakos, M., Nussing, S., Sant, S., Wang, Z., D’Souza, C., Almeida, G.F., Kostenko, L., Ekle, S.B., Meehan, B., Godfrey, D.I., Reading, P.C., Corbett, A.J., McCluskey, J., Klenerman, P., Kedzierska, K., Hinks, T.S.C., 2018. MAIT cells contribute to protection against lethal influenza infection in vivo. Nat. Commun. 9, Wang, H., D’Souza, C., Lim, X.Y., Kostenko, L., Pediongeo, T.J., Ekle, S.B., Meehan, B. S., Shi, M., Wang, N., Li, S., Liu, L., Mak, J.Y.W., Fairlie, D.P., Iwakura, Y., Gunning, J.M., Stent, A.W., Godfrey, D.I., Rossjohn, J., Westall, G.P., Kjer-Nielsen, L., Strugnell, R.A., McCluskey, J., Corbett, A.J., Hinks, T.S.C., Chen, Z., 2018. MAIT cells contribute to protection against pulmonary Legionella longbeachae infection. Nat. Commun. 9, 3350.

Wragg, K.M., Tan, H.X., Kristensen, A.B., Nguyen-Robertson, C.V., Kelleher, A.D., Parsons, M.S., Wheatley, A.K., Berzins, S.P., Pellicci, D.G., Kent, S.J., Juno, J.A., 2020. High CD26 and low CD94 expression identifies an IL-23 responsive Vdelta2(+) T cell subset with a MAIT cell-like transcriptional profile. Cell Rep. 31, 107773.