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MAIT cells: programmed in the thymus to mediate immunity within tissues

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MAIT cells are an evolutionarily conserved T cell subset recognizing ubiquitous microbial metabolites. Herein, we review recent literature showing that MAIT cells can be divided into type 1 and type 17 subsets, which acquire a tissue resident differentiation program in the thymus and localize in specific tissues. We also discuss the nature and *in vivo* availability of the different agonist and antagonist MAIT ligands with potential consequences for MAIT cell biology.

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Introduction

MAIT cells have become the subject of intense interest because of their abundance in humans, their recognition of most bacteria and yeasts, and their modifications in several diseases. In contrast with the large body of knowledge available on NKT cell biology, much less is known about the development of MAIT cells. Still, it appears more and more clear that murine MAIT and NKT cells share a number of key features. Both T cell subsets express the master transcription factor PLZF (encoded by Zbtb16), probably as a consequence of positive selection on $CD4^+CD8^+$ (DP) thymocytes expressing their restricting MHC molecules (MR1 and CD1d). Following positive selection, both MAIT and NKT cells engage in a complex differentiation process in the thymus, which results in the development of functionally distinct subsets expressing either RORγt or T-bet. Each functional program appears to preferentially target MAIT and NKT cells to specific locations in the body, suggesting an important role for thymic signals in prepositioning effector cells in designated tissues.

In this review, we will focus on recent advances on MAIT cell development both in mice and humans leading to the concept that MAIT cells are preset [1] in the thymus to reach specific tissues with unique functional features tailored to each tissue. Because the field has been particularly active since the landmark discovery that the vitamin B2 precursor, 5-A-RU is at the origin of the most active MAIT ligands [2,3], we will also discuss the different natural or drug-derived agonist and antagonist ligands and the potential consequences on MAIT cell biology.

Recent advances on human MAIT cell phenotype and development

MAIT cells were originally identified through the usage of an invariant TCRα chain associating TRAV1.2 with either TRAJ12, 20 or 33 with a 12 amino-acid length CDR3 (iTCRα), but can now be labelled with tetramers of their restricting molecule, MR1, loaded with 5-OP-RU, a derivative of the microbial vitamin B2 precursor 5-A-RU [2,4••,5,6]. For reasons previously discussed [7], we will restrict the term MAIT cells to T cells labelled with MR1:5-OP-RU tetramers and expressing a particular differentiation program marked by the expression of CD161 and PLZF. In the blood of adult humans, all MR1:5-OP-RU+ T cells express the iTCRα chain [5,8,9]. Other reviews have summarized in detail the phenotype of MAIT cells [10,11]. The phenotype of MAIT cells is extremely homogeneous with a memory phenotype and the concomitant expression of several transcription factors (RORγt, Tbet, Eomes and Helios) [12] that are usually mutually exclusive in other T cell subsets. Yet, some heterogeneity has recently been demonstrated with respect to CD56 expression [13•]. The $CD56^{hi}$ subset expresses higher levels of IL-12R and IL-18Rα as well as PLZF, Eomes, and T-bet and this is associated with higher frequency of IFN−γ secretion after IL-12+IL-18 stimulation. Heterogeneous expression of CD73, CD101 (BB27), CD226 (DNAM-1), CD328 (Siglec-7), and NKp80 was also observed [13•]. Single cell analysis will determine whether this heterogeneity corresponds to two or more distinct subsets.

In the human thymus, MR1:5-OP-RU+ T cells can be divided into three development stages: an immature stage ($CD27^-CD161^-$) followed by a stage 2 ($CD27^+CD161^-$) and finally a mature third stage ($CD27^+CD161^{++}$) [14••].

Thus, expression of CD161 is a late event in human MAIT ontogeny as previously suggested using sorted CD161^{hi} or CD161^{lo} thymic subsets and RT-QPCR for the iTCRα chain [15]. Importantly, in cord blood, most of the T cells expressing CD161 at high levels are not TRAV1.2^{+} [14••,16] and among the few CD161^{hi}TRAV1.2^{+} cells, only a small minority is labelled with MR1:5-OP-RU tetramers [8]. Still, all the CD161^{hi} T cells are also CD26^{hi} and IL-18Rα^{+} indicating the expression of a similar differentiation program [15,16]. MAIT cells in the cord blood display a naive CD45RA^{+}CD45RO^{-} phenotype and a naive (i.e. diverse) repertoire [8,11,14••,15–17], contrasting with the memory (CD45RO^{+}) phenotype and oligoclonality of adult human MAIT cells [6,8,18]. The frequencies of TRAV1.2^{+} and TRAV1.2^{-} T cells (including NKT cells) in the CD161^{hi} compartment are correlated to each other in cord blood, further suggesting a common differentiation pathway independently of antigen specificity [1,7,8]. The correlation of MAIT cells and other CD161^{hi} subset numbers between dizygotic twins also suggests that common, non-genetic but maternally derived factors determine their abundance [8]. A few months after birth, MR1:5-OP-RU+ T cells become memory (i.e. CD45RA^{-}CD45RO^{+}) while the other CD161^{hi} subsets decrease in relative proportion. It takes 5–20 years for MAIT cells to reach adult levels while the TRAV1.2^{-}CD161^{hi} subset becomes almost undetectable [8,11], indicating that antigen specificity is required for persistence and expansion, as previously outlined [7]. The tissue location of MAIT cell expansion in children, the nature of the antigen-presenting cells involved, the role of non-cognate cues as well as the commensal or pathogen origin of antigens are unclear at the moment.

In adults, MAIT cells are particularly abundant in the liver where they represent 20–40% of T cells [16,19,20]). Human MAIT cells in the liver express many genes associated with tissue residency [21•] as listed in Ref. [22••] including at the single cell level [23]. Importantly, NKT [24] and liver MAIT cells may have profibrogenic activity in certain pathological settings [20,25]. MAIT cells are also abundant in the genital tract [26] and in the skin [27], but their residency signature in these tissues has not been investigated.

The absence of CCR7 and CD62L expression by circulating blood MAIT cells is puzzling as these molecules are necessary to enter the lymph-node from the blood thereby allowing recirculation between blood, lymph-nodes and back to the blood through the lymphatic flow. As MAIT cell repertoire and phenotype are very similar in the lymph from the thoracic duct and in blood [28], MAIT cells may directly circulate between blood and peripheral tissues. Alternatively, MAIT cells may enter the lymph nodes through another mechanism than the one used by mainstream naive and memory T cells. As compared to other circulating T cells, human (but not murine) MAIT cells uniquely express the transcription factor CCAAT/enhancer-binding protein delta (C/EBPδ), controlling the expression of CCR6 and of the enzymes FUT7 and ST3GAL4, involved in the sialylation of several membrane molecules necessary for rolling and arrest on inflamed endothelium, steps that are necessary for CCR2-dependent extravasation [29]. Since CCR2 is highly expressed by MAIT cells in both humans and mice, this suggests that circulating MAIT cells are specifically poised to reach inflamed tissues.

MAIT subsets and tissue residency

The availability of MR1:5-OP-RU tetramers has opened the study of MAIT cells in mice [4••,14••,21•,30•]) without the caveats of TCR transgenic mice [15,31]. In contrast with NKT cells that encompass three subsets (NKT1 expressing T-bet, NKT2 secreting IL-4 and NKT17 expressing RORγt) [32••], only two subsets of MAIT cells (MAIT1 and MAIT17) can be identified in the thymus or the periphery of C57Bl/6 and Balb/c mice [14••,21•,30•]. However, the proportion of MAIT1/17 and NKT1/17 subsets are exactly the opposite: MAIT17 cells are much more abundant than MAIT1 cells while NKT1 cells are much more abundant than NKT17 cells.

Using a congenic B6-MAITCast strain containing increased numbers of MAIT cells [33], we recently showed that the transcriptomic programs of MAIT1 and MAIT17 subsets are quasi-identical to those of NKT1 and NKT17 subsets, respectively [21•]. Each functional program (type 1 or type 17) is associated with a specific tissue location: T-bet^{+} cells preferentially localize in the liver, while RORγt^{+} cells are positioned in mucosal tissues such as lung, skin and gut lamina propria [21•]. Each functional program also correlates with distinct patterns of tissue residency: spleen and liver MAIT and NKT subsets are resident and do not exchange between parabiotic mice [21•,34]; in the lungs, type 17 MAIT/NKT subsets are extravascular and do not exchange between parabiotic mice, while type 1 subsets are intravascular and do exchange between parabiotic mice [21•]. These distinct locations and circulatory properties are also associated with specific functions as the effector molecules expressed by type 1 versus type 17 subsets are very different. The relative frequency of MAIT1/17 and NKT1/17 probably explains the preferential location of NKT and MAIT cells in organs such as in the liver and lungs. Thus, a type 1 program would be operating in the spleen and liver, while a type 17 program would be present in mucosal tissues, adapting the class of the immune response to the organ.

MAIT cell development in mice

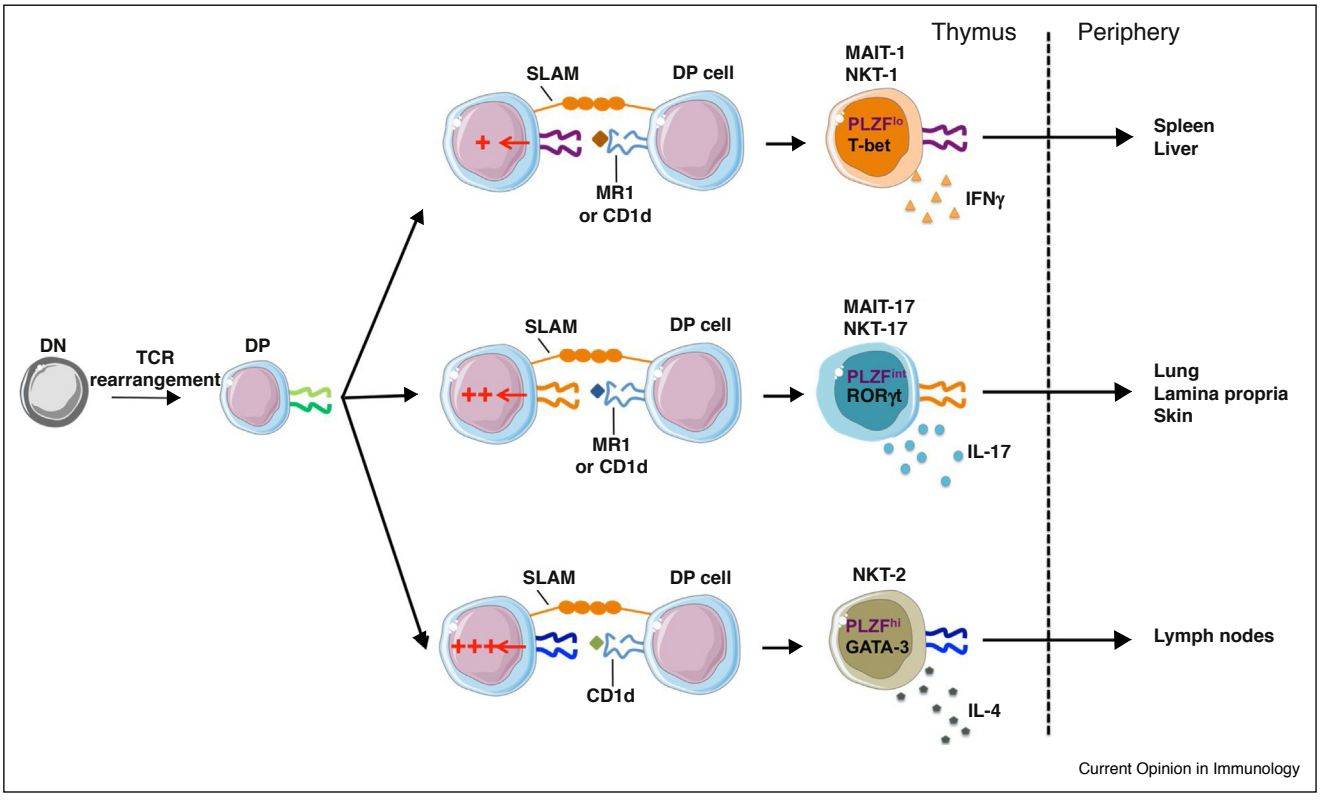
In mice, MAIT cell development was divided into three distinct stages in the thymus [14••]: an immature stage 1 (HSA^{hi}CD44^{lo}) corresponding to recently selected cells, an intermediary stage 2 (HSA^{lo}CD44^{lo}) and a mature

stage 3 ($HSA^{lo}CD44^{hi}$). Mature MAIT cells can be further divided into MAIT1 and MAIT17 subsets, the latter being more abundant [14••]). PLZF is expressed at stage 2 and is also necessary for the final maturation of MAIT cells [14••]. Most features of mature MAIT cells are already operational in the thymus as shown by the capacity to secrete either IFN-γ or IL-17 [14••]. The selection of both MAIT and NKT cells by DP thymocytes [31,35] likely explains their common features. DP thymocytes provide homotypic Slamf1 and Slamf6 interactions that are necessary for the acquisition of PLZF expression in NKT cells [36]. This is probably also true for MAIT cells as both NKT and MAIT cells are absent in mice deficient in the molecular adaptor, SAP, which is necessary to transduce Slam mediated signals ([36] and (Legoux *et al.*, CD1 and MR1 meeting, NAPA, 2017). PLZF in turn induces effector differentiation and suppresses the transcription of Bach2, a repressor of effector differentiation in naive cells [37].

The signals controlling T-bet or RORγt lineage choice in MAIT cells are unknown. Given that MAIT and NKT cells acquire very similar transcriptomic programs in the thymus, it is likely that identical signals are responsible for differentiation of both subsets (Figure 1). The TCR repertoire of NKT1, NKT2 and NKT17 subsets is biased toward different TCR Vβ segments [32••], suggesting that TCR affinity for a selecting self-ligand may instruct the lineage choice. The high expression of PLZF in NKT2 versus NKT1 subsets is likely related to high versus low TCR triggering during selection [32••,38•,39]. This hypothesis is supported by observations that each Vβ segment confers differential reactivity to self or to exogenous NKT ligands [40,41]. Moreover, expressing TCRβ chains from either NKT1, 2 and 17 subsets in T cell precursors results in increased development of NKT2 cells when TCRβ chains of high avidity for CD1d restricted antigens were used [42,43]. By contrast, decreased TCR signaling in ZAP70 mutant mice reduces the generation of NKT2 and NKT17 by affecting the persistence of Egr2, a known inducer of PLZF expression [44•,45].

Thus, it is probable that TCR avidity for an endogenous ligand presented by MR1 in the thymus is also involved in the lineage choice of MAIT cells (Figure 1). The absence of MAIT2 cells in Balb/c mice that are rich in NKT2 further suggests a role for TCR specificity, instead of cytokines (that would impact both NKT and MAIT lineage

Figure 1



Classical model of NKT and MAIT differentiation. TCR signal strength during positive selection may determine PLZF expression levels and lineage choice of NKT cells, and possibly of other preset T cell populations such as MAIT cells. Lineage choice in turn controls effector function and peripheral tissue localization of preset T cells [21•,49].

determination), in the choice of the IL-4-producing lineages. The strong variations in the proportions of each NKT subset in various mouse strains implies variations in the amounts or the nature of self-ligand(s) presented by CD1d in these mice. By contrast, the proportion of MAIT cell subsets is stable across mouse strains, suggesting that MR1 self-ligand(s) are produced and presented similarly in all strains. We have not yet been able to identify differences in TCR Vβ usage between MAIT1 and MAIT17 subsets [21•]. Given the capacity of MAIT TCRs to finely discriminate between different types of MAIT ligands [46•], deep sequencing of CDR3β may be necessary to precisely compare TCR repertoires between MAIT subsets.

The strength of TCR signaling during positive selection is also a proposed mechanism for lineage choice of $CD4^+$ T cells, which differentiate into $Foxp3^+$ Treg cells when receiving strong TCR signals in the thymus [47]. In the case of Treg, selection of TCRs with high affinity for self-peptides is seen as a way to ensure tolerance to self-antigens in the periphery. In the case of preset T cells, the reason (if any) for T cells selected by strong TCR signals to populate mucosal tissues, or for T cells selected by weaker TCR signals to seed spleen and liver, is unknown.

Other subsets related to MAIT cells

A subset of murine γδ T (Vγ1Vδ6.3–4) cells also expresses PLZF in a SAP-dependent manner [48] suggesting that like NKT and MAIT cells, this subset is selected by an agonist ligand presented by hematopoietic cells. Interestingly, PLZF is expressed at high level and is associated with a transcriptome similar to that of NKT2 cells, which are also located in lymphoid organs [39,49]. In humans, a Vδ2 subset expresses high levels of PLZF [50] and shares many phenotypic and functional characteristics with MAIT and NKT cells, except for a lower expression of IL-18Rα and CD161, probably related to lower expression of RORγt. This subset shares with the NKT and MAIT subsets a common transcriptome program [51] linked to PLZF expression indicating common functions including tissue residency. The two alternative models of NKT and MAIT development are discussed in Box 1 and displayed in Figures 1 and 2.

Differences between human and mouse MAIT cells

In both humans and mice, MAIT cells are tissue-resident T cells acquiring an effector phenotype during thymic development, and recognizing small metabolites presented by MR1. Despite these similarities, MAIT cells differ between humans and mice on several grounds:

- MAIT cells are highly abundant in human blood and tissues, but very rare in mice. Given the low frequency of MAIT cells in the human thymus, this discrepancy is likely due to a lack of expansion in the periphery of laboratory mice. This lack of expansion may be attributable to the cleanliness of laboratory mice. Alternatively, variations in the differentiation programs of human and mouse MAIT cells may result in different responses to antigen exposure.
- In the thymus and blood, individual human MAIT cells express both T-bet and RORγt at the same time, while mouse MAIT cells differentiate into either $T\text{-bet}^+$ or $ROR\gamma t^+$ cells. As a result, human MAIT cells all express NK receptors (such as CD161), while only $T\text{-bet}^+$ MAIT cells do so in mice. Since murine MAIT cells may lose RORγt and acquire T-bet upon antigen priming [52], the presence of well identified MAIT1 and MAIT17 subsets may reflect the too clean status of the laboratory mouse facilities or more basic physiological differences between mice and humans.
- MAIT cells fail to develop in mice deficient for the Slam-Associated Protein (SAP) (Legoux *et al.*, CD1 and MR1 meeting, NAPA, 2017), consistent with a key role for SLAM interactions in the acquisition of effector functions in the thymus. By contrast, MAIT cells expressing PLZF are found in the blood of SAP-deficient patients [15,53], suggesting additional pathways for PLZF induction in humans, as further supported by the expression of PLZF by mainstream CD8 T cells in the liver [21•].

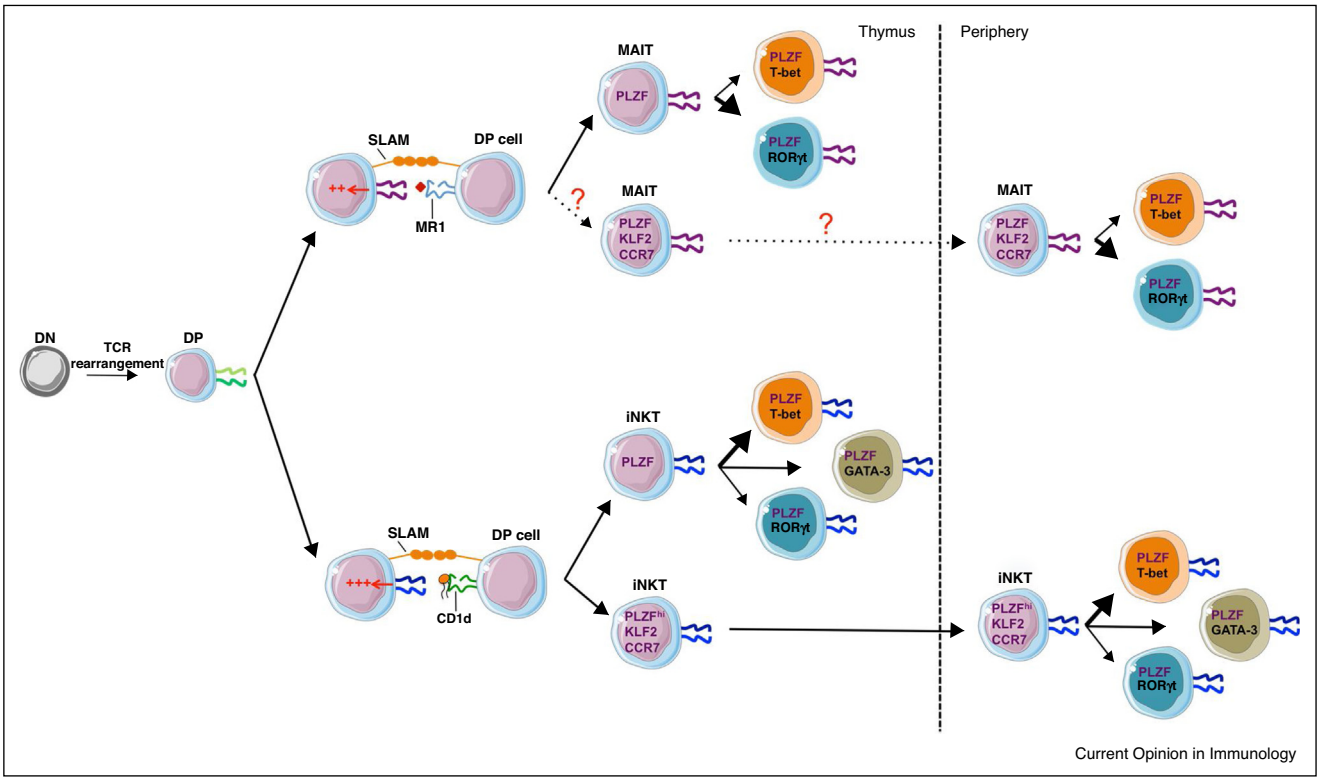
Box 1 Two alternative models of NKT and MAIT cell development

Classical model (Figure 1): Upon positive selection by DP thymocytes, MAIT and NKT precursors lose HSA and progressively acquire PLZF, together with CD44. Thymocytes then differentiate into $Tbet^+$ or $ROR\gamma t^+$ subsets for MAIT cells, and into $Tbet^+$, $ROR\gamma t^+$ and IL4-producing subsets for NKT cells. These mature populations then exit the thymus and seed peripheral organs according to their functional program.

Alternative model [30•] (Figure 2): Here, mature NKT thymocytes are resident of the thymus and only minimally contribute to the peripheral pool of NKT cells. Instead, peripheral NKT cells are produced through differentiation of a unique $PLZF^{hi}CCR7^+$ precursor, which exits the thymus in a Klf2-dependent manner. A similar $CCR7^+$ thymic precursor exists in MAIT cells, suggesting that MAIT cells may also undergo maturation and differentiation in the periphery.

The quantitative importance of the classical and alternative pathways for differentiation of preset T subsets remains unclear. The preferential use of a given pathway may vary in mice of different ages (after birth, at weaning, in young and old adult mice). Indeed, MAIT and NKT cells are also resident in the liver and the lungs and the input fluxes into tissues may be very low at steady state once the peripheral compartments have been generated. Given that mature MAIT cells in thymus do not express CCR7 or CD62L in bulk transcriptomic analyses [21•], it is possible that these T cells never leave this organ, in agreement with their expression of genes associated with tissue residency. The presence of MAIT cells in peripheral tissues would therefore rely on cells exiting the thymus before the induction of the tissue-residency program (likely controlled by PLZF). It would be of interest to measure the lag of time necessary for PLZF to shut down the expression of CCR7 and CD62L in newly selected thymocytes.

Figure 2



Alternative model of NKT differentiation, which may also apply to MAIT cells. Thymic differentiation of NKT cells results in mature subsets resident of the thymus. NKT cells found in the periphery differentiate in the periphery from a CCR7^{+}PLZF^{hi}CD44^{lo} precursor.

Microbial 5-A-RU as a platform for the production of MAIT ligands

5-A-RU, an unstable intermediate of the vitamin B2 (Riboflavin) biosynthetic pathway is at the origin of the main MAIT ligands [2,54]. This pathway is present in most bacteria and yeast as well as in *Aspergillus fumigatus* [55] but strikingly absent from all animal cells [56]. In three model organisms (*Escherichia Coli*, *Lactococcus Lactis and Salmonella typhimurium*), MAIT cell activation strictly relies on the synthesis of 5-A-RU. Recent studies were aimed at the identification of other MAIT agonist ligands [52,57•,58,59]). In a first study, 47 strains of bacteria from the human environment were tested for their capacity to activate MAIT cells [57•]. The ability to synthesize 5-A-RU was necessary for all microbial strains. The most active bacteria belong to the *Bacteroidetes* and *Proteobacteria* phyla that produce the largest amount of vitamin B2 [57•]. Since MAIT agonists 5-OP-RU and 5-OE-RU are produced through non-enzymatic condensation of methylglyoxal and glyoxal from the microbial intermediary metabolism, the growth conditions of bacteria modulate their amount and probably their relative proportions [58]. Although MAIT cells can be activated by other derivatives of 5-A-RU and even by vitamin B2 derivatives, these latter compounds require micromolar concentrations, while 5-OP-RU is active in the low nanomolar range [59]. In addition, a drug metabolite (from diclofenac) displays some agonist activity in the high micromolar range but the clinical impact on MAIT cells remains to be determined [60•]. In contrast, many natural or synthetic compounds are able to bind MR1 and to block the activation of MAIT cells [52,54,59,60•].

While MAIT agonist and antagonist ligands have been relatively well characterized *in vitro*, no data are available *in vivo* with regard to the amounts available in the gut lumen, tissue diffusion, and relative proportion of the different molecules binding to MR1 that would block or activate MAIT cells at steady state or during disease. It will be important to measure the rate of synthesis by microbes *in situ* at the mucosae, the relative proportion of the agonist and antagonist ligands as well as their relative affinity to MR1 and the nature of the cells expressing MR1 to which these MAIT ligands have access. We also need to characterize the way these ligands may circulate in the organism associated or not to bacteria. For the moment, these questions are difficult to address

since most agonist compounds are highly unstable. Moreover, the T cell-based bio-assays allowing their detection are much more sensitive than the available biophysical assays such as mass-spectrometry, but do not provide molecular identification and are sensitive to both agonist and antagonist compounds.

Conclusion

Taking into account the specific features of each subset, defined by the expression of transcription factors such as Tbet, RORγt or Gata3, most of the knowledge acquired on NKT cell biology during the past 25 years can now be translated to all related (PLZF-expressing) subsets, and specifically to MAIT cells. The particular intra-thymic development of all these subsets induces a tissue-residency program, which is modulated by the expression of additional transcription factors such as T-bet or RORγt. The functions of MAIT cells within tissues remain to be explored. It is also unclear whether MAIT cell function always requires antigen triggering once in tissues. Given the strong similarities in transcriptional profiles of MAIT and NKT subsets but also Innate Lymphoid Cells, perhaps in relation to common expression of PLZF [61], these various immune populations may therefore fulfill redundant functions.

Conflict of interest statement

Nothing delcared.

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