MAIT Cell Development and Functions: the Microbial Connection

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Abbreviations
5-A-RU: 5-amino-ribityl uracil
5-OE-RU: 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil
5-OP-RU: 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil
Ac-6-FP: acetyl-6-formyl-pterin
Areg: Amphiregulin
BM: Bone Marrow
CDR3: Complementary-Determining Region 3
DP: Double Positive
iNKT: invariant Natural Killer T
MAIT: Mucosal Associated Invariant T
MR1: MHC class I related molecule
PLZF: Promyelocytic Leukaemia Zinc Finger or Zbtb16: Zinc finger and BTB domain-containing protein 16
SAP: Slam Adaptor Protein
SPF: Specific Pathogen Free
TECs: Thymic Epithelial Cells
Tregs: regulatory T cells

Abstract (143 words)

Mucosal Associated Invariant T (MAIT) cells are an evolutionarily conserved T cell subset, which reacts to most bacteria through TCR-mediated recognition of metabolites derived from the vitamin B2 biosynthetic pathway. Microbiota-derived signals impact all stages of MAIT cell biology including intra-thymic development, peripheral expansion and functions in specific organs. In tissues, MAIT cells can integrate multiple signals and display effector functions involved in the defense against infectious pathogens. In addition to anti-bacterial activity, MAIT cells improve wound healing in the skin, suggesting a role in epithelium homeostasis through bi-directional interactions with the local microbiota. In humans, blood MAIT cell frequency is modified during several auto-immune diseases, which are often associated with microbiota dysbiosis further emphasizing the potential interplay of MAIT cells with the microbiota. Herein we will review how microbes interact with MAIT cells, from initial intra-thymic development to tissue colonization and functions.

(7961 words)

Introduction

Mucosal Associated Invariant T (MAIT) cells represent the most abundant T cell subset recognizing bacterial compounds (Franciszkiewicz et al., 2016). MAIT cell frequencies are very high in humans in the blood (1-10% of T cells), the liver (20-40%) and the lung and gut lamina propria (Dusseaux et al., 2011; Martin et al., 2009). They are much less abundant in mice (Rahimpour et al., 2015; Tilloy et al., 1999). They have been discovered almost 30 years ago following identification of an invariant T cell receptor-α (TCRα) chain (Vα7.2-Jα33) in $CD4^{-}CD8^{-}$ T cells from human blood (Porcelli et al., 1993). A T cell subset expressing a homologous TCRα chain has been then evidenced in mice and cattle (Tilloy et al., 1999), indicating evolutionary conservation and therefore important function(s). Selection by a non-polymorphic MHC class Ib molecule has also been demonstrated early on (Tilloy et al., 1999). This molecule has been identified as the evolutionarily-conserved MHC class I related molecule (MR1), which expression on hematopoietic cells is required for MAIT cell development (Treiner et al., 2003). MAIT cells have been rapidly characterized as T cells displaying immediate effector activities related to the expression of the master transcription factor PLZF (Zinc finger and BTB domain-containing protein 16, encoded by *Zbtb16*), also expressed by invariant natural killer T (iNKT) cells (Savage et al., 2008). MAIT cell dependency on commensal microbiota has been demonstrated as MAIT cells are absent from the periphery of germ-free mice (Treiner et al., 2003), indicating an intimate relationship between MAIT cells and bacteria. In the early 2010's, the ligand activating MAIT cells has been identified, first as a soluble microbial compound (Le Bourhis et al., 2010) and then as unstable vitamin B2 (riboflavin) precursor derivatives (Corbett et al., 2014; Kjer-Nielsen et al., 2012; Tastan et al., 2018). These molecules are only found in riboflavin-synthesizing bacteria and

yeasts (Mondot et al., 2016), conferring a broad anti-microbial reactivity to MAIT cells. This exquisite microbial specificity associated with the decrease of MAIT cell blood numbers during infections in humans (Gold et al., 2010; Grimaldi et al., 2014; Le Bourhis et al., 2013; Le Bourhis et al., 2010) and the protective effect of MAIT cells in several microbial infection experimental models ((Cui et al., 2015; Le Bourhis et al., 2013; Le Bourhis et al., 2010; Meierovics et al., 2013; Meierovics and Cowley, 2016; Wang et al., 2018), reviewed in (Godfrey et al., 2019; Salou et al., 2017)) support an anti-microbial function of MAIT cells.

Vitamin B2 metabolites produced by the microbiota travel through the body and control thymic development of MAIT cells (Legoux et al., 2019a). Microbial ligands may also be implicated in epithelial homeostasis, since MAIT cells promote tissue repair in response to TCR triggering (Constantinides et al., 2019; Hinks et al., 2019; Lamichhane et al., 2019; Leng et al., 2019). Thus, MAIT cells represent a direct way for the mammalian immune system to sense the microbiota, and to provide mucosal barrier protection. The role of vitamin B2 metabolites in tissue homeostasis is reminiscent of the way short chain fatty acids induce the expression of the Foxp3 transcription factor in T cells, with the acquisition of tissue-repair functions (Smith et al., 2013; Uchimura et al., 2018). Similarly, fMet peptides from some commensals expand H2-M3 major histocompatibility molecule-restricted T cells with both anti-microbial and tissue-repair functions (Harrison et al., 2019; Linehan et al., 2018). Although described only in primates, Vγ9δ2 γδ T cells represent another subset of abundant, effector T cells with specificity for phospho-antigen metabolites, produced by microbes or stressed cells (Fournie and Bonneville, 1996). Thus, mammalian T cells have evolved several mechanisms for sensing the production of metabolites by the microbiota and for mounting a tissue-repair response in return.

Herein, we will first review the unique features of MR1 and MR1 ligands. We will then summarize the role of bacterial metabolites in the ontogeny and anti-microbial functions of MAIT cells. Finally, we will discuss the contribution of MAIT cells to tissue remodeling upon injury and how MAIT cell ligands together with other microbial signals may be involved.

1. MR1 and its ligands have unusual properties.

In contrast with classical MHC or CD1 molecules that present peptides or glycolipids, respectively, the only known MHC protein presenting metabolites to αβ T cells is MR1. The *MR1* gene is absent from fish and birds, and appeared about 170 million years ago in the common ancestors of living marsupials and eutherians (Boudinot et al., 2016; Tsukamoto et al., 2013). *MR1* has been subsequently lost in several species, including *Lagomorphs* and all members of the *Carnivora* family (Boudinot et al., 2016). The MR1 protein shows remarkably high homology, with signs of purifying selection, indicating evolutionary pressure to maintain both the ligand binding groove and the external structure of the MR1 molecule (Boudinot et al., 2016; Huang et al., 2009). Associated with the absence of MR1 polymorphism, this suggests that the function of MR1 is to bind and present a limited number of ligands. Accordingly, only two molecules have

been described as strong agonists for MAIT cells, and both are derived from the vitamin B2 precursor 5-amino-ribityl uracil (5-A-RU) (Corbett et al., 2014; Soudais et al., 2015). The unstable 5-A-RU reacts with glyoxal or methylglyoxal, generated in the intermediary metabolism, and give rise to the short-lived adducts 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU) and 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), respectively. Crystallographic studies reveal that both 5-OE-RU and 5-OP-RU establish a Schiff base with Lysine 43 of the MR1 groove, stabilizing these compounds (Corbett et al., 2014). Unless bound to MR1, 5-OP- and -OE-RU spontaneously cyclize and, though being still able to bind MR1, become weak agonists for MAIT cells (Kjer-Nielsen et al., 2012; Mak et al., 2017).

The MAIT cell TCR recognizes MR1:5-OP-RU complexes using a docking mode similar to mainstream T cells on classical MHC molecules, with a key role for the complementary-determining region (CDR) 3 of the TCRα chain in contacting the antigen (Awad et al., 2020; Gherardin et al., 2016; Reantragoon et al., 2012). CDR3α sequences are composed of the junction of TRAV and TRAJ gene segments, with the stochastic addition or deletion of nucleotides in the TCR rearrangement process, resulting in extremely diverse sequences. Remarkably though, contact with 5-OP-RU is always mediated by the evolutionarily conserved Tyrosine 95 from the CDR3 of the TCRα chain. Indeed, in humans, cattle, pig (Xiao et al., 2019) and mice (Tilloy et al., 1999), the TCRα chain of MAIT cells is made of the evolutionarily conserved TRAV1 (official nomenclature of human Vα7.2) and TRAJ33 (and also TRAJ12 and TRAJ20 in humans) genes, associated with a CDR3 of fixed length (Reantragoon et al., 2013; Tilloy et al., 1999). Across mammals, the species which have lost the TRAV1 gene do not have a functional MR1 gene, which is either absent or pseudogenized (Boudinot et al., 2016). Conversely, when the *TRAV1* gene is present, the *MR1* gene is always functional. The CDR1 and CDR2 sequences of TRAV1 are also highly conserved when compared with other TRAV genes. Thus, strong selective pressure maintains TRAV1 and MR1 in a phylogenetic locked interaction, which is released when the TRAV1 locus is lost, as a consequence of the high variability of the TCRα locus during evolution (Mondot et al., 2016).

MR1 also binds a number of other small molecules, including other bacterial metabolites (Harriff et al., 2018), synthetic drugs (Keller et al., 2017a; Salio et al., 2020) and metabolites derived from human cell lysates (Lepore et al., 2017). Some MR1-bound metabolites can activate MAIT cells, but only at high concentrations. Of all the identified MR1 ligands, 5-OE-RU and 5-OP-RU exhibit a 100- to 1,000-fold higher potency than other MR1-binding molecules (Corbett et al., 2014; Harriff et al., 2018; Kjer-Nielsen et al., 2012).

In humans, very rare (< 1/1000 of MAIT cells) MR1:5-OP-RU reactive TRAV1^{neg} T cells also express PLZF (Zbtb16), hence their classification as MAIT-like cells (Koay et al., 2019a). In the four donors studied, these cells presented oligoclonal expansions and used TRAV36 associated with TRAJ34 or 37, and a CDR3 of constant length. Together with the use of a

predominant TCRβ chain (TRBV28-TRBJ2.5), this suggests strong structural constraints to recognize MR1:5-OP-RU. However, no TRAV36 ortholog exists in mice indicating that this subset is not under strong selective pressure. In addition, a few TRAV1^{neg} mainstream T cells (i.e. not expressing PLZF), also called non-MAIT-like, recognize bacterial metabolites presented by MR1 with high affinity (Gherardin et al., 2016; Harriff et al., 2018; Meermeier et al., 2016). These cells are mostly CD8^{+}, display a naive phenotype associated with a diverse repertoire. Finally, another TRAV1^{neg} TCR, identified in 2 individuals, recognizes a tumor-associated ligand presented by MR1 (Crowther et al., 2020). These T cells are neither activated by bacteria nor labelled with MR1:5-OP-RU tetramers. Interestingly, addition of the MR1-binding but non-agonist acetyl-6-formyl-pterin (Ac-6-FP) decreases their activation, suggesting the presence of an endogenous ligand (specific to tumor cells), with lower affinity than Ac-6-FP for MR1. In the absence of evolutionary conservation of a TCR shared between species, the physiological relevance of these TRAV1^{neg} MR1-restricted T cells is unclear.

Interestingly, a human TCRγδ T cell subset can bind the alpha 3 domain of MR1 underneath the antigen binding groove (Le Nours et al., 2019). Although very rare (less than 1% of the γδT cells and 100-fold less frequent than MAIT cells), MR1-specific γδT cells may sense any MR1 ligand able to induce cell-surface expression of MR1, thereby potentially playing important roles in immune responses. Thus, MR1-specific γδT cells likely survey a much broader repertoire of metabolic ligands than MAIT cells, which are only activated by vitamin B2-derived metabolites. However, co-evolution of MR1 with TRAV1 strongly suggests that the main function of MR1 is to present antigens to TRAV1^{+} TCRαβ MAIT cells. It is unclear whether MR1-specific γδT cells exist in other species.

The nature of MAIT antigens (i.e. small metabolites) likely affects the biology of MAIT cells. Indeed, in contrast with classical peptide or glycolipid antigens, 5-OP-RU applied onto the intact skin or delivered by oral gavage can be detected shortly after in the thymus (Legoux et al., 2019a). Hence, this metabolite can cross mucosal barriers, travel through the body and react with MR1 in the thymus (**Figure 1**). These results are consistent with known properties of other microbiota-derived metabolites, as microbial short chain fatty acids and tryptophan metabolites can reach distant host tissues such as the brain, thymus and foetus (Gomez de Aguero et al., 2016; Uchimura et al., 2018). Antibodies facilitate stability or transport of microbial metabolites (such as tryptophan metabolites) through the host (Gomez de Aguero et al., 2016). However, given that MAIT cell development requires 5-OP-RU to reach the thymus, and that MAIT cells develop normally in mice lacking B cells (Legoux et al., 2019a), antibodies are likely dispensable for 5-OP-RU transport. Whether 5-OP-RU requires a carrier in blood is unclear. The dynamic properties of 5-OP-RU *in vivo* may also affect the initiation and maintenance of MAIT cell responses (see below).

2. MAIT cell development is controlled by host intrinsic signals

MAIT cells develop in the thymus (Koay et al., 2016; Tilloy et al., 1999) in a process recently reviewed in details (Pellicci et al., 2020). Following positive selection, MAIT cells undergo intra-thymic differentiation and, contrary to mainstream T cells, acquire immediate effector functions associated with the related phenotype (Koay et al., 2016; Rahimpour et al., 2015) and the ability to migrate into non-lymphoid tissues (Salou et al., 2019). These original characteristics, shared with iNKT cells, are related to the expression of the master transcription factor PLZF (*Zbtb16*) during thymic development (Koay et al., 2016; Savage et al., 2008). PLZF (*Zbtb16*) is a known suppressor of the naïve T cell program and inducer of effector genes in iNKT cells (Mao et al., 2016; Savage et al., 2008). MAIT and iNKT subsets display highly similar transcriptomic programs once split according to expression of T-bet or RORγt, which define the iNKT1, MAIT1 and iNKT17, MAIT17 effector subsets, respectively (Salou et al., 2019). Accordingly, as for iNKT cells, positive selection of MAIT cells in mice strictly depends on the expression of the Signaling lymphocytic activation molecule (SLAM) Adaptor Protein (SAP), which signals downstream of SLAM molecules (Koay et al., 2019b; Legoux et al., 2019b). SLAM signaling can result in both inhibitory and stimulatory effects on the development of iNKT cells, but the molecular pathways involved are unclear (Lu et al., 2019; Tuttle et al., 2018; Zhao et al., 2012). Hence, positive selection of effector MAIT cells in mice requires signaling from the TCR and concomitant engagement of SLAM molecules at the surface of hematopoietic thymocytes (Legoux et al., 2019b) (**Figure 2**).

Bone marrow (BM) chimera experiments demonstrated that TRAV1$^+$ T cells recognizing MR1:5-OP-RU complexes can be selected by MR1 expressed on thymic epithelial cells (TECs) (Legoux et al., 2019b). TECs do not express SLAM molecules, and thymocytes undergoing positive selection on TECs differentiate into naïve CD4$^+$ T cells, which patrol secondary lymphoid organs but do not migrate into non-lymphoid tissues (**Figure 2**). In mice, we estimated that about half of the immature thymocytes specific for MR1:5-OP-RU are selected on SLAM$^+$ hematopoietic cells, the other half being selected on Slam$^-$ TECs (Legoux et al., 2019b).

Following positive selection on hematopoietic cells (Seach et al., 2013), MAIT precursors down-regulate CD24a and up-regulate *Klf2*, *Sell* and *S1pr1* (Legoux et al., 2019b), in a process similar to differentiation of mainstream T cells preparing for thymus egress (Weinreich and Hogquist, 2008). Some of these MAIT precursors may exit the thymus at this stage, as proposed for iNKT cells (Wang and Hogquist, 2018). Early thymic egress of MAIT cells is also suggested by the presence of MR1:5-OP-RU-specific T cells with remaining GFP expression in the spleen and lymph nodes of RAG-GFP mice (Winter et al., 2019). Following CD24a down-regulation, MAIT precursors begin to express the transcription factor PLZF (*Zbtb16*), with acquisition of high amounts of CD44 and subsequent effector differentiation into T-bet- or RORγt-expressing cells subsets (Legoux et al., 2019b; Salou et al., 2019). Effector differentiation requires *Satb1* and *Cxcr6* (Koay et al., 2019b). Although the sequence of molecular events associated with MAIT effector (PLZF (*Zbtb16*)$^+$) differentiation is emerging (Koay et al., 2019b; Legoux et al., 2019b),

the mechanisms controlling MAIT1 versus MAIT17 lineage choice remain obscure. TCR avidity for the selecting ligand appears involved in iNKT1, 2 and 17 commitment, with stronger signaling for iNKT2 and iNKT17 in comparison with iNKT1 subsets (Tuttle et al., 2018). Given the similarity between iNKT and MAIT cell development, TCR avidity for the selecting antigen probably determines MAIT1 versus MAIT17 choice. Consistent with a role of TCR signaling during MAIT cell development, MAIT cell numbers are decreased in *miR181*$^{-/-}$ (Winter et al., 2019) and in *Zap7*0-hypomorphic (SKG) mice (Koay et al., 2019b). Studying MAIT1 and MAIT17 proportions in these mice could be informative on the role of TCR avidity in MAIT subset commitment since the iNKT2 subset, which requires stronger TCR signal for selection, is absent (Tuttle et al., 2018). The role of TCR strength is also supported by the lower proportion of MAIT17 cells in the thymus of *Ccr7*$^{-/-}$ mice, which exhibit reduced TCR signaling (Davalos-Misslitz et al., 2007; Koay et al., 2019b).

Whether the developmental process of MAIT cells is conserved between mice and humans is unclear. Although some orthologous transcription factors are induced in both species upon thymic development (i.e. *ZBTB16*, *RORC*, *TBX21*), human MAIT cells exit the thymus as T-bet^{+}RORγt^{+} cells while mouse MAIT cells express either transcription factors. In addition, SAP-deficient patients have a normal frequency of blood MAIT cells, and these cells express normal amounts of PLZF (*ZBTB16*) (Martin et al., 2009) and Legoux and Lantz, unpublished) suggesting additional mechanisms by which MAIT cells may differentiate into effector cells in humans.

3. MAIT cell development is controlled by the microbiota

The absence of MAIT cells in the mesenteric lymph nodes of germ-free mice has been recognized early on using RT-qPCR for the MAIT iTCRα chain mRNA (Treiner et al., 2003). Reduced MAIT cell frequency has been then reported in the thymus of germ-free mice using MR1:5-OP-RU tetramers (Koay et al., 2016). As parabiotic experiments demonstrate that MAIT cells do not circulate from the periphery to the thymus (Legoux et al., 2019a), the effect of microbiota on MAIT cells is likely happening directly in the thymus. Both the number of mature MAIT17 cells and of CD24^{+} immature MR1:5-OP-RU tetramer-labelled T cells are reduced in germ-free mice. The residual tetramer-positive cells have reduced TCR signaling and proliferation as compared with specific pathogen free (SPF) mice, suggesting that the microbiota provides a TCR ligand for thymic MAIT cell selection and proliferation. Experiments with germ-free mice mono-colonized with *E. coli* strains mutated in the vitamin B2 pathway either up-stream (*RibD*-deficient) or downstream (*RibE*-deficient) of MAIT antigen production establish that thymic MAIT cell development is directly dependent upon *RibD* gene expression and 5-A-RU production by bacteria (Legoux et al., 2019a). Both immature CD24^{+} precursors and mature MAIT17 cells are restored upon microbial colonization, which suggests *de novo* positive selection of MAIT cells by a microbial antigen. Alternatively, given that residual MAIT cells exist in germ-free mice (discussed below), the microbial ligand might impact MAIT cell development mostly through

expansion of MAIT17 cells selected beforehand on a self-antigen or even on empty MR1 molecules. The 5-A-RU-derived 5-OP-RU antigen rapidly travels from mucosal surfaces (skin or stomach) to the thymus, where it induces expansion of MAIT cells. A variety of thymic cells can capture exogenous 5-OP-RU, including TECs, dendritic cells and $CD4^+CD8^+$ double positive (DP) thymocytes, but the latter cells express the highest amounts of MR1 (Legoux et al., 2019a; Seach et al., 2013). Interestingly, only MAIT17 cells - but not MAIT1 cells - respond to microbial colonization and expand in the thymus (**Figure 2**).

Importantly, positive selection of MAIT cells takes place in germ-free mice, as indicated by the presence of small numbers of mature, cytokine-producing MAIT1 and MAIT17 thymocytes in these mice (Legoux et al., 2019a). By contrast, *Mr1*$^{-/-}$ mice are completely devoid of mature MAIT cells, which indicates that MR1, either unloaded or loaded with unidentified self-ligand(s), mediates positive selection of MAIT cells. MR1-restricted self-metabolites in the thymus may be identical, or distinct, to the MR1 ligands suggested but not yet identified in human cell lysates (Lepore et al., 2017) or involved in the human MR1-dependent recognition of tumour cells by a T cell clone (Crowther et al., 2020).

Intriguingly, microbiota colonization of adult mice restores thymic MAIT cell development, but newly generated MAIT cells do not populate peripheral tissues such as lung (Legoux et al., 2019a) and skin (Constantinides et al., 2019). MAIT and iNKT subset frequencies in mice are highly correlated in different tissues (Salou et al., 2019), as are iNKT and MAIT cell frequencies in human cord blood (Ben Youssef et al., 2018). This suggests a competition for peripheral niches between T cell subsets expressing similar effector programs, such as iNKT or γδ T cells. The increased MAIT cell frequency in the skin of γδ T cell-deficient mice or in the spleen of *Cd1d*$^{-/-}$ mice supports this hypothesis (Constantinides et al., 2019; Koay et al., 2016). Alternatively, the tissues or its endothelium may be permissive for colonization only for a short period after birth, as previously suggested for conventional T cells (Alferink et al., 1998). Finally, early life colonization may produce MAIT cells with different tissue-homing properties. Interestingly, 5-OP-RU alone is not sufficient for MAIT cell development when administered to adult mice (Constantinides et al., 2019; Legoux et al., 2019a), but enables MAIT cell development and skin homing in neonates (Constantinides et al., 2019). Thus, in addition to 5-A-RU-derived antigens, microbiota-induced signals are necessary to support MAIT cell maturation in the thymus of adults, but not in neonates. MAIT cell development is not affected in MyD88-deficient mice (Legoux et al., 2019a), which excludes a role for TLR signals and for IL-1, IL-18 and IL-33. IL-23 alone is not sufficient to induce thymic MAIT cell development (Constantinides et al., 2019), but may act in conjunction with 5-OP-RU to control MAIT cell development in adult SPF mice.

In humans, mature T cell development starts in utero, and $V\alpha7.2^+CD161^+$ T cells are found in the thymus at early age. As 5-OP-RU cross mucosal barriers in mice and microbial metabolites

can influence the development of innate lymphoid cells in utero (Gomez de Aguero et al., 2016), vitamin B2 metabolites produced by the microbiota of the mother might be able to reach the fetal thymus and contribute to MAIT cell selection and expansion. MR1:5-OP-RU-specific thymocytes identified with tetramer express high amounts of PLZF (*Zbtb16*), CD161 and IL-18Rα (Koay et al., 2016), demonstrating intra-thymic acquisition of PLZF (*Zbtb16*). Contrary to mouse MAIT cells that become fully mature and memory in the thymus, only the most mature MAIT cells in the human thymus express CD161 and IL-18Rα (Martin et al., 2009) as well as PLZF (*Zbtb16*) (Koay et al., 2016; Koay et al., 2019b; Martin et al., 2009). Accordingly, thymic MAIT cells exhibit many features of adult MAIT cells with expression of tissue homing chemokine receptors such as CCR6, and CXCR6. However, thymic mature and cord blood MAIT cells remain negative for the memory marker CD45RO (Ben Youssef et al., 2018; Martin et al., 2009). Still, thymic mature MAIT cells are able to secrete TNF-α and IFN-γ after PMA+ionomycin stimulation, but to a lesser extent than adult blood MAIT cells (Koay et al., 2016). According to RNAseq analysis, the cytotoxic capacity seems to be similarly acquired in the thymus (Koay et al., 2019b). This suggests that additional events occurring in the periphery, such as microbiota encounter, might be pivotal for the final maturation steps.

In cord blood, about 1% of T cells express high amounts of CD161 and IL-18Rα as well as *Zbtb16* and *Rorc* (Walker et al., 2012), which are strongly associated to the MAIT differentiation program. However, only a minority uses the Vα7.2 TCRα chain among which less than 10% is labelled with MR1:5-OP-RU tetramers (Ben Youssef et al., 2018) suggesting that a large set of T cells with various specificities acquires a MAIT-like differentiation program in the thymus during fetal life, possibly through selection on DP thymocytes expressing various endogenous ligands (Lantz and Legoux, 2018). Notably, only the MR1:5-OP-RU tetramer-labelled T cells acquire a memory phenotype in the few weeks following birth and subsequently expand in 5 to 6 years to reach adult frequencies (Ben Youssef et al., 2018; Gherardin et al., 2018b; Koay et al., 2016).Among $CD161^{hi}$ T cells, only iNKT and MAIT cells are found in adult blood (Ben Youssef et al., 2018). Associated with the oligoclonality of adult MAIT cells (Lepore et al., 2014; Tilloy et al., 1999), these data suggest that only T cells with the highest avidity for 5-OP-RU expand, which depends on their particular TCRβ chain (Awad et al., 2020; Eckle et al., 2014). The other $CD161^{hi}$ T cells which do not recognize microbial ligands presented in the right context would disappear or be diluted out by MAIT cells. Thus, like for mice in the thymus, the microbial ligands and maybe other microbiota-derived signals are key for the development of human MAIT cells in the periphery.

We hypothesize that the different locations (thymus versus periphery) where the microbial ligands impact MAIT cell development in mice versus humans is related to body size. As microbiota-produced 5-OP-RU seems to freely diffuse in the body, and is highly unstable unless

bound to MR1, its concentration in fluids is directly related to the rate of diffusion from the microbiota. This rate is proportional to mucosal surface, but the resulting concentration is also inversely proportional to the volume of distribution. Thus, in the absence of specific transporters or epithelial layer disruption, 5-OP-RU concentration should be inversely correlated with body size: concentration = k × surface ($size^2$) ÷ volume ($size^3$) = k ÷ size where k is the production rate per surface unit. Thus, the ability of 5-OP-RU to reach the thymus is probably higher in mice than in humans, which would rely more on endogenous ligands. The analysis of MAIT cell development in the thymus of phylogenetically close species with different body size may resolve this issue.

4. MAIT cells reside in peripheral tissues at steady state

MAIT cells are present in non-lymphoid tissues in both humans and mice, with highly variable percentages between individuals. The microbiota seems to be a strong determinant of MAIT cell numbers at steady state as the percentage of skin and lung MAIT cells is strongly cage-dependent in mice (Constantinides et al., 2019). While MAIT1 cells localize preferentially to the spleen, lymph nodes and liver (Rahimpour et al., 2015; Salou et al., 2019), MAIT17 cells also home to barrier tissues such as lung, skin, and gut (**Figure 3**), probably in relation with the differentiation program associated with RORγt vs T-bet expression.

Tissue tropism seems to be an intrinsic feature of the MAIT effector program. Indeed, in mice, both MAIT1 and MAIT17 thymocytes express gene signatures associated with tissue residency, rather than blood circulation (Salou et al., 2019). Thymic MAIT cells express the *Runx3*-related transcription program (Milner et al., 2017; Salou et al., 2019) controlling the establishment of conventional tissue-resident memory $CD8^+$ T cells. Accordingly, as compared to single-positive thymocytes, thymic MAIT cells preferentially seed non-lymphoid organs such as lung and liver upon adoptive transfer (Salou et al., 2019). In B6 mice, MAIT cells represent only 0.03% of T cells in the thymus, but up to 1-3% of T cells in the liver, lung and gut lamina propria (Rahimpour et al., 2015). The MAIT program alone may be sufficient for tissue colonization as antigen recognition in lymph-nodes is dispensable for the recruitment of conventional memory $CD8^+$ T cells into non-lymphoid tissues (Mackay et al., 2012). Accordingly, MAIT cells expand normally following *S. epidermidis* association in mice devoided of peripheral lymph nodes ($Lta^{-/-}$) (Constantinides et al., 2019). Still, BM chimera using $Mr1^+$ donors into $Mr1^{-/-}$ recipients results in decreased numbers of MAIT cells in the lungs (Wang et al., 2019), but this could also be explained by impaired thymic exit in $Mr1^{-/-}$ recipients. Similarly to iNKT cells (Thomas et al., 2011), very few MAIT cells found in spleen, liver and lung (except lung MAIT1) recirculate between 5-week long parabiotic pairs (Salou et al., 2019), which indicates that MAIT cells are long-term resident cells in these tissues. In spleen and liver, MAIT1 cell residency relies on LFA1 and ICAM1 interactions (Salou et al., 2019). In spleen and lung, MAIT17 cells are located in the

extravascular space and do not rely on these interactions. In skin, while IL-23 is necessary for MAIT17 development or maintenance (Constantinides et al., 2019), the mechanisms of tissue retention are unknown.

In humans, the liver MAIT transcriptome is enriched in a residency signature (Milner et al., 2017) as compared to blood, suggesting that the tissue resident property is shared with mouse MAIT cells (Salou et al., 2019). In the buccal mucosa, MAIT cells express the residency markers CD103 and CD69 (Lu et al., 2020; Sobkowiak et al., 2019). However, MAIT cells from matched thoracic duct lymph and blood samples have a shared TCR repertoire, suggesting recirculation between compartments (Voillet et al., 2018). As MAIT cells in the blood lack CCR7 expression, the more likely scenario is migration from blood to tissues and then to the lymph and back to the blood, similarly to classical effector memory T cells (Beura and Masopust, 2014). As in mice, human MAIT cells appear targeted to tissues during thymic development, since tissue-homing molecules such as *CCR6*, *CCR5* and *CXCR6* are expressed in thymic MAIT cells (Lantz and Legoux, 2019; Salou et al., 2019). *RUNX3* and *CEBPD* (see below) are also upregulated in the more mature human thymic MAIT cells (Koay et al., 2019b). Accordingly, human MAIT cells are abundant in the lung (2-4%), liver (20-50%) and intestine (up to 60% of $CD4^-$ T cells in the jejunum) (Dusseaux et al., 2011; Kurioka et al., 2016). Thus, as for mouse MAIT cells, the tissue-homing phenotype of human MAIT cells is acquired in the thymus.

Whether MAIT cells are homogeneous or not can be debated, as studied for other resident cells with similar effector functions such as innate lymphoid cells (Gury-BenAri et al., 2016). In mice, two single cell RNA-seq datasets suggest minimum heterogeneity in the thymus, once MAIT1 and MAIT17 subsets are separated (Koay et al., 2019b; Legoux et al., 2019b). In humans, blood MAIT cells appear very homogenous with high expression of IL-18Rα, CD161 and CD26 allowing their identification by cytometry (Dusseaux et al., 2011; Martin et al., 2009). Together with anti Vα7.2 staining, these markers faithfully identify MR1:5-OP-RU tetramer-positive MAIT cells except in a few cases (Gherardin et al., 2018a). In tissue, single-cell RNAseq on broncho-alveolar lavage MAIT cells from pneumonia patients identifies three MAIT subpopulations with varying expression of *RORC*, *TBX21* and *GATA3*, but still transcriptionally very close (Lu et al., 2020). In the absence of comparison to reference cell populations such as naive and memory mainstream T cells, the biological meaning of distances on the UMAP graph is unclear (Kiselev et al., 2019). Similarly, cytometry analysis of 332 immune receptors on human blood MAIT cells revealed limited heterogeneity despite bimodal expression of few markers such as CD56, CD84 and CD94. High expression of these markers is associated with higher lymphokine secretion after IL-12 and IL-18 stimulation (Dias et al., 2017).

Altogether, MAIT cells from mice and humans are programmed in the thymus to seed and reside in barrier tissues. Once there, local cues probably modulate the MAIT transcription

program, as MAIT cells isolated from different organs express distinct effector gene sets ((Salou et al., 2019) and Salou and Lantz, unpublished observations). Thus, MAIT cells seem to display specific properties according to the organ, suggesting that their function is tailored to the tissue, as previously proposed for mainstream T cells (Matzinger and Kamala, 2011).

5. MAIT cells are protective during various infectious diseases

The strong evolutionary conservation of the MAIT-MR1 axis indicates important functions for MAIT cells. These functions could be related to particular properties of MAIT cells: secretion of specific effector molecules, antigen specificity, positioning in specific tissues or ability to be directly activated by certain cytokines. Rapid production of IFN-γ, TNF-α, IL-17, GM-CSF and cytotoxic molecules is a key characteristic of MAIT cells, shared with mainstream memory T cells (reviewed in (Franciszkiewicz et al., 2016; Godfrey et al., 2019; Salou et al., 2017)) (**Figure 4**). Rapid response may rely on preformed RNAs instead of de novo transcription, as shown by PMA-Ionomycin-induced IFN-γ secretion experiments in which transcription or translation is inhibited (Gutierrez-Arcelus et al., 2019). MAIT cells are able to detect the presence of bacteria through their TCR and they also express various cytokine receptors such as receptors for IL-18, IL-12, IL-7, IL-1, IL-15 and IL-23 (Franciszkiewicz et al., 2016). The integration of these signals modulates the pattern of secreted effector molecules, resulting in context-dependent functions (**Figure 4**). For instance, IL-23R-deficient mice have reduced expansion of MAIT cells upon lung bacterial infection (Wang et al., 2019). Recently, type 1 IFN and the gut-derived TL1A have been added to the list of cytokines modulating MAIT cell functions (Lamichhane et al., 2020; Leng et al., 2019).

Because of their antigen specificity, the involvement of MAIT cells in infections by 5-A-RU-producing bacteria has been extensively investigated, both in mice and humans. Briefly, the absence of MAIT cells results in defective or delayed clearance of several intra-cellular bacteria such as *Mycobacterium abscessus* (Le Bourhis et al., 2010), *M. bovis* BCG (Chua et al., 2012), *Francisella tularensis* (Meierovics et al., 2013; Meierovics and Cowley, 2016) and *Legionella longbeachae* (Wang et al., 2018).. MAIT cells are also important for the clearance of urinary tract infection (Cui et al., 2015) or during *E. coli* peritonitis (Georgel et al., 2011). In humans, the frequency of MAIT cells in the blood is decreased in several bacterial infections (Grimaldi et al., 2014; Le Bourhis et al., 2010) with concomitant increase at the infected sites in few reports (reviewed in (Godfrey et al., 2019; Salou et al., 2017)). Longitudinal follow-up of human blood samples after *Salmonella enterica* Typhi infection or a *Shigella* strain vaccine challenge support the hypothesis of a migration towards inflammatory sites, as blood MAIT cells decrease in numbers, appear activated, and exhibit clonal expansion (Grimaldi et al., 2014; Howson et al., 2018; Le Bourhis et al., 2010; Salerno-Goncalves et al., 2017). MAIT activation is also associated with increased CCR6 and CCR9 expression, which favor homing to inflamed tissues and gut, respectively. Moreover, C/EBPδ, important for transmigration into inflamed tissues (Lee et al., 2018), is highly expressed by human MAIT cells. However, whether the main

function of MAIT cells is the defense against microbial pathogens remains unclear. Indeed, no susceptibility to a particular bacterial pathogen has yet been ascribed to a genetic deficiency of the MAIT-MR1 axis, besides a polymorphism of the *Mr1* gene in a Vietnamese cohort that has been associated to disseminated tuberculosis susceptibility (Seshadri et al., 2017).

Interestingly, MAIT cells seem to be involved in the defense against viruses. MAIT cell frequencies are selectively decreased in the blood of patients during several viral infections ((Loh et al., 2016; van Wilgenburg et al., 2016) reviewed in (Godfrey et al., 2019; Ussher et al., 2018)) and MAIT cells protect from experimental influenza infection, with an increased mortality in *Mr1*$^{-/-}$ animals without, however, modifications in the viral load (Wilgenburg et al., 2018). As viruses do not produce 5-A-RU, TCR-independent stimulation of MAIT cells is probably pivotal in these settings. Accordingly, MAIT cells are activated *in vitro* by the IL-12 and IL-18 secreted by influenza-infected monocytes (Loh et al., 2016).

The mechanisms of MAIT cell anti-infectious immunity are still unclear. *In vitro*, MAIT cells kill infected cells through granzymes and perforin (Kurioka et al., 2015; Le Bourhis et al., 2013). MAIT cells produce IFN-γ, TNF-α, IL-17 and (G)M-CSF (Franciszkiewicz et al., 2016; Godfrey et al., 2019; Salou et al., 2017), which likely stimulates anti-infectious responses through myeloid cells. MAIT cells also secrete various proinflammatory chemokines (Hinks et al., 2019; Lamichhane et al., 2019), suggesting a role in orchestrating the immune response. The loss of protection imparted by IFN–γ-deficient MAIT cells transferred into MAIT-deficient animals suggests that IFN-γ is crucial for anti-Legionella and -influenza immunity (Wang et al., 2018; Wilgenburg et al., 2018). However, in these studies, MAIT cells were expanded through Salmonella infection of the donor animals, to obtain the large numbers of MAIT cells required for adoptive transfers. This procedure induces a RORγt^{+}T-bet^{+} profile (Chen et al., 2017), distinct from the predominant RORγt^{+}T-bet^{neg} profile at steady state but more similar to the phenotype of human MAIT cells. In *F. tularensis* infection, MAIT cells improve bacterial clearance and mainstream T cell recruitment (Meierovics and Cowley, 2016). Differentiation of monocyte-derived macrophages is delayed in *Mr1*$^{-/-}$ mice, probably due to the absence of early GM-CSF production by MAIT cell. Transfer of monocyte-derived macrophages in *Mr1*$^{-/-}$ animals restored CD4^{+} T cell recruitment (Meierovics and Cowley, 2016). Importantly, when fighting bacteria, MAIT cells release inflammatory mediators able to induce tissues damage, such as Granulysine, Granzyme B and K, implicated in killing cells infected by intracellular bacteria (Kurioka et al., 2015; Ussher et al., 2014; Ussher et al., 2016), and IL-4i (leading to the formation of H_2O_2) (Turtle et al., 2011).

Thus, the exact mechanisms, direct or indirect, leading to MAIT cell-dependent protection against infectious agents remain to be fully dissected. Recent analysis of MAIT transcriptome following various activation strategies unraveled a previously unappreciated tissue repair function (see below).

6. MAIT cells are involved in both anti-microbial immunity and tissue repair

When compared to cytokine-activated or resting conditions, *in vitro* TCR-mediated activation of human blood MAIT cells results in an up-regulation tissue repair mediators, including *IL22*, *AREG*, *VEGFA* and *TGFA*, together with inflammatory molecules (Hinks et al., 2019; Lamichhane et al., 2019; Leng et al., 2019). Accordingly, supernatant from human MAIT cells cultured with *E. coli*-infected THP1 cells promotes closure in an *in vitro* scratch assay (Leng et al., 2019). Stimulation of human blood MAIT cells with IL-2, IL-7, mitogen and CD28 for a long period (8 to 10 days) induces high amounts of IL-13 and IL-5 (Kelly et al., 2019), two cytokines involved in tissue repair (Van Dyken and Locksley, 2013). A tissue-repair signature is also enriched in mouse lung MAIT cells following *Legionella* infection (Hinks et al., 2019) and in skin MAIT cells from *S. epidermidis*-associated animals (Constantinides et al., 2019). A role for MAIT cells in wound healing has been directly demonstrated by measuring the epidermal tongue length 5 days after a punch biopsy of the skin, with faster wound healing in C57Bl/6 mice pretreated topically with 5-OP-RU (Constantinides et al., 2019). The same beneficial effect of MAIT cells has been observed in mice associated with *S. epidermidis* prior to wounding, but only in γδ T cell-deficient mice, suggesting redundancy between γδ T cells and MAIT cells in the skin (Constantinides et al., 2019). At steady state, MAIT cells in the lung express more tissue repair mediators than in liver or spleen (Salou et al., 2019). This is also true for human MAIT cells in liver as compared to blood (Salou et al., 2019). MAIT cells may also regulate intestinal epithelium tissue integrity, as gut permeability to FITC-Dextran after gavage is increased in NOD mice deficient for MR1 (Rouxel et al., 2017).

Apart from these cytokines, and the putative mediators of tissue repair described for commensal-specific H2-M3-restricted $CD8^+$ T cells (Linehan et al., 2018), how MAIT cells repair tissues remains unknown. One important effector molecule in several tissue-repair models is amphiregulin (Areg). Areg is involved in the tissue repair function of $Foxp3^+$ regulatory T cells (Tregs) following influenza infection or muscle injury (Arpaia et al., 2015; Burzyn et al., 2013). MAIT cells may influence various pathways to promote repair including mediators regulating angiogenesis or tissue repair according to a MAIT transcriptome analysis(Hinks et al., 2019).

Consistent with the production of pro-angiogenesis molecules and epithelial growth factors, MAIT cells may have a pro-tumoral role (Yan et al., 2020). While the described mechanism is impairment of IFN-γ secretion by NK and $CD8^+$ T cell, the MAIT tissue repair function is also likely involved. This has been previously described for Treg cells (Green et al., 2017) or γδT cells (Jin et al., 2019). In three different models of transplanted tumors, *Mr1*$^{-/-}$ mice develop reduced numbers of metastases, a phenotype reversed by MAIT cell adoptive transfer (Yan et al., 2020). The pro-tumoral effect of MAIT cells requires MR1 expression on the tumor cells. *In vivo*, the nature and the origin (endogenous or bacterial) of the MAIT ligand presented by tumor cells remains unknown, although tumoral colonization by bacteria is a possibility (Nejman et al., 2020). Similarly, exacerbated or uncontrolled tissue-repair function could have unwanted effects such as the

fibrotic gastritis induced by *H. pylori* infection once MAIT cells have been expanded with 5-OP-RU and TLR ligands (D'Souza et al., 2018). MAIT cells could also promote liver fibrosis (Bottcher et al., 2018; Hegde et al., 2018), maybe through direct activation by bacterial products reaching the liver by the portal vein (Riva et al., 2017). Similarly, MAIT cells have been implicated in kidney fibrosis (Law et al., 2019).

Thus, MAIT cells are able to exert various effector functions (**Figure 4**), consistent with the co-expression of several master transcription factors such as RORγt, T-bet, Helios and Eomes in humans (Leeansyah et al., 2015). The expression of these usually mutually exclusive transcription factors is reminiscent of the commensal-specific H2-M3-restricted $CD8^+$ T cells populating the mouse skin following *S. epidermidis* colonization (Harrison et al., 2019; Linehan et al., 2018). In MAIT cells, the factors triggering one effector function over others are beginning to be unraveled as described in the next section.

7. MR1 ligands activate MAIT cells at steady state and during diseases

Recent *in vitro* evidence suggests a pivotal role of MAIT TCR signaling in eliciting tissue-repair function (Hinks et al., 2019; Lamichhane et al., 2019; Leng et al., 2019). Given that most microbes produce 5-A-RU (Mondot et al., 2016), and as microbiota-derived 5-OP-RU travels rapidly into the organism (Legoux et al., 2019a), tissue repair functions are likely constantly triggered (**Figure 4**). Therefore, MAIT cells would be involved both in tissue homeostasis, and recovery of tissue function after infection with 5-A-RU-producing pathogens. Infections with 5-A-RU-deficient pathogens might still activate MAIT cells via the commensal microbiota-derived 5-OP-RU. In thymic MAIT cells, microbiota-derived 5-OP-RU triggers MAIT17- but not MAIT1-proliferation, suggesting that MAIT17 may be the main actors of reparation.

When and in which circumstances the MAIT TCR is triggered *in vivo* is unclear. MR1 expression might not be high enough for MAIT cell activation in peripheral tissues, or MR1 may be loaded with non-agonist ligands. Indeed, DP thymocytes express 2-3 times more *Mr1* RNA than the alveolar macrophages and 5-6 times more than other cells in the body (Heng et al., 2008). MR1 is mostly intra-cellular at steady state and ligand availability seems to be the limiting factor for cell surface translocation (McWilliam et al., 2016). In HSV-1 infection, MR1 expression is suppressed and the protein is targeted for proteosomal degradation, preventing MR1 surface expression (McSharry et al., 2020). Similar mechanisms might occur at steady state or during other infections to prevent MAIT cell activation. Another layer of regulation may lie in the presence of other MR1 ligands, derived or not from bacteria, and binding MR1 without activating MAIT cells (Awad et al., 2020; Eckle et al., 2014; Harriff et al., 2018; Keller et al., 2017b; Soudais et al., 2015). Poor or null agonists, such as 5-OP-RU derived lumazines, may also regulate MAIT cell activation by preventing MR1 binding to stronger MAIT agonists. Since the relative proportion of these agonists or antagonists may vary according to microbiota composition and metabolic status (Schmaler et al., 2018), this would allow MAIT cells to sense microbiota ecology.

Whether TCR triggering is required for MAIT functions in pathological settings remains unclear as MAIT cells can be activated independently of TCR stimulation, through cytokines, either alone or in combination as reviewed elsewhere (Franciszkiewicz et al., 2016; Godfrey et al., 2019). In iNKT cells, TCR expression is dispensable for IFN-γ secretion in response to LPS stimulation *in vivo* (Vahl et al., 2013). TCR expression is also dispensable to maintain the iNKT cell lineage identity, including PLZF (*Zbtb16*) expression, in peripheral tissues. As MAIT and iNKT subsets have an almost identical transcriptional program (Salou et al., 2019), MAIT cell IFN-γ production may similarly be independent of TCR triggering. Moreover, for the Treg cells implicated in lung repair following influenza infection, Areg secretion is TCR-independent (Arpaia et al., 2015). In contrast, TCR signaling induces MAIT responses in several settings. Blocking MR1 decreases the number of metastasis, as well as NK and CD8 T cell secretion of IFN-γ (Yan et al., 2020). TCR triggering seems necessary but not sufficient for MAIT cell proliferation: 5-OP-RU inhalation increases MAIT cell numbers in the lungs only when associated with TLR 2, 3, 6 or 9 ligands (Chen et al., 2017). 5-OP-RU, together with IL-23, drives MAIT cell expansion and improves protection against legionella infection (Wang et al., 2019). Similarly, 5-A-RU-deficient salmonella does not induce MAIT cell proliferation, while 5-OP-RU addition does (Chen et al., 2017). Finally, deletion of MR1 before *S. epidermidis* association prevents MAIT cell increase in the skin (Constantinides et al., 2019). In this model, IL-18 blockade demonstrates an additional IL-18-dependency for the proliferation, while IL-17 secretion required IL-1 (Constantinides et al., 2019). Thus, TCR triggering seems important for proliferation following infection and for the pro-tumoral effect of MAIT cells. However, as MAIT cells can be triggered either through their TCR or by microbial-induced lymphokines, the need for bacterial ligands is still unclear for MAIT cell control of bacterial infection.

This issue is also complicated by the possible difference in TCR or lymphokine dependency of MAIT1 versus MAIT17 subsets as most of the *in vivo* data were generated in barrier organs in which MAIT17 cells are the great majority. MAIT1 vs MAIT17 activation requirements and subsequent effector functions are likely very different as MAIT1 cells express more NK receptors and cytotoxic molecules than MAIT17 cells. In the thymus, triggering MAIT1 TCR results in lower Nur77 expression in comparison to MAIT17 cells (Legoux et al., 2019a; Legoux et al., 2019b). Thus, in contrast to MAIT17 cells that mainly express tissue repair mediators following TCR stimulation, MAIT1 cells may preferentially respond to danger signals and produce cytotoxic molecules. However, in a pathological context in which IL-1, IL-12, IL-18 as well as MAIT ligands are present, MAIT17 cells upregulate T-bet, and secrete type-1 effector molecules (IFN-γ or TNF-α) together with tissue repair mediators (Chen et al., 2017; Hinks et al., 2019; Wang et al., 2018).

Thus, it is unclear how MAIT cells arbitrate between anti-bacterial functions with the associated cost of inflicting tissue damage and tissue repair functions (Salou and Lantz, 2019) (**Figure 4**). Anti-infectious immunity and tissue repair are tightly interweaved processes as

infection and the subsequent immune response result in tissue damage, the resolution of which is key to maintain tissue integrity and functions. MAIT cells are likely involved in both processes through the variety of effector molecules they produce (Hinks et al., 2019; Lamichhane et al., 2019; Leng et al., 2019). Moreover, physiologic (such as stomach acidity) or physical stress (due to breathing in the lungs for instance (Solis et al., 2019), or little traumatic disruptions of barrier tissues) may put in contact the organism with usually excluded microbiota. As seen above, MAIT cells can be activated through TCR-dependent or -independent pathways, and a tight integration of these signals is pivotal in triggering one function versus another. In mice at steady-state, both functions appear segregated in two distinct subsets expressing either T-bet or RORγt. However, upon bacterial infection, single MAIT cells can co-express T-bet and RORγt. In such MAIT cells, as in humans, both effector functions may be achieved by the same cell sequentially or according to the lymphokine context.

8. Concluding remarks

Besides infectious diseases, the frequency of blood MAIT cells is modified during several auto-immune diseases: diabetes (Magalhaes et al., 2015; Rouxel et al., 2017), multiple sclerosis (Annibali et al., 2010; Mekinian et al., 2017; Mexhitaj et al., 2019; Miyazaki et al., 2011; Treiner and Liblau, 2015; Willing et al., 2018; Willing et al., 2014), inflammatory bowel disease (Serriari et al., 2014) as well as obesity (Magalhaes et al., 2015). All these diseases are associated with important dysbiosis (Honda and Littman, 2016). Interestingly, bariatric surgery leads to a rapid (3 months) recovery of MAIT cell blood numbers (Magalhaes et al., 2015). One of the first changes observed after bariatric surgery is microbiota modifications (Liu et al., 2017) although this is controversial (Aron-Wisnewsky et al., 2019). Thus, MAIT cells could sense the changes in microbiota ecology and the modifications of their blood frequency would reflect the dysbiosis which is common in these diseases.

Tissue location, antigen specificity and context-dependent effector functions of MAIT cells explain their versatile properties and their involvement in various settings such as anti-bacterial defense, tissue repair and epithelium homeostasis. Bacterial infections can inflict considerable damage to host tissues as pathogen clearance by immune cells involves the release of mediators which are also associated with cell death and loss of tissue integrity. Following pathogen elimination, tissue repair processes enable the reconstruction of the vasculature, intercellular matrix, epithelium and stromal cell networks. Through the expression of both anti-microbial and wound-healing effector molecules, MAIT cells are well equipped to contribute to both of these overlapping phases of immune responses to infections according to the lymphokine milieu and ligand availability. Future studies will elucidate the dynamics of vitamin B2-derived metabolites *in vivo*, at steady state and along the course of infections and wound healing processes. A better understanding of MAIT cell triggers, together with the development of stable agonists, will pave the way for harnessing the clinical potential of these abundant T cells.

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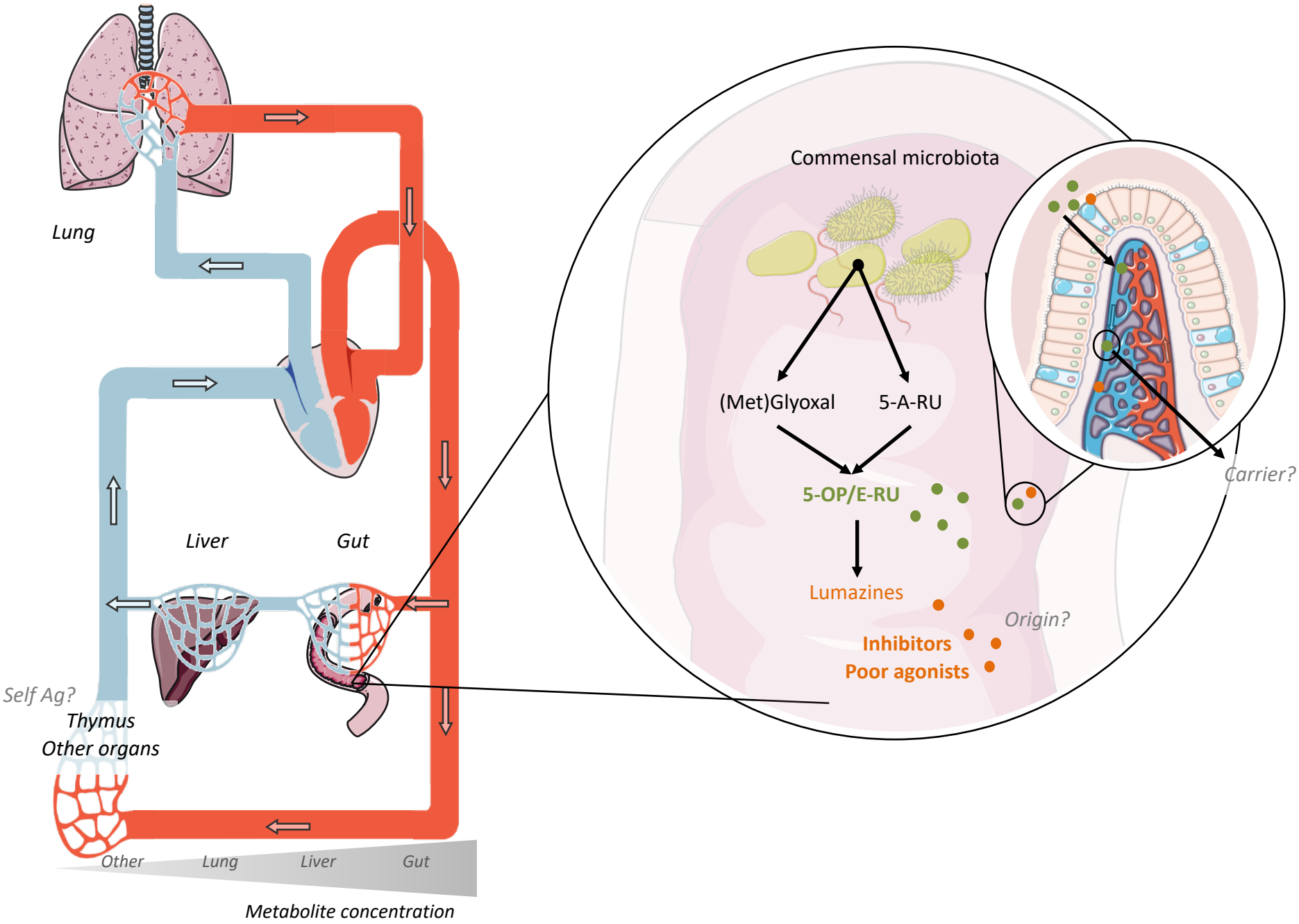
Figure legends.

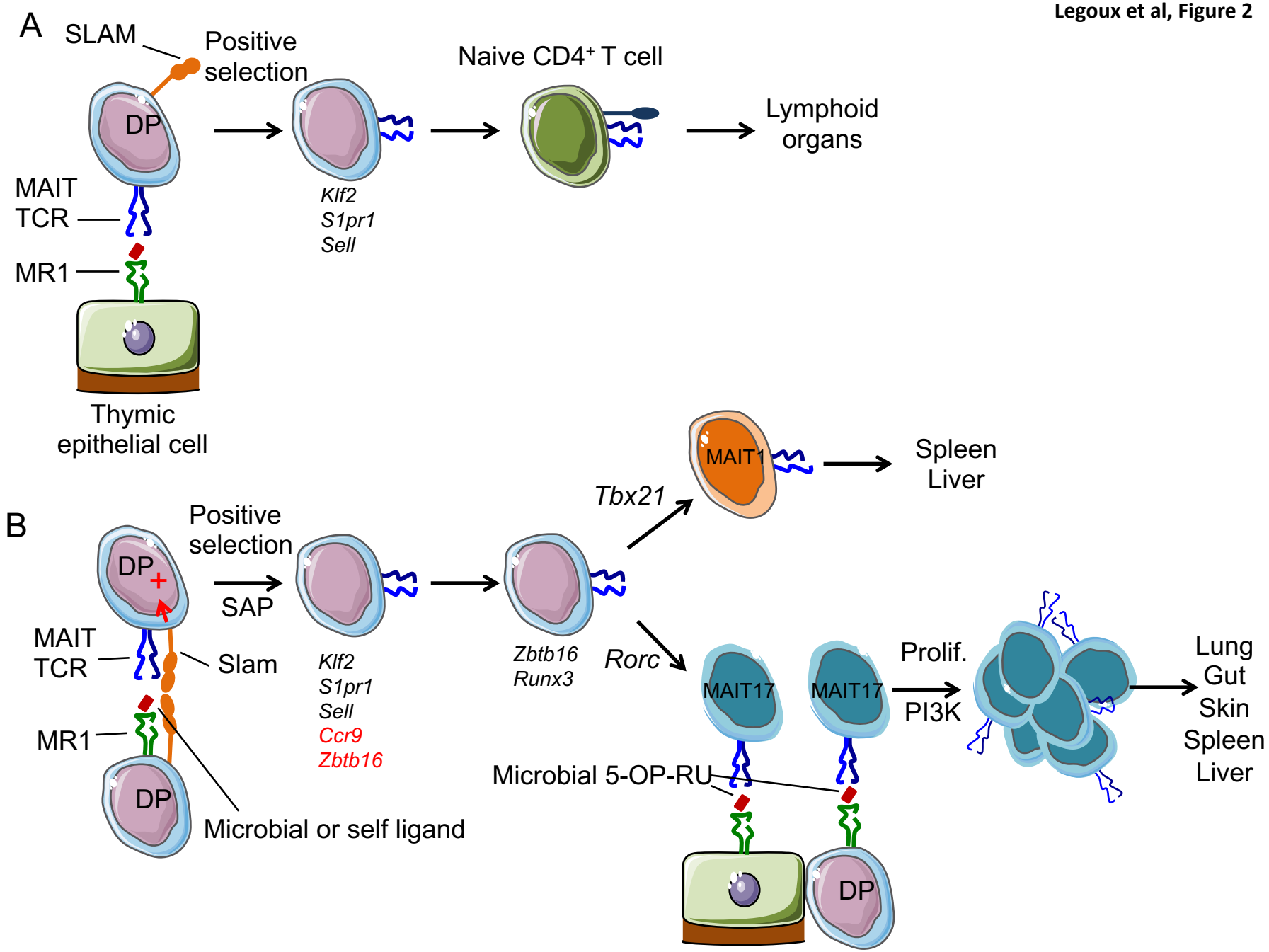
Figure 1: **Model of MAIT antigen circulation**. In gut bacteria, the unstable riboflavin precursor 5-A-RU reacts with methylglyoxal or glyoxal from the intermediary metabolism to generate 5-OP-RU or 5-OE-RU, respectively. Bacteria may also produce weak agonists or antagonists that may also bind to MR1 and prevent MAIT activation. The unstable 5-OP/E-RU rapidly crosses the epithelial layer to reach the portal blood and the liver before going through the lung and follow the general blood circulation. This pathway leads to a progressive dilution of 5-OP/E-RU by the afferent blood arriving at each step from other organs. The concentration of 5-OP/E-RU in the arterial blood reaching the thymus is still high enough to generate MR1:5-OP-RU complexes on DP thymocytes able to select MAIT cells, in a process also related to the high expression of MR1 by DP thymocytes. How 5-OP-RU crosses the epithelial layers and whether it is bound to a carrier in the blood are unknown.

Figure 2: Development of 5-OP-RU-specific T cells in the thymus. Upon rearrangement of a TCR with specificity for the 5-OP-RU:MR1 complex, immature thymocytes undergo positive selection by recognizing MR1 at the surface of either TECs (A) or DP thymocytes (B). Positive selection on TECs results in differentiation into naïve conventional-like $CD4^+$ T cells. Positive selection on DP thymocytes results in co-stimulation with Slam and engagement of the SAP signaling pathway, which drives expression of PLZF (*Zbtb16*) and effector differentiation into either MAIT1 or MAIT17 cells. Vitamin B2 metabolites such as 5-OP-RU can be captured by several cells in thymus, including DP thymocytes and TECs. Microbiota-derived antigens mediate positive selection of effector MAIT cells and intra-thymic proliferation of MAIT17 cells. Upon thymic egress, MAIT17 cells preferentially localize in mucosal tissues.

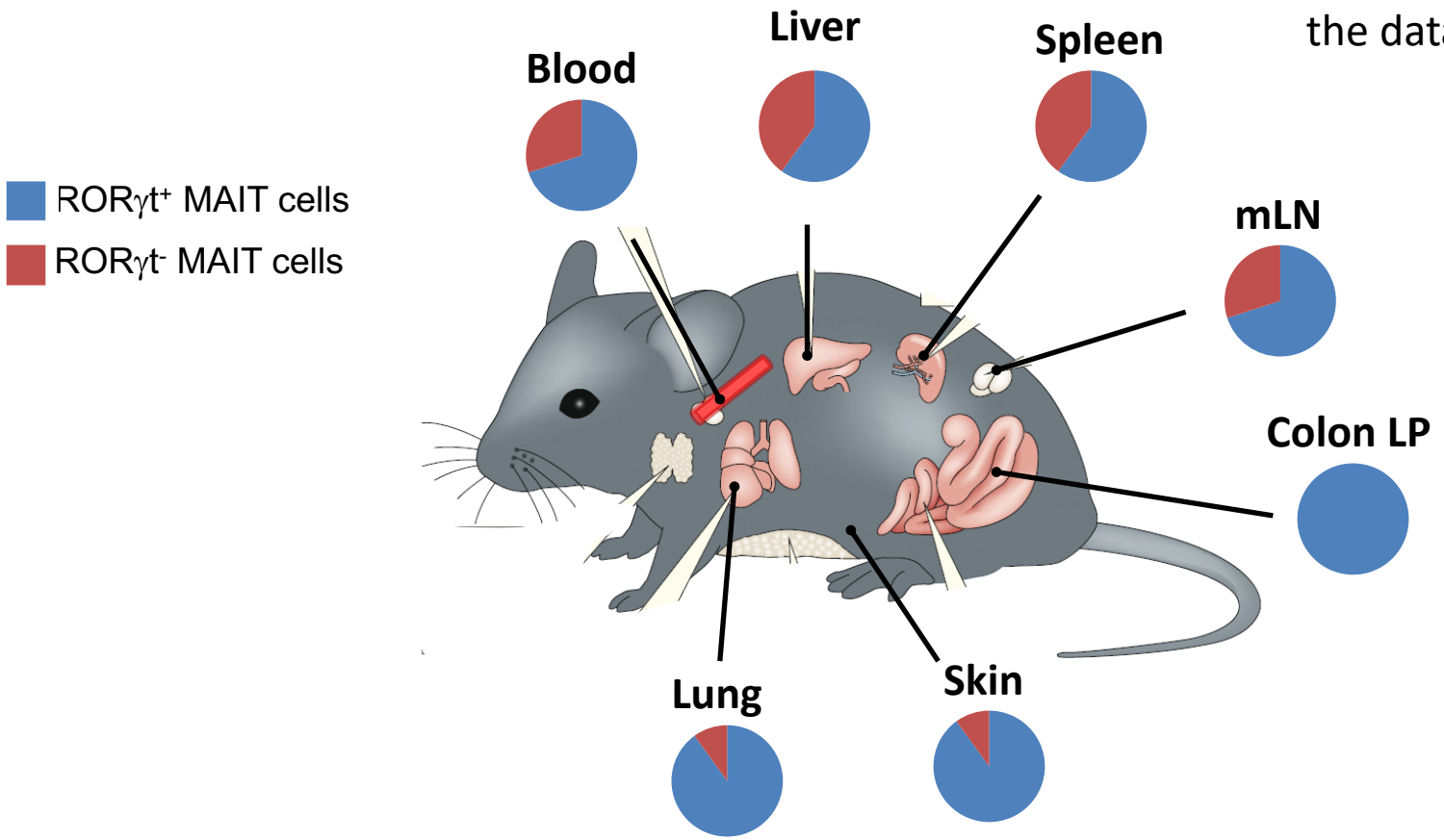
Figure 3: MAIT subset positioning in tissues of B6 or B6-MAIT^{Cast} mice. Percentages of MAIT1 (in red) and MAIT17 (in blue) cells among total MAIT cells are indicated.

Figure 4: MAIT17 cell potential effector functions in mucosal barriers including skin, lamina propria and lungs. At steady state, whether MAIT cell are stimulated or not and whether they exert effector functions remain uncertain. In an inflammatory context, depending on the stimulation mode, MAIT cells secrete various effector mediators. How these mediators vary according to the tissue and whether they are secreted by various subpopulations remains unknown. Another hypothesis is that different functions are sequentially triggered.
MAIT1 cell functions are less well described as they represent a small fraction of the total population in mucosal barriers


Lung
Liver
Gut
Self Ag?
Thymus
Other organs
Other
Lung
Liver
Gut
Metabolite concentration
Commensal microbiota
(Met)Glyoxal
5-A-RU
5-OP/E-RU
Lumazines
Origin?
Inhibitors
Poor agonists
Carrier?

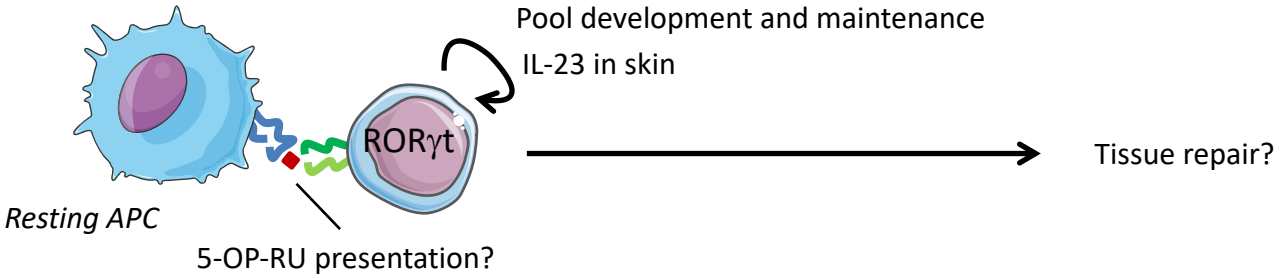

A
SLAM
Positive selection
DP
Naive CD4+ T cell
Lymphoid organs
MAIT TCR
MR1
Klf2
S1pr1
Sell
Thymic epithelial cell
B
Positive selection
SAP
DP
MAIT TCR
Slam
MR1
DP
Microbial or self ligand
Klf2
S1pr1
Sell
Ccr9
Zbtb16
Zbtb16
Runx3
Tbx21
MAIT1
Spleen
Liver
Rorc
MAIT17
MAIT17
Prolif.
PI3K
Lung
Gut
Skin
Spleen
Liver
Microbial 5-OP-RU
DP

The mouse picture and the organs were retrieved from a published paper (the drawings, not the data)



Steady state/homeostasis

Legoux et al, Figure 4



Inflammatory context (infection, tumor…)