

Les cellules MAIT et la riboflavine dans les interactions hôte-microbiote

Yara El Morr

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Préparée à l'Institut Curie

MAIT cells and riboflavin in host-microbiota interactions

Les cellules MAIT et la riboflavine dans les interactions hôte-microbiote

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Le 23 juin 2021

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ABSTRACT

Mucosal associated invariant T (MAIT) cells are an evolutionary conserved T cell subset, which recognize riboflavin precursor derivatives presented by MR1. Intestinal bacteria from the *Bacteroidetes* and *Proteobacteria* phyla can produce MAIT antigens, suggesting a direct interplay between MAIT cells and the microbiota. In humans, MAIT cells have been implicated in pathologies associated with intestinal dysbiosis such as inflammatory bowel diseases, but the role of MAIT cells in these diseases remains unknown. Increased levels of oxygen and oxidative stress are hallmarks of intestinal inflammation, and riboflavin contributes to bacterial respiration and to oxidative stress resistance, suggesting increased needs for riboflavin during intestinal inflammation. Supporting this hypothesis, bacteria possessing the riboflavin biosynthesis pathway are enriched in Crohn's disease patients.

In the first part of this work, we explored the hypothesis that MAIT cells monitor a bacterial metabolic pathway associated with altered gut ecosystem. We showed that hypoxia disruption in the colon, upon antibiotic treatment or dextran sodium sulfate (DSS)-induced colitis, increased MAIT antigen production by the microbiota. We sequence the 16S rRNA genes from the cecum of antibiotic- and mice, and analyzed expression of the riboflavin pathway genes in the *Helicobacter hepaticus* model of colitis. Dysanaerobiosis was associated with expansion of *Enterobacteriaceae* upon vancomycin treatment, and *Bacteroidaceae* and *Enterobacteriaceae* in colitic mice. Riboflavin production provided a fitness advantage to *Escherichia coli* in the inflamed intestine. Both *Bacteroidaceae* and *Enterobacteriaceae* can produce high amounts of MAIT antigens *in vitro*. In the *H. hepaticus* model of colitis, *ribD*, which controls MAIT antigen production, was over-expressed by *Bacteroidaceae*, *Clostridiaceae* and *Enterobacteriacea*e during colitis. MAIT antigens crossed the intestinal barrier and induced T cell receptor (TCR) signalling in MAIT cells, which produced the tissue-repair mediator amphiregulin and reduced colitis severity. In the second part of this work, we characterized MAIT cells in the ileal environment, wherein high levels of oxygen have been observed at steady state. A range of bacteria that encode the riboflavin pathway populated the ileum, however ileal MAIT cells were not activated through TCR engagement and expressed distinct phenotypic profiles. We discuss the future studies that will be needed to understand MAIT-microbiota interactions in the ileum.

Collectively, in the colon, MAIT cells directly sense and react to changes in bacterial metabolism associated with intestinal inflammation and provide host protection in return. This new host-microbiota interaction may explain MAIT cell activation in other pathologies associated with dysbiosis such as colorectal cancer.

KEYWORDS

MAIT cells, lamina propria, microbiota, riboflavin, colitis, mice.

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List of abbreviations

MAIT: Mucosal-associated invariant T (MAIT) cells

IBDs: Inflammatory bowel diseases

MHC: Major histocompatibility complex

MR1: Major histocompatibility complex class I-related gene protein

CD1: Cluster of differentiation 1 related to the class I MHC molecules

TRAV: T cell receptor alpha variable

β_2-m: Beta-2 microglobulin

ER: Endoplasmic reticulum

K43: Lysine 43

Rib: Riboflavin

***E. coli*:** *Escherichia coli*

GTP: Guanosine-5'-triphosphate

5-A-RU: 5-amino-6-(1-D-ribitylamino)uracil

HAD: Haloacid dehalogenase

5-OP-RU: 5-(2-oxopropylideneamino)-6-d-ribitylaminouracil

5-OE-RU: 5-(2-oxoethylideneamino)-6-d-ribitylaminouracil

FMN: Flavin mononucleotide

FAD: Flavine adenine dinucleotide

ATP: Adenosine triphosphate

mRNA: Messenger RNA

NADH: Nicotinamide adenine dinucleotide

GSSG: Glutathione disulfide (Oxidized GSH)

GSH: Glutathione

H_2O_2: Hydrogen peroxide

***F. prausnitzi*:** *Faecalibacterium prausnitzii*

DMSO: Dimethyl sulfoxide

DHA: Dihydroxyacetone

TCR: T cell receptor

TRAJ: T cell receptor alpha joining

TRBV: T cell receptor beta variable

CDR: Complementarity-determining region

Tyr95: Tyrosine 95

6-FP: 6-Formylpterin

DP: Double positive

TECs: Thymic epithelial cells

SLAM: Signaling lymphocytic activation molecule

SAP: SLAM-associated protein

PLZF: Promyelocytic leukemia zinc finger

iNKT: Invariant natural killer T cells

Tbet: T-box expressed in T cells

Rorγt: Retinoic acid-related orphan receptor gamma t

CD: Cluster of differentiation

GF: Germ-free

SPF: Specific-pathogen-free

CAST: Castaneous

CCR: C-C chemokine receptor

CXCR: C-X-C chemokine receptor

IL: Interleukin

IL-R: Interleukin receptor

IFN: Interferon

CCL: Chemokine (C-C motif) ligand

CXCL: C-X-C motif chemokine ligand

TNF: Tumor necrosis factor

GM-CSF: Granulocyte-macrophage colony-stimulating factor

PMA: Phorbol 12-myristate 13-acetate

TLR: Toll-like receptor

AREG: Amphiregulin

VEGF-A: Vascular endothelial growth factor A

TGFα: Transforming growth factor alpha

M. tuberculosis: Mycobacterium tuberculosis

P. aeruginosa*: Pseudomonas aeruginosa*

HCV: Hepatitis C virus

PD-1: Programmed cell death protein 1

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

COVID19: Coronavirus disease 2019

WT: Wild-type

MS: Multiple sclerosis

VLA-4: Very late antigen-4

CNS: Central nervous system

EAE: Experimental autoimmune encephalomyelitis

T1D: Type 1 diabetes

NOD: Non-obese diabetic

UC: Ulcerative colitis

CD: Crohn's disease

GI: Gastrointestinal tract

Th: T helper cells

IEC: Intestinal epithelial cells

AMPs: Antimicrobial peptides

FGF2: Fibroblast growth factor

STAT3: Signal transducer and activator of transcription 3

NKG2D: Natural killer group 2 member D

BTLA: B- and T-lymphocyte attenuator

SCFAs: Short-chain fatty acids

PPAR-γ: Peroxisome proliferator-activated receptor gamma

pO2: Partial pressure of oxygen

EPR: Electron paramagnetic resonance

HIF: Hypoxia-inducible factor

H_2S: Hydrogen sulfide

ROS: Reactive oxygen species

LEfSe: Linear discriminant analysis effect size

PICRUSt: Phylogenetic investigation of communities by reconstruction of unobserved states

rRNA: Ribosomal RNA

CO2: Carbon dioxide

DSS: Dextran sodium sulfate

DUOX2: Dual oxidase 2

APOA1: Apolipoprotein A1

NADPH: Dihydronicotinamide-adenine dinucleotide phosphate

NLRs: NOD-like receptors

IgA: Immunoglobulin A

BAs: Bile acids

O2: Oxygen

HDACs: Distone deacetylases

DCs: Dendritic cells

FoxP3: Forkhead box P3

GPR: G-protein-coupled-receptor

Ahr: Aryl hydrocarbon receptor

ILC: Innate lymphoid cells

B6: C57BL/6

Nr4a1: Nuclear receptor subfamily 4 group A member 1

GFP: Green fluorescent protein

mLN: Mesenteric lymph nodes

I+ B LN: Inguinal and brachial lymph nodes

PCA: Principal component analysis

***B. thetaiotaomicron*:** *Bacteroides thetaiotaomicron*

LSL: Lox-Stop-Lox

HBSS: Hanks balanced salt solution

EDTA: Ethylenediaminetetraacetic acid

DTT: Dithiothreitol

FCS: Fetal calf serum

OVA: Ovalbumin

ASV: Amplicon sequence variant

iMGMC: Integrated Mouse Gut Metagenomic Catalog

Ct: Threshold cycle

C. difficile: *Clostridioides difficile*

C. perfringens: *Clostridium perfringens*

C. butyricum: *Clostridum butyricum*

PCR: Polymerase chain reaction

CFUs: Colony forming units

LB: Luria broth

PMDZ: Pimonidazole

DAPI: 4′,6-diamidino-2-phenylindole

H&E: Hematoxylin and eosin

MyD88: Myeloid differentiation primary response 88

Nlrp6: NOD-like receptor family pyrin domain containing 6

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Introduction

Through an enduring mutualistic partnership, humans have co-evolved with the trillions of microbes that inhabit different sites of our bodies. Host-specific microbiota is a result of this continuous mutual selection between the host and the microbes (Ley et al. 2008). The highest concentration of mutualistic microorganisms is found in the gastrointestinal tract with the colon being colonized with the densest and most metabolically active community (Rooks and Garrett 2016). Research from the past 20 years has revealed critical functions of the gut microbiota, which is often considered as a new organ system.

In the gut, the microbiota relies on the host environment and nutrients for its survival, and in return provides benefits to the host in many ways. Indeed, commensal bacteria influence host nutrient uptake, food metabolism and support host resistance to invading pathogens (Wu and Wang 2019). In recent years, several studies have revealed that the gut microbiota provides the host with essential metabolites and components for host immune development and regulation (G. Wang et al. 2019; Ahern and Maloy 2020). There is an increasing awareness that microbial communities can modulate locally the gut immune system, which encounters more antigens than any other part of the body. Changes in the composition or function of the microbiota (dysbiosis) are associated with many pathologies, but how the intestinal immune system monitors such alterations remains poorly understood. This question is of particular importance for mucosal-associated invariant T (MAIT) cells, which are predominantly found in tissues colonized by the microbiota and recognize metabolites broadly produced by many bacteria and fungi.

In this introduction, I will first review what is currently known about MAIT cells. Second, I will describe the interplay between the gut microbiome and the host immune system at steady state and in diseases with a particular focus on the inflammatory bowel diseases (IBDs).

Chapter I: Mucosal associated invariant T cells

MAIT cells represent the most abundant αβT cells recognizing a given bacterial compound. Unlike conventional T cells, which recognize peptide antigens presented by highly polymorphic MHC Class I or MHC Class II molecules, MAIT cells recognize microbial-derived metabolites presented by the MHC-I related protein, MR1.

1. MR1 genomic organization and transcripts

MR1 is a non-classical member of the MHC class I family of proteins that is encoded by the non-polymorphic *Mr1* gene. This *Mr1* gene appeared in mammals and is the most highly conserved *Mhc* gene between human and mouse, with ≈90% identity in coding sequences for the α1 and α2 domains that bind the ligand (S. Huang et al. 2009). In addition, the similarity scores of the α1 and α2 domains of MR1 across mammals show that MR1 sequences have evolved more slowly compared with CD1 or MHC1 (Fig. 1) (Boudinot et al. 2016). This high evolutionary conservation of MR1 reflects the conserved structure of the antigen presented.

Interestingly, *Mr1* has been pseudogenized or lost in carnivores, lagomorphs and the armadillo. Very interestingly, the TRAV1 TCR gene segment used by MAIT cells (see section 2 below) is absent in all species in which a functional *Mr1* gene is also lacking (Boudinot et al. 2016) (Fig. 2). This observation strongly suggests a co-evolution between *Mr1* and TRAV1 and indicates that the main function of MR1 is to present antigens to $TRAV1^{+}$ cells.

The Mr1 message and encoded protein are expressed in various cell types and tissues. In the steady state without antigen, the majority of MR1 resides in an incompletely folded conformation weakly associated with β2-microglobulin (β2m) in the endoplasmic reticulum (ER). MR1 antigens access the ER via an unknown process and form a Schiff base with the residue Lysine 43 (K43) of the MR1 groove. The covalent bound between MR1 and the ligands allows the complete folding of MR1, β2m association, and trafficking of the MR1-antigen complexes to the cell surface by a mechanism to be defined. The complexes are subsequently internalized for degradation or recycling (Fig. 3) (McWilliam et al. 2016).

In accordance with the above-mentioned endosomal trafficking of MR1, using anti-MR1 antibodies (clones 26.5 and 12.2), which only recognize MR1 in a folded form, MR1 appears to have limited surface expression and is more readily detectable at the cell surface in the presence of ligand (Eckle et al. 2014; Soudais et al. 2015). Despite considerable effort, surface expression of endogenous MR1 remains challenging. Nonetheless, MR1 is likely to be expressed on different human and mouse cells (double positive thymocytes, monocytes, dendritic cells, macrophages, and epithelial cells) that have been shown to activate MAIT cells *in vitro* in an MR1-dependent manner (Gold et al. 2010; Le Bourhis et al. 2010; Ussher et al. 2016; Legoux, Bellet, et al. 2019; Harriff et al. 2016).

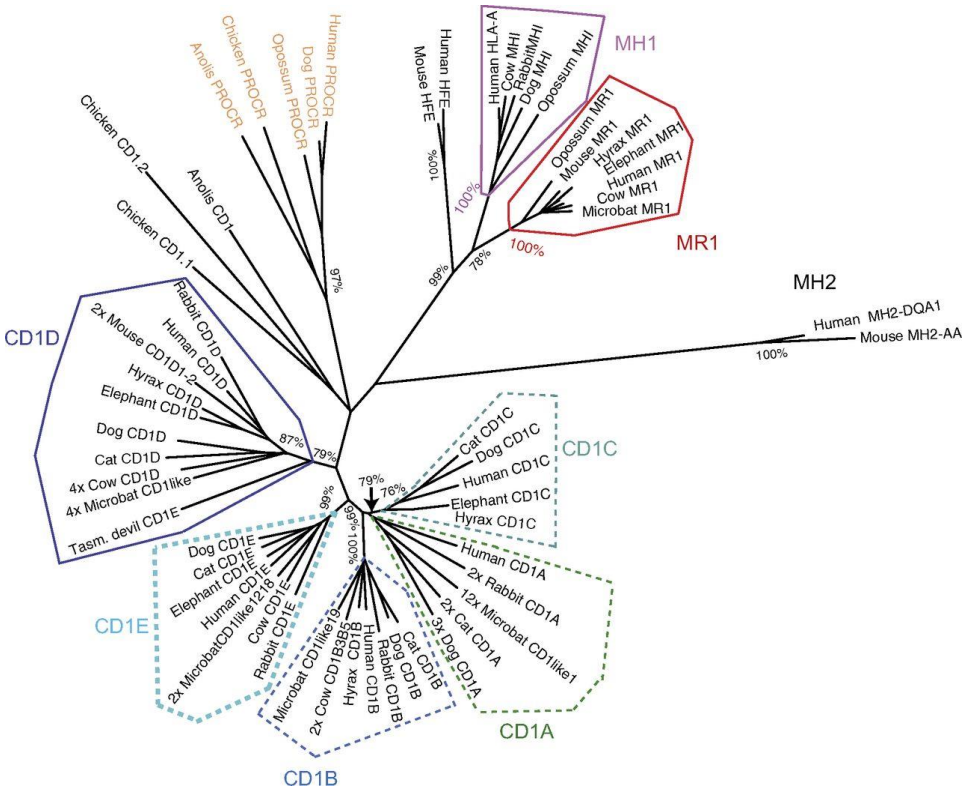


Figure 1: Neighbor-joining tree based on similarity scores of the α1-α2 domains of MR1, CD1 and classical MHC proteins in mammals. Boudinot et al showed a strong conservation of MR1 sequences evolving more slowly than other MCH1-like proteins (Boudinot et al. 2016).

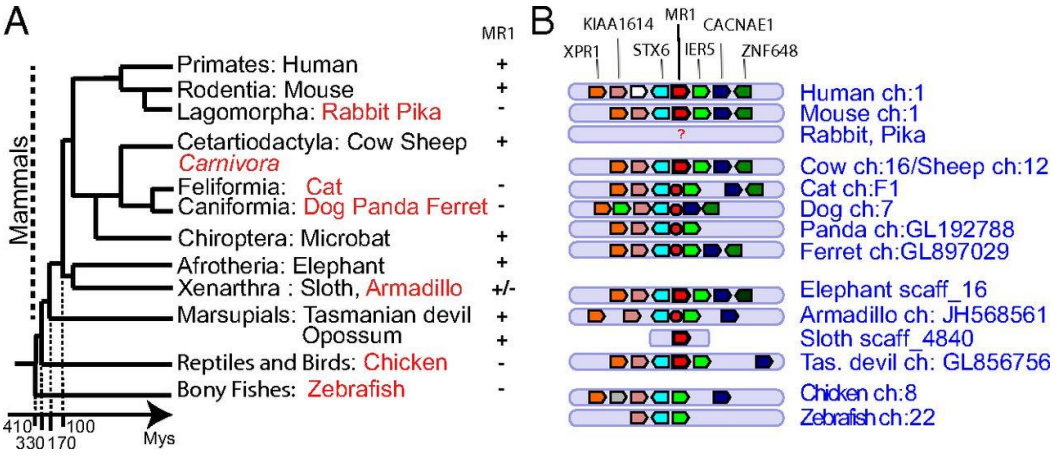


Figure 2: Inactivation or loss of *Mr1* in several groups of mammals.
In Carnivora (Cat, Dog, Panda, Ferret) and Armadillo, the MR1 region is conserved but MR1 is a pseudogene (red sphere). A functional Mr1 is also missing in the rabbit and pika in which the MR1 region has been lost. Chicken and Zebrafish do not have Mr1.

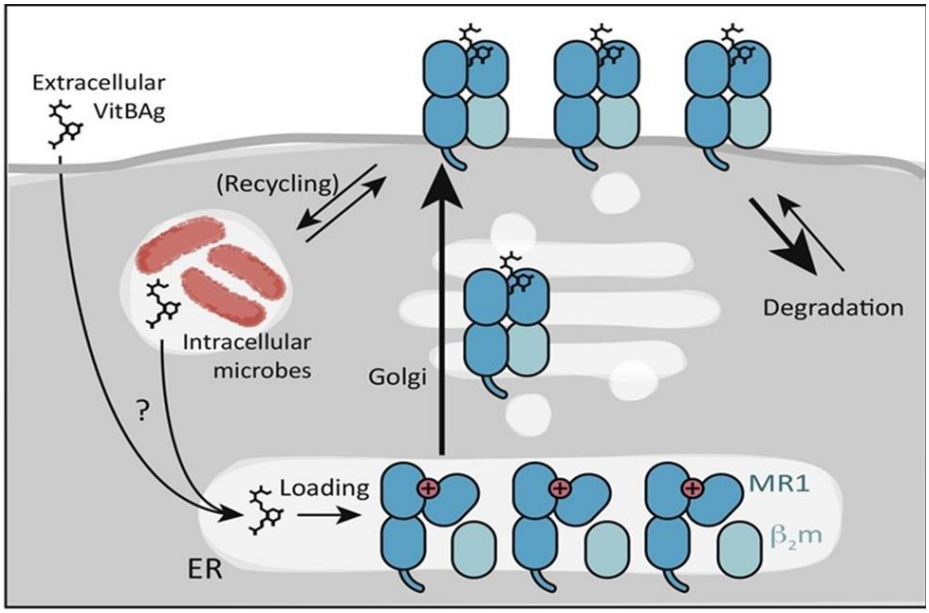


Figure 3: The Intracellular Pathway of MR1.
MR1 surface expression and antigen presentation are almost strictly dependent on antigen availability within the ER (McWilliam and Villadangos 2017).

2. Ligands for MR1

The MR1 binding groove is characterized by an A' pocket and an F' pocket. The A' pocket is lined with aromatic residues that facilitate the binding of small organic metabolites derived from two vitamin B sources: metabolites derivatives from the riboflavin (vitamin B2) biosynthesis pathway that activate MAIT cells, and metabolites derivatives from folate (vitamin B9) that act as competitive inhibitors of MAIT cell activation (Kjer-Nielsen et al. 2012; Corbett et al. 2014; Eckle et al. 2015; Kjer-Nielsen et al. 2018).

2.1. MR1 antigens that activate MAIT cells

The MAIT cell activating metabolite antigens are generated during the synthesis of riboflavin inside all plants and fungi and most bacteria. Almost all *Bacteroidetes*, *Fusobacteria* and *Proteobacteria* are able to synthesize riboflavin. However, half of the *Firmicutes* species and very few *Actinobacteria* are predicted to be riboflavin producers (Magnúsdóttir et al. 2015). To note, animal cells and the non-producing riboflavin microorganisms rely on external riboflavin which they take up using specialized transporter proteins (Abbas and Sibirny 2011; Kanehisa et al. 2002). The pathways of riboflavin synthesis are identical in eubacteria and plants but different in fungi and archaea (Abbas and Sibirny 2011; Garcia-Angulo 2016).

2.1.1. Riboflavin synthesis pathway in Escherichia coli and formation of MAIT antigens

Riboflavin is made through several enzymatic steps encoded by *rib* genes (Fig. 4). In *E. coli* and all Gram-negative bacteria, *rib* genes are scattered throughout the genome which is not the case in most Gram-positive bacteria *(Bacillus spp, Lactococcus spp.)* where the *rib* genes are grouped into a single operon.

Riboflavin synthesis starts from one guanosine-5'-triphosphate (GTP) derived from the purine biosynthesis pathway and two ribulose-5-phosphate molecules from the pentose-phosphate pathway. This first reaction is catalyzed by the GTP cyclohydrolase II encoded by *ribA* and produces 2,5-diamino-6-hydroxy-4-(5' -phosphoribosylamino)pyrimidine.

The next two reactions, the deamination produces 5-amino-6-(5’ -phosphoribosylamino)uracil and the reduction produces 5-amino-6-(5’ -phosphoribitylamino)uracil. Those steps are catalyzed by a bifunctional pyrimidine deaminase/ reductase enzyme, encoded by the *ribD* gene.The *ribD* product undergoes further dephosphorylation giving 5-amino-6-(1-D-ribitylamino)uracil (5-A-RU). The mechanism of dephosphorylation remained unknown until recently. Multiple broad-spectrum hydrolases belonging to the haloacid dehalogenase (HAD) superfamily are able to mediate this dephosphorylation. 5-A-RU is the direct precursor of MAIT cell antigens. It gives rise to lumazine derivatives (Fig. 4, violet) or pyrimidine adducts (5-OP-RU and 5-OE-RU, red) which are formed from the non-enzymatically spontaneous reaction of the unstable 5-A-RU with methylglyoxal or glyoxal, respectively (Fig. 4) (Corbett et al. 2014). Methylygyoxal and glyoxal are produced by several metabolic pathways, in particular glycolysis and therefore can be provided by both prokaryotic and eukaryotic cells.

Next, 5-A-RU condensates with 3,4-dihydroxy-2-butanone-4-phosphate (3,4-DHBP) by lumazine synthase encoded by *ribE*. The condensation leads to 6,7-dimethyl-8-ribityllumazine (RL-6,7-diMe), the riboflavin immediate precursor. The 3,4-DHBP is synthesized from ribulose 5-phophate by the 3,4-DHBP synthase coded by *ribB*. The final step is the dismutation of 6,7-dimethyl-8-ribityllumazine mediated by riboflavin synthase coded by *ribC.* In this step, riboflavin and 5-A-RU, the substrate of the lumazine synthase reaction, are generated.

All organisms, including animal cells, generally do not use free riboflavin but riboflavin is utilized to synthesize flavin nucleotides; flavin mononucleotide (FMN) and flavine adenine dinucleotide (FAD). FMN is produced by specific phosphorylation of riboflavin by the riboflavin kinase and is then converted to FAD by the action of FAD synthetase or FMN adenylyltransferase in the presence of ATP (Abbas and Sibirny 2011; Garcia-Angulo 2016; Kjer-Nielsen et al. 2018).

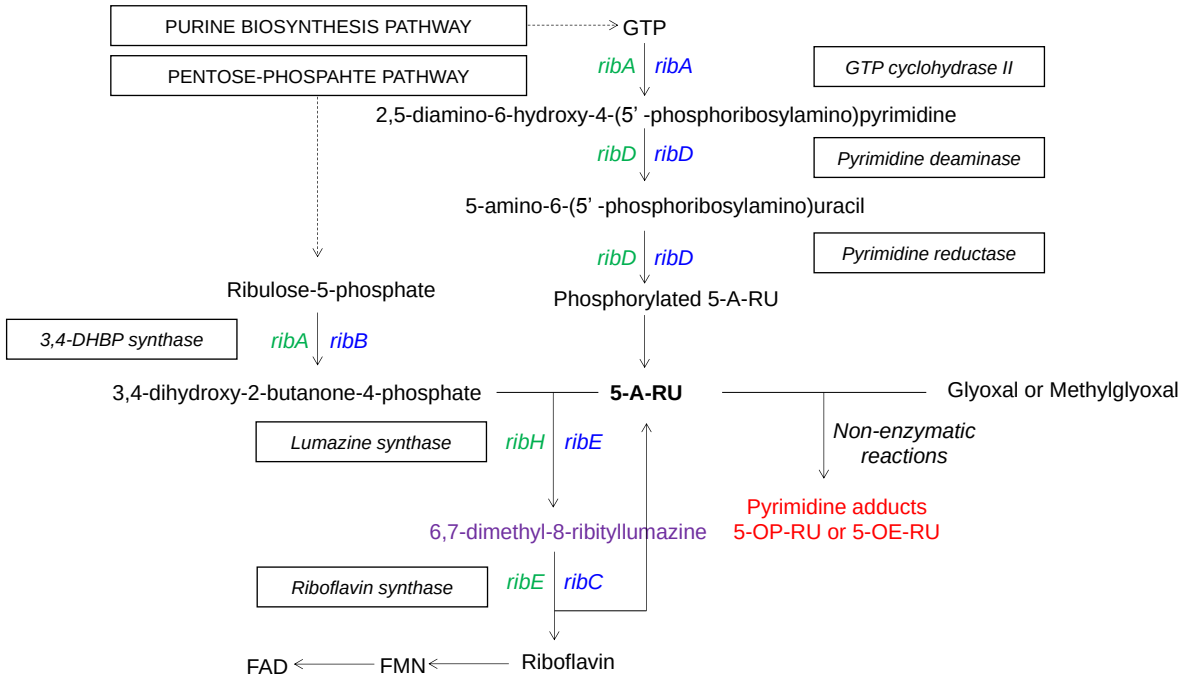


Figure 4: Riboflavin biosynthesis pathway and formation of MAIT antigens in bacteria (KEGG database). Riboflavin biosynthesis proceeds through different enzymatic steps starting from GTP and ribulose 5-phosphate. This pathway is conserved in many species with few variations in enzymes nomenclature. Bacillus gene names are shown in green and *E. coli* gene names are shown in blue. Note: In fungi and archaea, despite few variations in some reaction's mechanisms (Abbas and Sibirny 2011), the intermediary compound 5-A-RU is still produced and used for the spontaneous generation of 5-OP-RU and 5-OE-RU.

2.1.2. Regulation of riboflavin synthesis

The details of riboflavin synthesis regulation are still emerging. It occurs at the level of enzyme activity and synthesis and differs in bacteria, yeasts, and fungi. However, the physiological role of these regulations is not known.

In yeasts, different possible feedback regulations of riboflavin biosynthetic enzymes have been observed. FAD and other nucleotides containing an adenylic moiety can allosterically inhibit GTP cyclohydrolase II. In addition, riboflavin has been shown to inhibit the activity of lumazine synthase from *Pichia guilliermondii* (Abbas and Sibirny 2011).

In bacteria, production of riboflavin is regulated by the FMN riboswitch (also known as RFN element). This is found upstream several *rib* operons and monocystronic *rib* genes. The binding of FMN to riboswitch element (noncoding RNA) of the nascent mRNA causes transcription termination of *rib* genes. In fact, the conformation of the FMN riboswitch element changes resulting in terminator hairpin formation. In the absence of FMN, the conformation of the FMN element forms an antiterminator structure that allows normal transcription of downstream genes. By competing RNA structures, the FMN riboswitch attenuates also translation mechanisms by sequestering of the ribosomal binding site (also called the shine-Dalgarno (SD) box) in bacterial mRNA (Fig. 5) (Pedrolli et al. 2015; Abbas and Sibirny 2011; Vitreschak et al. 2002). Thus, riboflavin gene expression levels in some eubacteria but also in few archaea and eukaryotes can be directly coupled with the intracellular availability of FMN.

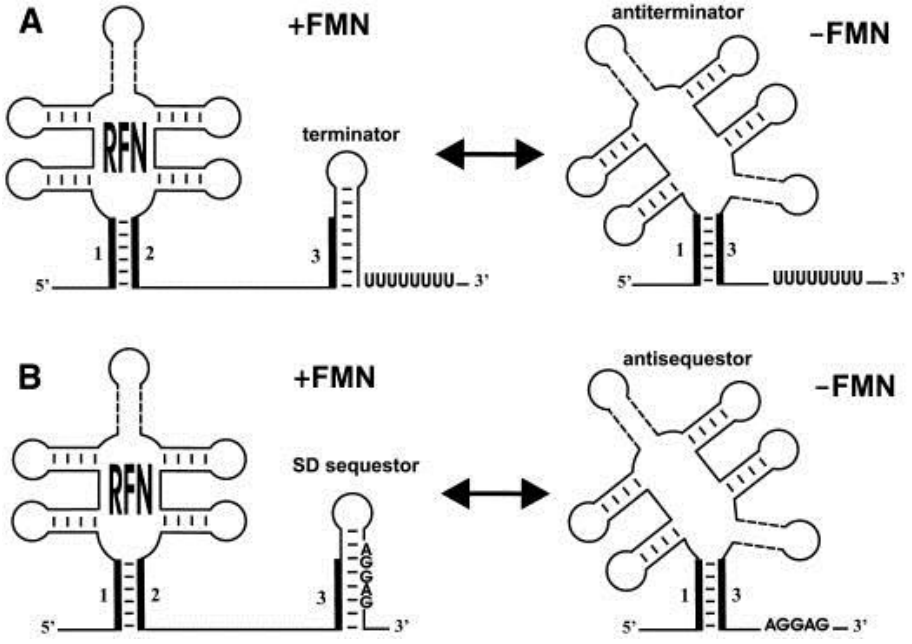


Figure 5: The FMN riboswitch regulates riboflavin genes in bacteria.
(A) Transcription attenuation; (B) translation attenuation (Vitreschak et al. 2002)

2.1.3. Biological role of riboflavin

Except for a direct function as a redox cofactor for the Na+ -pumping NADH: quinone oxidoreductase (Juárez et al. 2008), riboflavin usually does not have direct metabolic functions in the living cell. Indeed, the biological role of riboflavin, in most cases, is connected with the functions of FMN and FAD which are the most important organic cofactors. FMN and FAD bind to proteins, producing flavoproteins (flavoenzymes). It is currently estimated that around 2% of the genes in bacterial and eukaryotic genomes code for flavin-binding proteins (Abbas and Sibirny 2011). Because of their three redox states (oxidized, one-electron reduced or two-electron reduced) (Fig. 6), flavins are involved in several oxidation-reduction reactions that occur in plants, animals, and bacteria.

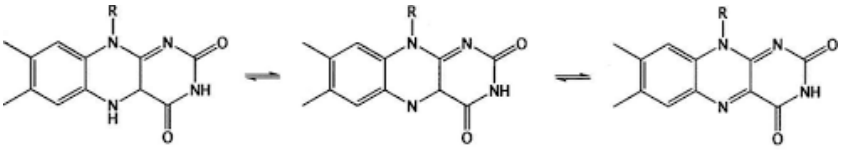


Figure 6:The three redox states of flavin mononucleotide.

FMN and FAD are co-factors for oxidoreductases involved in aerobic respiratory chain (NADH dehydrogenase) (J. Chen and Strous 2013), in microaerobic respiration (fumarate reductase), and in oxidative stress reduction (glutathione reductase, alkyl hydroperoxide reductase). The fumarate reductase uses FAD and allows the strict anaerobe *Bacteroides fragilis* to grow in the presence of nanomolar concentrations of oxygen (Baughn and Malamy 2004). The glutathione reductase utilizes FAD to catalyze the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress (Ashoori and Saedisomeolia 2014). The alkyl hydroperoxide reductase, which is overproduced in bacteria following H_2O_2 treatment or oxygen exposure, requires FAD as cofactor to mediate bacterial protection against oxidative damage (Poole 2005; Rocha and Smith 1999). Taking these implications into consideration, researchers observed that strict anaerobe *Clostridioides difficile* extensively synthesize FMN and FAD after oxygen exposure possibly due to an increased demand for cofactors in redox reactions (Neumann-Schaal et al. 2018).

In bacteria, in addition to their role as ubiquitous intracellular cofactors, flavins are involved in extracellular electron transfer mechanisms. For example, the human gut anaerobe *Faecalibacterium prausnitzi* employs an extracellular electron shuttle of flavins and thiols to transfer electrons to oxygen. This mechanism allows *F. prausnitzi* to survive in the mucosal-luminal interface in the presence of some oxygen (Khan et al. 2012).

2.1.4. Discovery of MAIT antigens

In 2009, more than 10 years after the discovery of MAIT cells, MAIT ligands were discovered using a combination of methodologies. An important step in identifying MAIT ligands was the discovery that various strains of bacteria and yeasts and not viruses can activate MAIT cells in a MR1-dependent manner (Gold et al. 2010; Le Bourhis et al. 2010). It was suggested by Lantz's team that MAIT ligand could be an extremely conserved compound among microbes (Le Bourhis et al. 2010).

Subsequent experiments based on refolded MR1 with the culture supernatant of *Salmonella Typhimurium* lead to the discovery of several MR1 stimulatory ligands. High resolution mass spectrometry of the ligands eluted from MR1, allowed determination of ligands from the riboflavin biosynthetic pathway: 7-hydroxy- 6-methyl-8-d- ribityllumazine (RL-6-Me-7-OH) and 6,7-dimethyl-8-d- ribityllumazine (RL-6,7-diMe) (Kjer-Nielsen et al. 2018). In accordance, it was noted that only riboflavin producing microbes can activate MAIT cells (Gold et al. 2010; Le Bourhis et al. 2010).

Further mass spectrometry experiments (Corbett et al. 2014) allowed the determination of the most potent organic MAIT antigens identified to date, the 5-OP-RU and 5-OE-RU which are active in the low nanomolar range. However, these metabolites are very unstable in water unless they are stabilized by the covalent bound with K43 of the MR1 groove (Corbett et al. 2014). They have a short half-life in water where they spontaneously cyclize to form weak agonists for MAIT cells ($t_{1/2}$ 1.5 h, 37 °C) (Kjer-Nielsen et al. 2012; Mak et al. 2017). A synthesis was therefore devised to prepare 5-OP-RU in dimethylsulfoxide (DMSO), where it was indefinitely stable in water (Mak et al. 2017). Compared to aqueous solution, 5-OP-RU in DMSO is a potentially useful new reagent for a better understanding of MAIT cell recognition and activation *in vitro*. Nevertheless, understanding the biology of microbial 5-OP-RU remains hampered by the low stability of free 5-OP-RU *in vivo*.

To further confirm the major role of 5-A-RU as a precursor for MAIT cell ligands, culture supernatant from *Lactococcus lactis* (Gram-) or *E. coli* (Gram+) with individual mutation in genes of riboflavin pathway either upstream or downstream of MAIT antigen production were tested for activation of MAIT cells (Soudais et al. 2015; Corbett et al. 2014). Lantz group and McCluskey group showed that MAIT activation is directly dependent upon *ribA* and *ribD/G* genes expression and 5-A-RU production by bacteria.

In another series of experiments, it was found that 5-A-RU can react through nonenzymatic reaction with dihydroxyacetone (DHA), another intermediate from glycolysis pathway, to form less potent MAIT antigens (Soudais et al. 2015). This was explained by probably an intracellular conversion of DHA into methylglyoxal, given that DHA was able to react with 5-A-RU in the cells and not in the medium. Besides metabolites from the riboflavin pathway, synthetic small molecules can also bind the MR1 groove and react with the MAIT TCR. These chemical agonists, such as diclofenac, present a 100 000 fold lower ability to activate MAIT cells than 5-OP-RU (Keller et al. 2017).

The discovery of the potent activators of MAIT cells enabled the construction of antigen-loaded MR1 tetramers. The resulting 5-OP-RU:MR1 tetramer (available at the NIH tetramer facility) allowed, in both humans and mice, the identification and study of MAIT cells replacing less specific approaches (Rahimpour et al. 2015; Koay et al. 2016).

2.1.5. Interaction between the MR1-antigen complex and MAIT TCR

In contrast to conventional αβ T cells, MAIT cells express semi-invariant T cell receptors (TCR) made of a non-variable TCR-α chain paired with a β-chain of limited diversity. In humans (Xiao et al. 2019), the TCR-α chain of MAIT cells is made of the evolutionarily conserved TRAV1 (Vα7.2) and TRAJ33/12/20 (Jα33/12/20) genes, preferentially joined to Vβ2 or Vβ13 (TRBV20 or TRBV6). In mice (Tilloy et al. 1999), MAIT cells express an orthologous TCR-α chain consisting of TRAV1 and TRAJ33, which pairs with TRBV13 and TRBV19 TCR-β chains.

The MAIT TCR-α and -β chains specially the CDR1α, CDR2α and CDR2β loops straddle the α2 and α1 helices of MR1, placing the invariant CDR3α at the apex of the A′ antigen-binding pocket of MR1. This allows CDR3α to interact directly with the proximal hydroxyl of the ribityl tail that is common to the riboflavin-derivative antigens.

The interaction is mediated by the evolutionarily conserved CDR3α-encoded Tyrosine at position 95 (Tyr95α) (Fig. 7A and Fig. 8A-B) (Corbett et al. 2014; Eckle et al. 2014; 2015). Despite the varied TRAJ and TRBV usage in the MAIT TCR repertoire, Tyr95α is conserved making conserved interactions with MAIT antigens (Eckle et al. 2014).

Interestingly, a human TCRγδ T cell subset was found recently to exhibit MR1 autoreactivity and to sense with moderate affinity any MR1 ligand able to induce surface MR1 expression. However, these cells adopt distinct and unusual binding modes to the a3 domain of MR1 (Le Nours et al. 2019) (Fig. 7B).

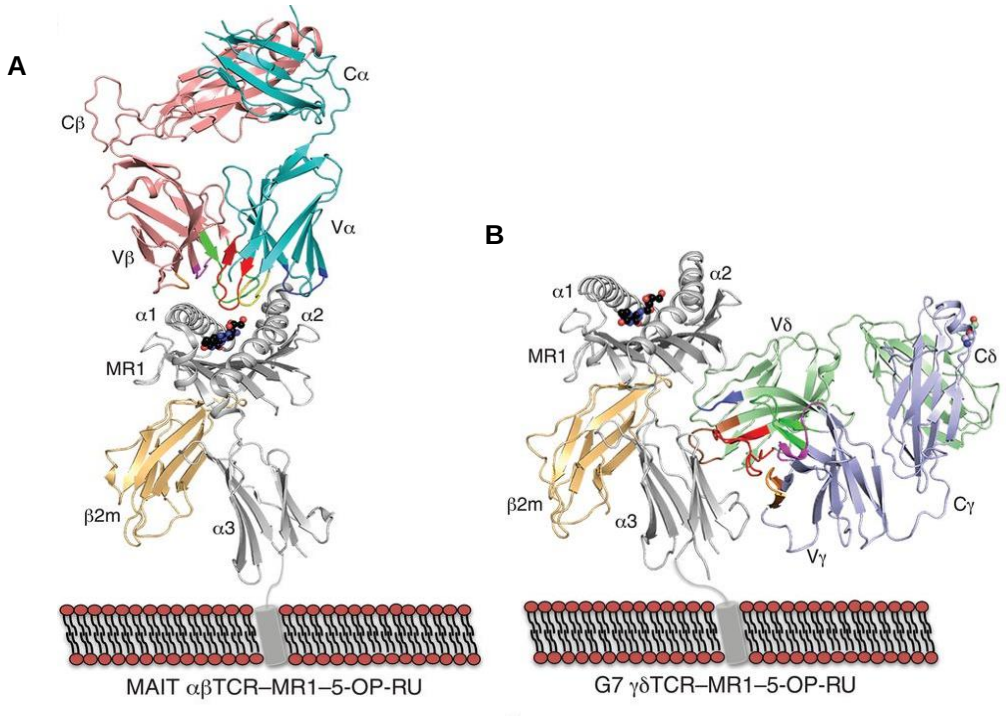


Figure 7: TCR recognition of MR1–5-OP-RU (Le Nours et al. 2019). (A) MAIT αβTCR and (B) γδTCR footprint on surface MR1.

2.2. MR1 antigens that act as competitive inhibitors of MAIT cell activation

The discovery of MAIT non-stimulatory metabolites was based on interesting observation of MR1 refolded in the presence of cell culture medium RPMI 1640 (Kjer-Nielsen et al. 2012). This observation suggested that RPMI medium contained a ligand that could bind to MR1 in the absence of bacterial culture.

MR1 folded in the presence of RPMI medium analyzed by mass spectrometry revealed the 6-Formylpterin (6-FP) (Kjer-Nielsen et al. 2012). The 6-FP is a photo-degradation product derived in ultraviolet light from the folates (vitamin B9) present in the culture medium. Analogs of 6-FP have been synthesized such as acetyl-6-FP and other derivatives of 6-FP (Eckle et al. 2015; Soudais et al. 2015). These compounds can form a covalent bond with MR1, specifically a Schiff-base bond between the formyl group and the residue K43 of MR1 (Kjer-Nielsen et al. 2012; Eckle et al. 2014; 2015). Although these compounds up-regulate MR1 cell surface expression (Eckle et al. 2014; Soudais et al. 2015), they fail to promote MAIT TCR recognition and activation (Eckle et al. 2014; 2015). Given that the ribityl chain is crucial for MAIT activation through direct TCR contacts (Awad et al. 2020), the ribityl-less 6-FP forms a single hydrogen bond with the TCR Tyr95α and fails to stimulate MAIT cells (Eckle et al. 2015) (Fig. 8C). 6-FP and their analogs are competitive antagonists of MAIT cells (Eckle et al. 2014; Soudais et al. 2015) and they can be used to modulate pharmacologically the MAIT cell activation.

Interestingly, folate is mainly provided in the diet and also can be made by fungi and some prokaryotes in the body. In fact, vitamin B9 biosynthesis pathway is present in nearly all *Bacteroidetes* genomes as well as in most *Fusobacteria* and *Proteobacteria* and in few *Firmicutes* and *Actinobacteria* (Revuelta et al. 2018; Magnúsdóttir et al. 2015). However, whether folate-based metabolites are present in biological fluids remains unknown.

Finally, all structures to date have shown that the antigens fill only the A' pocket of recombinant MR1 which contains K43 residue for Schiff base formation. Thus, the F' pocket remains empty suggesting that bigger molecules might occupy both A' and F′ pockets. However, whether these ligands exist, and whether they would be of bacterial or other origins are unknown.

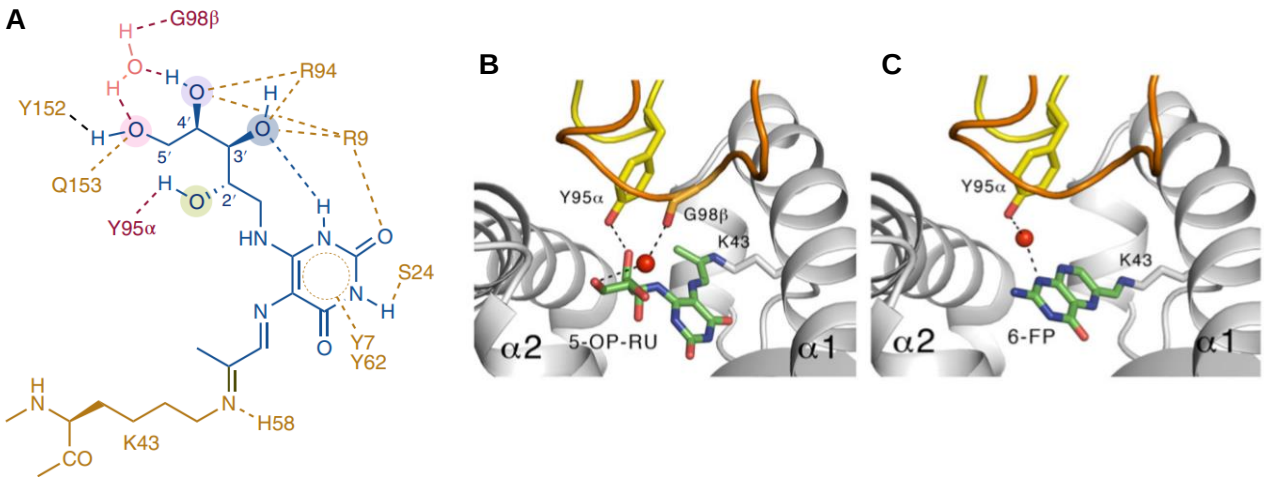


Figure 8: MAIT TCR recognition of MR1-binding ligands derived from riboflavin. Two-dimensional diagram interaction summary of the crystal structure of the MAIT TCR–MR1–5-OP-RU complex. MR1 and TCR residues and their hydrogen bonds are colored in orange and maroon, respectively (Awad et al. 2020). Structural characterization of MAIT TCR recognition of MR1-binding (B) 5-OP-RU, (C) 6-FP (Eckle et al. 2015).

3. Thymic development of MAIT cells

MAIT cells arise in the thymus from a $CD4^+CD8^+$ double-positive (DP) precursor (Martin et al. 2009; Koay et al. 2016). $TRAV1^+$ immature thymocytes undergo positive selection by MR1 expressed at the surface of either DP thymocytes (Seach et al. 2013) or thymic epithelial cells (TECs) (Legoux, Gilet, et al. 2019) (Fig. 9).

Positive selection on hematopoietic cells (Fig. 9A) results in the engagement of SLAM molecules and the activation of the SAP signalling pathway (Legoux, Gilet, et al. 2019), which drives expression of the master transcription factor promyelocytic leukemia zinc finger PLZF (also known as zinc finger and BTB domain-containing protein 16, encoded by Zbtb16). PLZF in turn suppresses the naive T cell program and controls the “preset” phenotype of MAIT cells. As for iNKT cells (Koay et al. 2016; Savage et al. 2008), PLZF induces in MAIT cells the acquisition of effector programs associated with the transcription factors Tbet or RORγt which define the iNKT1 and MAIT1 and iNKT17 and MAIT17 effector subsets, respectively (Legoux, Gilet, et al. 2019; Salou et al. 2019).

Those cells patrol the non-lymphoid tissues for immediate immunity, contrary to mainstream T cells. Although, the development of MAIT cells strongly resembles that of iNKT cells, mature mouse MAIT cells do not display a phenotype of NKT2 cells (Rahimpour et al. 2015; Salou et al. 2019). This could result from weaker TCR signals during MAIT cells positive selection, possibly in relation with lower amounts of MR1 as compared to CD1d.

Positive selection on TECs (Fig. 9B) does not engage the SLAM-SAP pathway, and results in the differentiation of 5-OP-RU-specific T cells into naïve conventional-like $CD4^+$ T cells that remain in secondary lymphoid organs (Legoux, Gilet, et al. 2019).

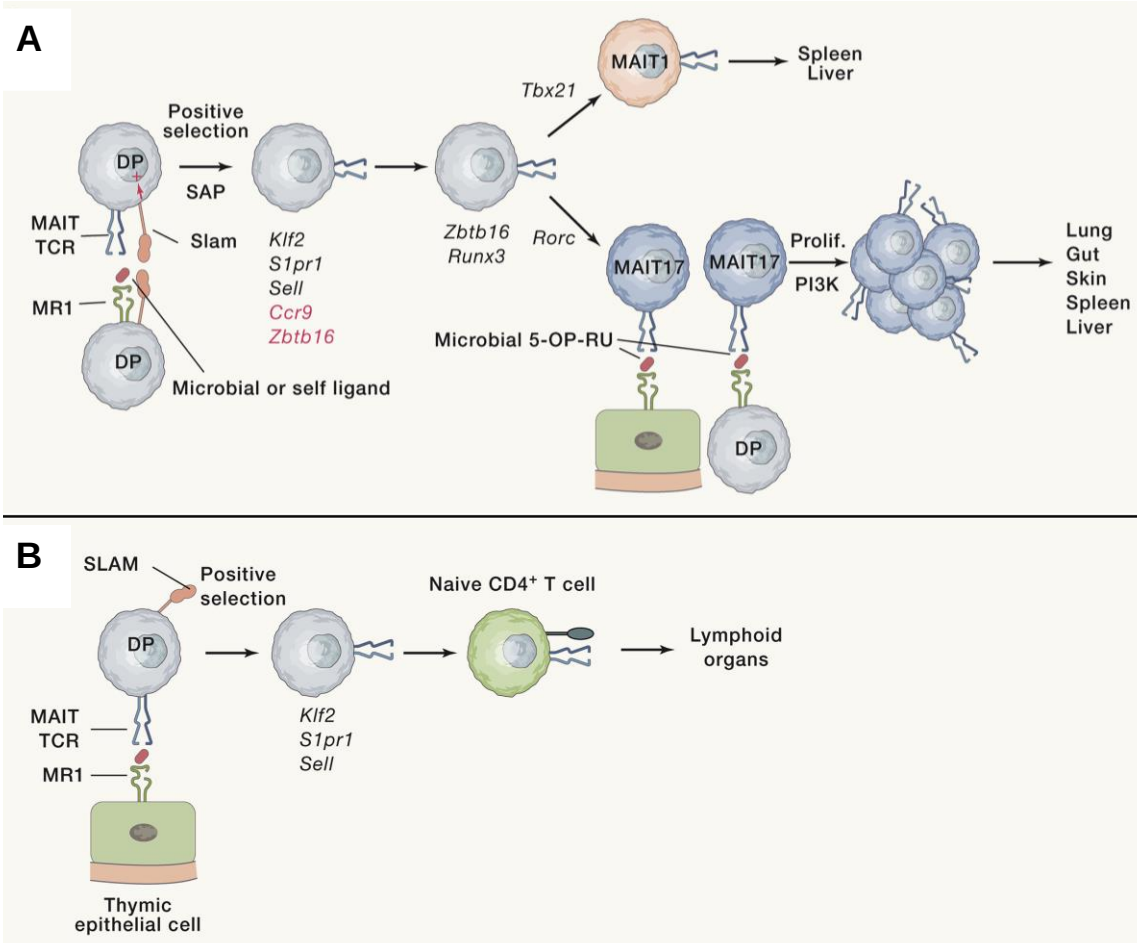


Figure 9: Intra-thymic development of MAIT cells (Legoux, Salou, and Lantz 2020). During thymic development, both (A) TECs and (B) DP thymocytes can mediate positive selection of 5-OP-RU-specific thymocytes, but with distinct outcomes.

Following positive selection on hematopoietic cells (Seach et al. 2013), MAIT cells develop in the thymus through a three-stage process (Koay et al. 2016). In mice, the stage 1 immature MAIT cells downregulates CD24 and develops into stage 2 $CD24^{-}$ $CD44^{-}$ MAIT cells. PLZF expression begins at stage 2 and is highest at stage 3 characterized by the high expression of CD44. The third mature stage differentiates subsequently into Tbet- or RORγt-expressing cell subsets (Salou et al. 2019; Legoux, Gilet, et al. 2019). The mechanisms controlling MAIT1 versus MAIT17 lineage choice remain unknown.

Ontogeny studies of human MAIT cells have identified three similar stages defined as $CD27^{-}$ $CD161^{-}$, $CD27^{+}$ $CD161^{-}$, and $CD27^{+}$ $CD161^{+}$ cells respectively (Koay et al. 2016). Interestingly, analysis of SAP-deficient patients revealed normal frequency of blood MAIT cells expressing PLZF (Martin et al. 2009) suggesting the contribution of additional mechanisms in human MAIT cell development. Furthermore, in humans, stage 3 MAIT cells exit the thymus as $Tbet^{+}$ $ROR\gamma t^{+}$ cells (Leeansyah et al. 2015) whereas mouse MAIT cells express either Tbet or RORγt (Rahimpour et al. 2015).

4. Role of bacterial metabolites in the ontogeny of MAIT cells

In the thymus of germ-free (GF) mice, both immature $CD24^{+}$ and mature $CD44^{+}$ MAIT cells are reduced (Koay et al. 2016; Legoux, Bellet, et al. 2019). As MAIT cells do not recirculate from the periphery to the thymus (Legoux, Bellet, et al. 2019), MAIT intrathymic development is likely directly controlled by the microbiota. Experiments with GF mice monocolonized with either ΔribD or ΔribE *E*. *coli*, showed that 5-A-RU production by bacteria is essential for positive selection and complete thymic maturation and proliferation of MAIT17 cells (Legoux, Bellet, et al. 2019) (Fig. 9A). These findings indicate that microbial riboflavin derivatives travel from the gut and reach mouse thymus with enough concentration to select MAIT cells. In agreement, results showed that 5-OP-RU applied onto intact skin or delivered by oral gavage is detected shortly after in the thymus (Legoux, Bellet, et al. 2019). This is due to the nature of 5-OP-RU which is a metabolite and not lipid or peptide antigen.

In the thymus, numbers of both immature and mature MAIT cells increase and reach normal levels 2 weeks after colonization of GF mice with a complex microbial flora (Legoux, Bellet, et al. 2019). However, these newly generated mature MAIT cells do not populate peripheral tissue when the bacterial colonization occurs in adults (Legoux, Bellet, et al. 2019; Constantinides et al. 2019).

By contrast, early life 5-OP-RU administration restores MAIT cell development and enables their skin homing (Constantinides et al. 2019).

Importantly, the absence of the microbiota and the colonization of GF mice with 5-OP-RU-producing *E. coli* had no effect on the number of MAIT1 cells, which was explained by a possible inhibition of the TCR signalling in this subset (Legoux, Bellet, et al. 2019). A small number of mature and functional MAIT17 cells develop in GF mice, indicating that positive selection still occurs in these mice (Legoux, Bellet, et al. 2019). It was suggested that MR1, which is required for each stage of MAIT development (Legoux, Gilet, et al. 2019), binds unidentified self-ligands to govern the positive selection of MAIT cells.

In humans, Vα7.2+ CD161+ T cells are found in fetal thymus (Leeansyah et al. 2014). It is unclear whether they are selected on DP thymocytes expressing endogenous self-antigen(s) presented by MR1, or whether riboflavin metabolites produced by the microbiota of the mother influence MAIT cell development in the fetal thymus. In fact, as shown for other microbial metabolites (Agüero et al. 2016), riboflavin metabolites produced by the microbiota of the mother might be able to reach the thymus.

In contrast to mouse MAIT cells that become antigen-experienced memory cells in the thymus, human mature MAIT cells exit the thymus as “naïve” cells and acquire a memory phenotype and expand in the periphery (Ben Youssef et al. 2018). This suggests that a final maturation occurs in the periphery likely derived by the commensal microbiota. Due to the human body size, it was suggested that the concentration of microbial metabolites reaching the thymus is not sufficient to affect MAIT cells in the thymus (Legoux, Salou, and Lantz 2020).

5. Tissue tropism and residency properties of MAIT cells

MAIT cells are far more abundant in humans compared with common laboratory mouse strains and the percentages are significantly variable within individuals and between organs (Franciszkiewicz et al. 2016). Constantinides *et al*. reported variability in the proportion of lung and skin MAIT cells between genetically identical mice housed in distinct cages. Thereby, they suggested a role of the microbiota in determining MAIT cell numbers at steady state (Constantinides et al. 2019).

In mice, each functional program associated with RORγt versus Tbet expression seems to correlate with a specific tissue location. MAIT1 are preferentially positioned in the spleen, lymph nodes, and liver, while MAIT17 cells are present in mucosal tissues such as skin (Constantinides et al. 2019) and lung (Rahimpour et al. 2015; Salou et al. 2019) (Fig. 9). In the spleen, liver and lung, MAIT cells are resident. They exhibit a tissue residency transcriptomic signature and do not exchange between 5-week-long parabiotic pairs, except for MAIT1 in the lungs being mostly intravascular (Salou et al. 2019). The tissue residency signature of both MAIT1 and MAIT17 is expressed in the thymus suggesting that the tissue tropism is acquired during thymic development (Salou et al. 2019).

In C57BL/6J mice, MAIT cells represent 0.08% in the spleen, 0.6% in the liver, 0.7% in the lamina propria and 3.3 % of T cells in the lung (Rahimpour et al. 2015). However, in Castaneus inbred SPF mouse strain, MAIT cells are 20 times more abundant compared to C57BL/6J strain (Cui et al. 2015). This increase in MAIT cell frequency results from one CAST genetic factor mapped to the TCRα locus leading to higher frequency of Vα19 transcripts in the thymus and thus an increased generation of the iVα19-Jα33 rearrangements. Accordingly, B6-MAIT^{CAST} congenic mouse strain, bearing the CAST locus associated with high numbers of MAIT cells on a B6 background, was generated by backcrossing the MAIT^{CAST} trait to C57BL/6J mice for more than 10 generations (Cui et al. 2015). This model facilitates phenotype analysis and functional studies of MAIT cells in mice.

In humans, as in mice, thymic MAIT cells exhibit tissue homing properties given that they express already in the thymus tissue-homing molecules such as CCR5, CCR6, and CXCR6 (Legoux, Salou, and Lantz 2020). In human thymus and in cord blood, the frequency of MAIT cells is very low (0.07% of T cells). MAIT cell frequency increases in the blood during the first 5-6 years of life, to reach 1-8% in adults (Ben Youssef et al. 2018). Accordingly in adults, human MAIT cells comprise 2%– 4% of T cells in the lung, 20%–50% in the liver, and up to 60% of CD4^{-} T cells in the jejunum (Dusseaux et al. 2011; Ayako Kurioka et al. 2016). In addition, MAIT cells from human liver (Salou et al. 2019) and buccal mucosa (Sobkowiak et al. 2019) display tissue resident property compared with the blood (Salou et al. 2019).

However, how resident MAIT cells respond to tissue microenvironment, and how they are affected in different disease conditions are unclear.

6. MAIT cell response to stimulation

In humans and mice, MAIT cells may be activated either through their TCR, which recognizes a ligand presented on MR1, or by inflammatory cytokines (Franciszkiewicz et al. 2016). MAIT cells are likely activated through TCR triggering during bacterial infection, given that most bacteria can make 5-A-RU (Mondot, Boudinot, and Lantz 2016). However, TCR-independent MAIT cell activation occurs during viral and non-riboflavin synthesizing bacterial infections and any pro-inflammatory conditions (van Wilgenburg et al. 2016; 2018; Paquin-Proulx et al. 2018; Loh et al. 2016). Once activated MAIT cells secrete various cytokines and chemokines and alter their cytotoxic granule content and transcription factor expression. However, MAIT cell effector functions vary according to the context of stimulation (Hinks et al. 2019; Lamichhane et al. 2019; Leng et al. 2019). MAIT cell responses following various activation protocols, at the RNA and/or the protein levels, are developed below.

6.1. TCR-independent activation

Resting human and mouse MAIT cells harbor several interleukin-receptors such as IL-18Rα, IL-12Rβ, IL-23R and IL-7Rα and can be activated by corresponding cytokines independent of their TCR (Rahimpour et al. 2015; Dusseaux et al. 2011). Given that IL-15 may trigger IL-18 production, MAIT cells can also be activated by IL-15 (van Wilgenburg et al. 2016). Moreover, type I interferons (IFN-α or -β) and the gut-associated pro-inflammatory cytokine TNF-like protein 1A (TL1A), were found to potentiate cytokine-mediated MAIT cell activation (van Wilgenburg et al. 2016; Leng et al. 2019). The TCR-independent activation of human MAIT cells induces a modest cytokine response dominated by IFN-γ production which is consistent with the upregulation of Tbet in blood MAIT cells (Jo et al. 2014; Lamichhane et al. 2019; Leng et al. 2019). In addition, the cytokine activation induces the production of perforin, granzyme and the expression of chemokines involved in lymphocyte trafficking to the site of infection particularly CCL3, CCL4, CXCL10, CXCL9 and CXCL11 (Hinks et al. 2019; Lamichhane et al. 2019; Leng et al. 2019) (Fig. 10A).Therefore, the TCR-independent MAIT cell activation is an important mechanism potentially involved in response to viral and bacterial infections.

6.2. TCR mediated activation

In human, in contrast to cytokine stimulation, MAIT TCR triggering results in a rapid, polyfunctional and proinflammatory response (Hinks et al. 2019; Lamichhane et al. 2019; Leng et al. 2019) (Fig. 10B). Following TCR activation, blood MAIT cells robustly and rapidly upregulate RORγt and Tbet and the transcription of multiple inflammatory cytokines including IL-17A, IL-22, TNF-α, IFN-γ, IL-1B, IL-6, IL-21, and GMCSF (Hinks et al. 2019; Lamichhane et al. 2019; Leng et al. 2019). At the protein level, TCR stimuli result in production of TNF-α and to a lesser extent IFN-γ by MAIT cells from the blood and the gut (Lamichhane et al. 2019; Leng et al. 2019). Although MAIT cell phenotype strongly suggests their potential to secrete IL-17, TCR stimulation does not result in IL-17 secretion. However, in response to phorbol 12-myristate 13-acetate (PMA) and ionomycin stimulation, peripheral blood MAIT cells produce IL-17 in addition to TNF-α, IFN-γ and IL-22 (Billerbeck et al. 2010; Dusseaux et al. 2011). This suggests that MAIT TCR stimulation alone is insufficient to fully activate MAIT cells and requires co-stimulation. Co-stimulation may include TLR agonists (Z. Chen et al. 2017), which promote MAIT cell activation mainly in an indirect way through the activation of antigen presenting cells (Hinks and Zhang 2020). Moreover, CD28 (Turtle et al. 2011) and cytokines (Z. Chen et al. 2017; Tang et al. 2013; Leng et al. 2019) have been shown to maximize MAIT cell activation and to make MAIT cells potent early responders. Indeed, costimulation with IL12, IL-18 and IL-15 markedly increases TNF-α and IFN-γ secretion by MAIT cells from the blood and the gut (Leng et al. 2019). Likewise, increased IFN-γ and IL-17 secretion by blood and liver human MAIT cells was seen following stimulation through TCR and IL-7, as well as IL-1β and IL-23 (Tang et al. 2013).

In mice, in accordance with the abundance of MAIT17 subset, TCR stimulation of MAIT cells from the spleen using anti-CD3/CD28-coated bead, leads to the production of large amounts of IL-17, lower levels of TNF-α and GM-CSF, but almost no IFN-γ. Notably, murine MAIT cells from the spleen also secrete IL-4 and IL-10 (Cui et al. 2015).

Interestingly, in both human and mouse, TCR stimulation either with or without cytokines results in MAIT cell expression of genes linked with the tissue-repair program including IL-22, AREG, VEGF-A, and TGFα and various growth factors. This provides evidence that TCR-dependent activation could allow MAIT cells to exhibit tissue repair functions (Hinks et al. 2019; Lamichhane et al. 2019; Leng et al. 2019; Salou and Lantz 2019).

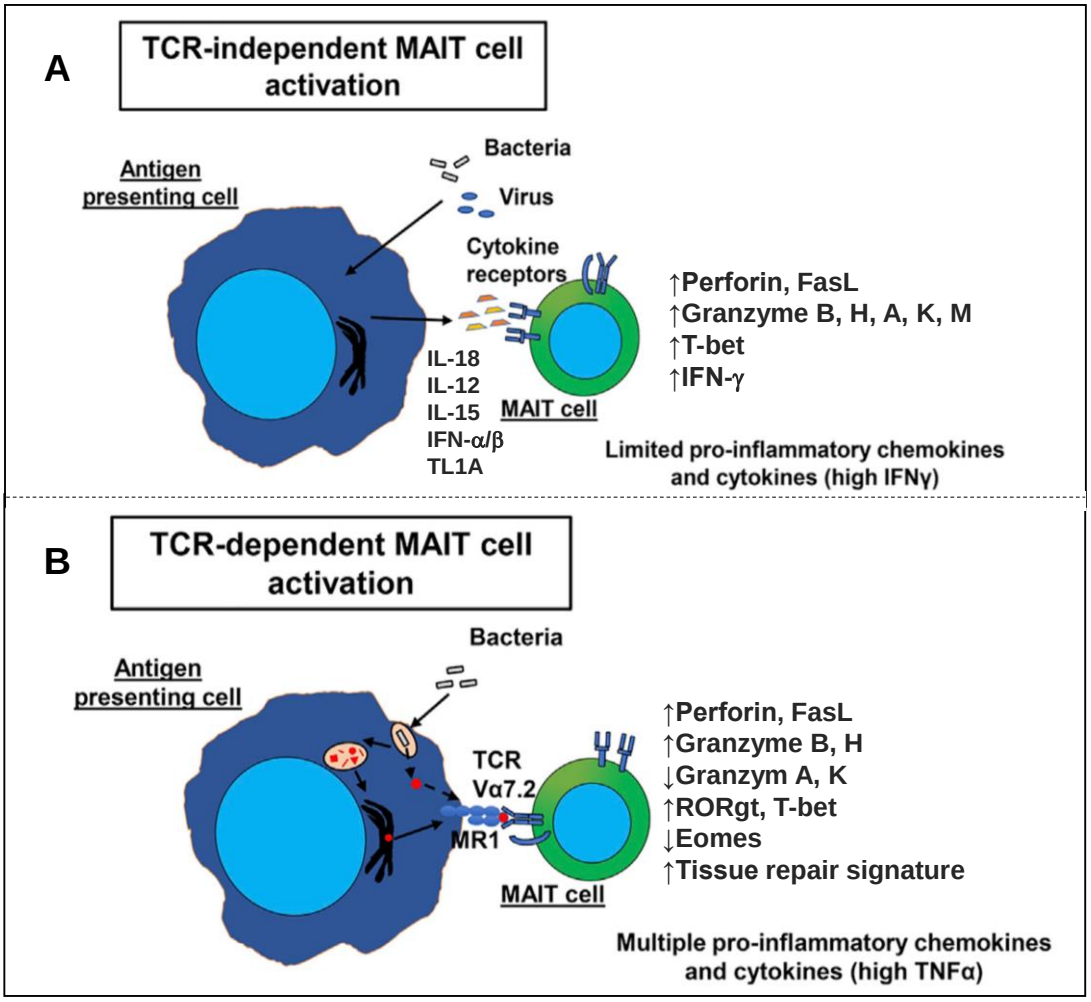


Figure 10: Responses of human MAIT cells to TCR and cytokine stimulation (Figure adapted from (Lamichhane et al. 2019)). (A) TCR-independent activation results in a slow and modest cytokine response dominated by IFN-γ under the control of Tbet. (B) TCR-dependent activation induces a rapid and stronger response. TCR stimuli produces broad pro-inflammatory cytokines and chemokines dominated by TNF-α under the control of Rorγt and Tbet. TCR and not cytokine triggered MAIT cells results in the expression of tissue repair signature.

7. MAIT cell function

7.1. MAIT cells in infectious diseases

7.1.1. Bacterial infections

Given their antigen specificity, their ability to be directly activated by certain cytokines and their rapid polyfunctional response, MAIT cells represent the most abundant T cell subset reacting against bacteria in humans. MAIT cell activation and functions have been addressed in various bacterial infectious diseases, both in humans and mice (reviewed in Salou, Franciszkiewicz, and Lantz 2017).

In various riboflavin-producing bacterially infected patients, the frequency of MAIT cells in the blood is decreased and seems to accumulate at the inflammatory sites (Salou, Franciszkiewicz, and Lantz 2017). The hypothesis of migration toward infected sites is supported by the decreased number of MAIT cells and the alteration in their proliferation and homing characteristics in the blood after *Salmonella typhimurium* infection (Salerno-Goncalves et al. 2017) or in response to *Shigella* vaccine (Le Bourhis et al. 2013). Similarly, decreased blood MAIT cells has been observed in patients with *Mycobacterium tuberculosis* infection (Harriff et al. 2014; Jiang et al. 2014; 2016; Saeidi et al. 2015; Yang et al. 2015), *Pseudomonas aeruginosa* pulmonary infection (D. J. Smith et al. 2014), children with *Vibrio cholerae* infection (Leung et al. 2014) as well as septic patients with severe bacterial infections (Grimaldi et al. 2014). Interestingly, septic patients who exhibit higher frequencies of circulating MAIT cells have better health outcomes.

Functional in vitro studies highlighted the ability of MAIT cells to kill APCs infected with *E. coli* (Le Bourhis et al. 2013; A. Kurioka et al. 2015; Lamichhane et al. 2019), *Shigella flexneri* (Le Bourhis et al. 2013), *M. tuberculosis* (Gold et al. 2010; Le Bourhis et al. 2010), *P. aeruginosa* (Jo et al. 2014; Tang et al. 2013) in a TCR- and cytokine dependent manner through the release of cytokines and cytotoxic effector molecules. Moreover, human MAIT cells were shown to react against *Enterococcus faecalis*, a bacteria that does not make 5-A-RU. In this case, IL-12 and IL-18 secretion by infected cells is only involved (Jo et al. 2014; Ussher et al. 2014).

The most convincing data supporting the antibacterial function of MAIT cells have been obtained in experimental infection mouse models. Briefly, mice deficient for MR1 and thus devoid of mature MAIT cells show defective or delayed bacterial clearance. For example, *Mr1*$^{-/-}$ mice are more susceptible to *E. coli* (Georgel et al. 2011), *Mycobacterium abscessus* (Le Bourhis et al. 2010), *Legionella longbeachae* (H. Wang et al. 2018) and *Francisella tularensis* (A. Meierovics, Yankelevich, and Cowley 2013; A. I. Meierovics and Cowley 2016) as compared to their MR1^{+} counterparts. Depending on the type of infection, MAIT cells produce large amounts of cytokines. Interestingly, during legionella infection, lung MAIT cells acquire a particular differentiation program. They express the tissue repair signature (Hinks et al. 2019) and both RORγt and Tbet, contrasting to the profile observed at steady state (RORγt^{+} Tbet^{-}).

Altogether, these findings highlight the protective role of MAIT cells against various pathogenic bacteria in humans and mice.

7.1.2. Viral infections

MAIT cells function as innate sensors of viral infection via cytokines activation. In the blood of patients infected with influenza, dengue, hepatitis C viruses (HCV), MAIT cells are selectively decreased compared to healthy controls. Residual MAIT cells are activated with high expression of CD38, CD69 and PD-1 (van Wilgenburg et al. 2016; Bolte et al. 2017; Beudeker et al. 2018; Loh et al. 2016; Hengst et al. 2016). Studies showed an inverse correlation between MAIT cell frequencies and severity of disease in patients infected with influenza or HCV (van Wilgenburg et al. 2016; Loh et al. 2016). Likewise, circulating MAIT cells are decreased in patients with human T lymphotropic virus type 1 infection (Paquin-Proulx et al. 2017) and human immunodeficiency virus (HIV) infection (Beudeker et al. 2018; Cosgrove et al. 2013; Fernandez et al. 2015; Leeansyah et al. 2013). Interestingly, two studies reported a marked reduction of MAIT cells in the blood of patients with SARS-CoV-2 infection (Parrot et al. 2020; Flament et al. 2021). Parrot et al., observed an enrichment of MAIT cells among T cells infiltrating the airways in patients with COVID19 patients. Flament et al., showed a strong activated and cytotoxic phenotype of MAIT cells in the blood and pulmonary fluids in all patients with COVID-19, particularly in patients with fatal outcomes. Therefore, it was suggested a possible involvement of MAIT cells in COVID-19 immunopathogenesis.

Murine experimental studies support the role of MAIT cells in viral infections. In mice infected with influenza virus, MAIT cells are activated with upregulation of CD25, CD69 and Granzyme B and accumulate in the lungs. *Mr1*$^{-/-}$ mice are more susceptible to the virus as compared to wild type mice, suggesting a protective role of MAIT cells against influenza virus. (van Wilgenburg et al. 2018). Because viruses do not produce 5-A-RU, MAIT cell activation during viral infections depends on IL-18 in synergy with IL-12, IL-15, and IFN-α/β. When co-cultured with influenza-infected cells, MAIT cells produce IFN-γ, TNF and granzyme B that likely inhibit viral replication (Loh et al. 2016; van Wilgenburg et al. 2016).

7.2. MAIT cells in non-infectious diseases

7.2.1. Wound

A role for MAIT cells in cutaneous wound healing has been observed in the absence of compensatory responses by γδT cells. Indeed, skin wounds heal faster in MAIT cell-sufficient *Tcrd*$^{-/-}$ mice as compared to MAIT cell-deficient *Mr1*$^{-/-}$ *Tcrd*$^{-/-}$ mice (Constantinides et al. 2019). Importantly, topical application of 5-OP-RU MAIT antigen to the skin of WT mice before wounding promotes wound healing (Constantinides et al. 2019). These findings are corroborated by the results of an *in vitro* wound-healing assay combining the intestinal epithelial cell line Caco2 with supernatants from human TCR-activated MAIT cells. Interestingly, MR1 blockade abolished the accelerating effect that these supernatants had on the closure of a scratch on Caco2 monolayer (Leng et al. 2019). These findings confirm the TCR-dependent tissue repair potential of MAIT cells. However, when and under which circumstances MAIT TCR signalling is triggered *in vivo* is unclear.

7.2.2. Obesity and type 2 diabetes

Similar to several infectious diseases, in obese and/or type 2 diabetes (T2D) patients, MAIT cells dramatically decline in the blood as well as in adipose tissue (Magalhaes et al. 2015; Carolan et al. 2015). In both obese and T2D patients, MAIT cells more frequently produce IL-17 in the blood and IL-10 and IL-17 in adipose tissue (Magalhaes et al. 2015; Carolan et al. 2015). Given that IL-17 is known to induce insulin resistance in adipocytes (Zúñiga et al. 2010), IL-17-producing MAIT cells were suggested to contribute to obesity-related morbidities. Accordingly, bariatric surgery in obese patients increase circulating MAIT cell frequency, which is associated with amelioration in patient's metabolic and inflammatory status (Magalhaes et al. 2015).

These findings in humans are in line with more recent results in obese mice (Toubal et al. 2020). Indeed, MAIT cells are decreased in the blood, adipose tissue and ileum of obese mice, associated with increased apoptosis in MAIT cells. Moreover, MAIT cells display an activated phenotype that is associated with elevated pro-inflammatory cytokines in adipose tissue and ileum, lipid and glucose metabolism deregulation and increased gut leakiness in obese mice (Toubal et al. 2020).

7.2.3. Autoimmune diseases

As discussed above, MAIT cell characteristics including responsiveness to inflammatory cytokines, expression of chemokine receptors and their localization at mucosal surface imply that they could be involved in immune- mediated diseases. Numerous reports, mostly in human samples and sometimes in mouse models, have described alterations in the frequency, location, phenotype and/or functions of MAIT cells within various autoimmune pathologies (Fig. 11).

Multiple sclerosis

Three studies reported decreased frequency of MAIT cells in the blood of multiple sclerosis (MS) patients correlating with the clinical activity of the disease. Salou *et al* showed decreased frequency of blood MAIT cells in the progressive form of the disease as compared to relapsing ones and to healthy controls (Salou et al. 2016). Miyazaki *et al*. observed a dramatic reduction in the frequency of blood MAIT cells in MS patients with active disease, which increases in parallel with clinical recovery (Miyazaki et al. 2011). Finally, Willing *et al* reported a reduction of MAIT cells in the blood of MS patients compared with healthy donors, which significantly correlated with IL-18 serum levels in MS patients (Willing et al. 2014).

In addition to CCR5 expressed at steady-state, blood MAIT cells from MS patients upregulate the expression of homing molecules involved in migration across the blood–brain barrier such as VLA-4 (Willing et al. 2014), and the homing receptors P- selectin glycoprotein ligand 1 and αL integrin (Salou et al. 2016). Consistent with these results, MAIT cells are found by immunohistochemistry within the inflamed central nervous system (CNS) lesions from MS patients, while they are almost absent in the cerebrospinal fluid (Willing et al. 2014; Salou et al. 2016). Over-expression of MR1 transcripts as well as IL-18 and IL-23 in MS lesions, suggests that infiltrating MAIT cells could be activated in situ in CNS lesions of MS patients (Salou et al. 2016). However, the role of MAIT cells in the inflamed brain remains to be addressed.

In experimental autoimmune encephalomyelitis (EAE), the mouse model of MS, Vα19$^+$ cells seem to protect mice from clinical EAE. In fact, adoptive transfer of invariant Vα19 transgenic cells into C57BL/6 mice with EAE reduces the disease severity. In addition, similar regulatory effect was observed in Va19 transgenic mice compared to MR1-/- mice. The suppression of EAE was associated with reduced expression of proinflammatory cytokines (IFN-γ, TNF, IL-2 and IL-17) and increased production of IL-10 in the draining lymph nodes and spleen (Croxford et al. 2006).

Type 1 diabetes

MAIT cell are decreased and altered in the blood of children with recent-onset type 1 diabetes (T1D) as compared to age-matched control children (Rouxel et al. 2017). Indeed, blood MAIT expression of CD25 and PD-1 and production of TNF-α, IL-4 and granzyme B are increased in children with recent onset T1D (Rouxel et al. 2017).

The non-obese diabetic (NOD) mouse *Mr1*$^{-/-}$ mice allowed to directly investigate the role of MAIT cells in T1D. Importantly, *Mr1*$^{-/-}$ NOD mice have accelerated diabetes and increased gut permeability to FITC-Dextran after gavage (Rouxel et al. 2017). Even though MAIT cells infiltrate in the pancreas and could contribute to islet destruction in diabetic stage (Rouxel et al. 2017), the exacerbation of diabetes development in *Mr1*$^{-/-}$ NOD mice suggested a protective role of MAIT cells against diabetes. Accordingly, MAIT cells were found enriched in the ileum at the prediabetic stage associated with increased level of IL-17 and IL-22 mRNA in MAIT cells, suggesting a capacity for regulating intestinal epithelium tissue integrity (Rouxel et al. 2017). Of note, a protective role of IL-17 and IL-22 has been established in several intestinal inflammation models as discussed below.

Further work is needed to determine whether impaired functions of MAIT cells triggers loss of gut integrity, which would increase MAIT cell activation in the pancreas probably due to the translocation of bacterial compounds from the gut to pancreatic lymph nodes.

7.2.4. Inflammatory bowel diseases

Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are chronic inflammatory conditions of the gastrointestinal (GI) tract. In contrast to CD, which can affect any part of the GI tract, UC is characterized by inflammation that is usually limited to the colon and/or the rectum.

The exact etiology of IBD has not yet been fully understood. The prevailing hypothesis is that IBD is a result of inappropriate immune response to antigens from the intestinal lumen in genetically susceptible individuals. In fact, IBD is associated with a breach in the intestinal barrier (Michielan and D'Incà 2015), which results in the development of innate and adaptive immune responses targeting commensal antigens (Shen et al. 2008). Furthermore, it is the dysregulated intestinal immune responses to these organisms that result in chronic intestinal inflammation. Indeed, IBD patients exhibit reduced numbers of Treg cells in the blood and gut mucosa and increased numbers of peripheral Th17 cells (Li, Sun, and Zhao 2006; Boden and Snapper 2008; Eastaff-Leung et al. 2010). Given that Treg cells are extremely important for intestinal homeostasis through mainly the release of IL-10 and TGF-β, dysfunction of Treg cells may play a crucial role in the development of IBD lesions (Ishimaru et al. 2008). Antigen presenting cells and effector T cells secrete cytokines that have a complex role in the development of IBD. Elevated levels of TNF-α, IL-12, IL-18, IL-23, IL-1 and IL-6 are detected in IBD and were identified to have important role in the pathogenesis of the disease (Neurath 2014). Accordingly, targeting IL-12, IL-23 or TNF-α alleviates symptoms in IBD patients (Sandborn et al. 2012; Sands et al. 2017; Lichtenstein 2013). Other cytokines such as IL-17 and IL-22 are also increased in IBD (Fujino et al. 2003; Kobayashi et al. 2008; Andoh et al. 2005; Brand et al. 2006).

While IL-17 is mostly a pro-inflammatory cytokine that provides a protective inflammatory response towards pathogens, it has been shown to be protective in several models of intestinal inflammation (Eyerich, Dimartino, and Cavani 2017). Clinical trials designed to block IL-17 signalling using anti-IL-17A or anti-IL-17RA antibodies actually worsened inflammation in patients with IBD (Hueber et al. 2012). These results were in line with the effect of IL-17 blockade in mouse models of colitis (O'Connor et al. 2009; Ogawa et al. 2004; Song et al. 2015; Maxwell et al. 2015). Subsequent studies revealed that IL-17 preserves the intestinal epithelial

barrier through the increase expression of claudin 1 and claudin 2, that are involved in the formation of tight junctions between T84 cell line (Kinugasa et al. 2000), and by enhancing the epithelial release of antimicrobial peptides (AMPs) (Maxwell et al. 2015) during epithelial injury. Moreover, IL-17 cooperates with the growth factor FGF2 to induce genes in mouse primary intestinal epithelial cells to repair damaged intestinal epithelium (Song et al. 2015).

Likewise, the function of IL-22 is difficult to generalize and continue to be under further investigation (Sonnenberg, Fouser, and Artis 2011). Some studies have reported that IL-22 exerts proinflammatory role after *Toxoplasma gondii* infection and in mouse models of arthritis and psoriasis (Y. Zheng et al. 2007; Ma et al. 2008, 17; Geboes et al. 2009; Muñoz et al. 2009). In contrast, other studies in mouse models of IBD, have demonstrated tissue-protective properties of IL-22. Indeed, IL-22 activates STAT3 in intestinal epithelial cells (IEC) and therefore promotes IEC proliferation and migration (Brand et al. 2006) and the production of mucin which is one of the major components in the intestinal mucus (Sugimoto et al. 2008). These findings suggest important functions of IL-22 in mucosal healing and in preserving the mucus layer to prevent bacterial invasion. Like IL-17, IL-22 induces the production of AMPs by IEC and thus limits bacterial replication and dissemination. Accordingly, IL-22 attenuates the intestinal inflammation in mice (Sugimoto et al. 2008, 22), while blockade of IL-22 signalling using anti-IL-22 antibodies or in IL-22 knockout mice worsens colonic injuries (Cl et al. 2013).

Interestingly, IL-17-and TNF-α-producing MAIT cells are significantly increased in the blood of CD patients accompanied by a decreased IFN-γ secretion. In addition, IL-22 producing MAIT cells trend toward increasing in UC (Serriari et al. 2014; Haga et al. 2016; Tominaga et al. 2017). In the blood of IBD patients, MAIT cells identified as CD161hi Vα7.2+ CD3+ are significantly reduced and accumulate in the inflamed intestinal mucosa (Serriari et al. 2014; Haga et al. 2016; Tominaga et al. 2017). These findings, together with the increased proportion of cycling MAIT cells in the blood of IBD patients (Serriari et al. 2014) and their expression of CD69, NKG2D or BTLA, suggest a chronic state of activation of MAIT cells in IBD patients (Serriari et al. 2014; Haga et al. 2016; Tominaga et al. 2017) (Fig. 11). However, studies are required to address the question of the role of MAIT cells upon intestinal inflammation.

MS, T1D and IBDs are associated with important alterations in the composition and function of the gut microbiota (Honda and Littman 2016), which may impact on the production or availability of riboflavin metabolite antigens for MAIT cells. Because of the strong link between microbial groups imbalance and health impairment or diseases, the following section will discuss the current understanding of the host-microbiota interactions.

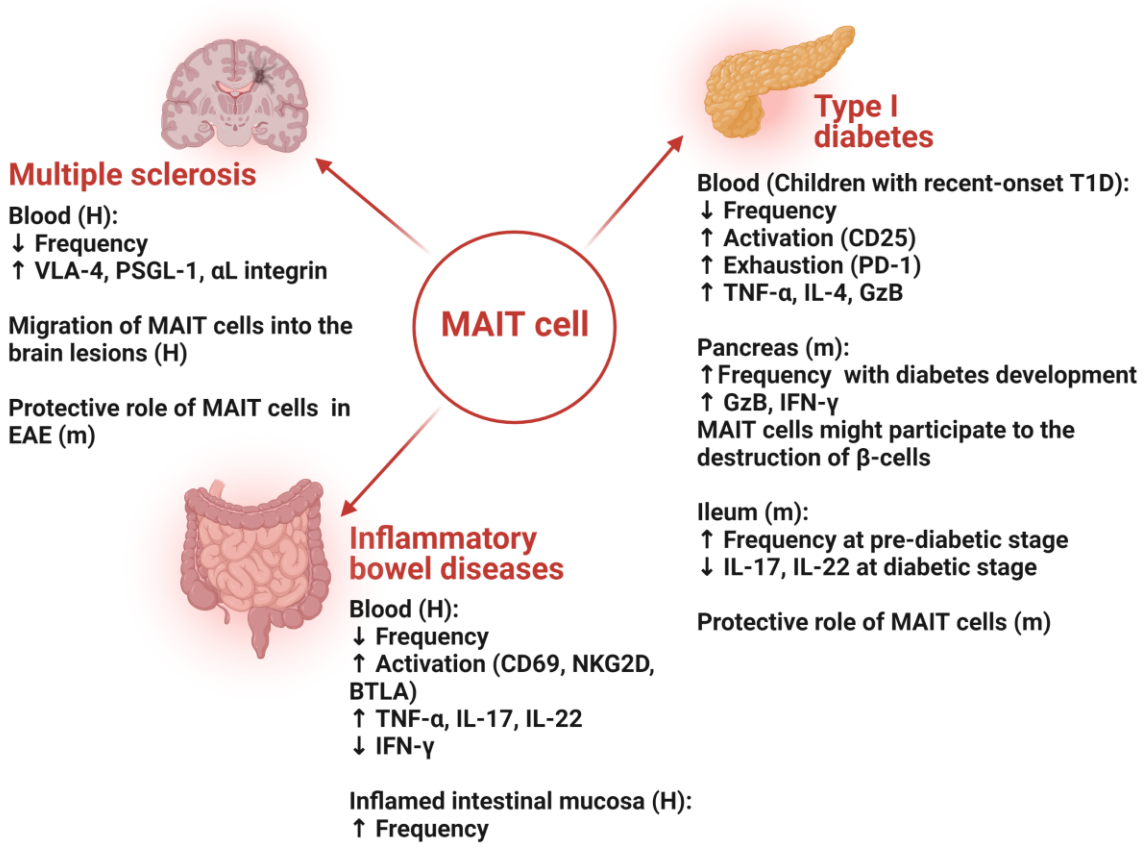


Figure 11: MAIT cells in autoimmune and immunological diseases. MAIT cells are reduced in the blood and accumulate in inflamed tissues, including central nervous system, pancreatic islets and intestine. The protective roles of MAIT cells were demonstrated in animal models of multiple sclerosis and type 1 diabetes. The possible contribution of MAIT cells to pancreatitis was demonstrated in T1D. (H= Human; m= mouse).

Chapter II: Intestinal microbiota and interactions with the host

1. Intestinal microbiota composition

In terms of bacterial composition, the healthy human fecal microbiome is composed of five dominant bacterial phyla. Strict anaerobic bacteria from the phyla *Firmicutes* and *Bacteroidetes* are the most relatively abundant while *Actinobacteria*, *Proteobacteria* and *Verrumicrobia* constitute a minority in healthy individuals (Tap et al. 2009). To note, the low abundance of the facultative anaerobic *Proteobacteria* is indicative of a healthy gut microbiota (Hollister, Gao, and Versalovic 2014).

As in humans, *Firmicutes* and *Bacteroidetes* are the two most abundant bacterial phyla in mice feces (Gu et al. 2013). Even though this general profile remains constant, clear differences can be observed between the murine and human gut microbiota when exploring deeper taxonomic classifications (Nguyen et al. 2015). Despite that, mouse models remain a powerful tool for studying gut-microbiota-associated diseases (Nguyen et al. 2015).

Several factors influence the composition and functionality of the microbiota within the gut. The abundance of oxygen is considered to be one of the most important forces by which the host shapes the microbiota in the gut. Indeed, intestinal oxygen is regulated by the host and altered in many situations. Evidences for oxygen to shape the configuration of the microbiome are developed below.

2. Role of oxygen in shaping the microbiota

2.1. From birth to adulthood

It is well established that the postnatal gut environment is different from the mature adult gut environment. Notably, the neonatal gut is characterized by a high abundance of oxygen while mature adult gut is almost completely hypoxic (Sanidad and Zeng 2020). Accordingly, immediately after birth, the neonatal gut becomes dominated by the facultative anaerobes *Enterobacteriaceae* and *Lactobacillaceae* in both human (Chu et al. 2017; Combellick et al. 2018) and mouse (Constantinides et al. 2019). The expansion of the facultative anaerobes leads to a gradual consumption of oxygen, which create a new environment suitable for the

colonization by the obligate anaerobes *Bifidobacteriaceae* and *Clostridiaceae* originating from the milk (Sanidad and Zeng 2020) (Fig. 11).

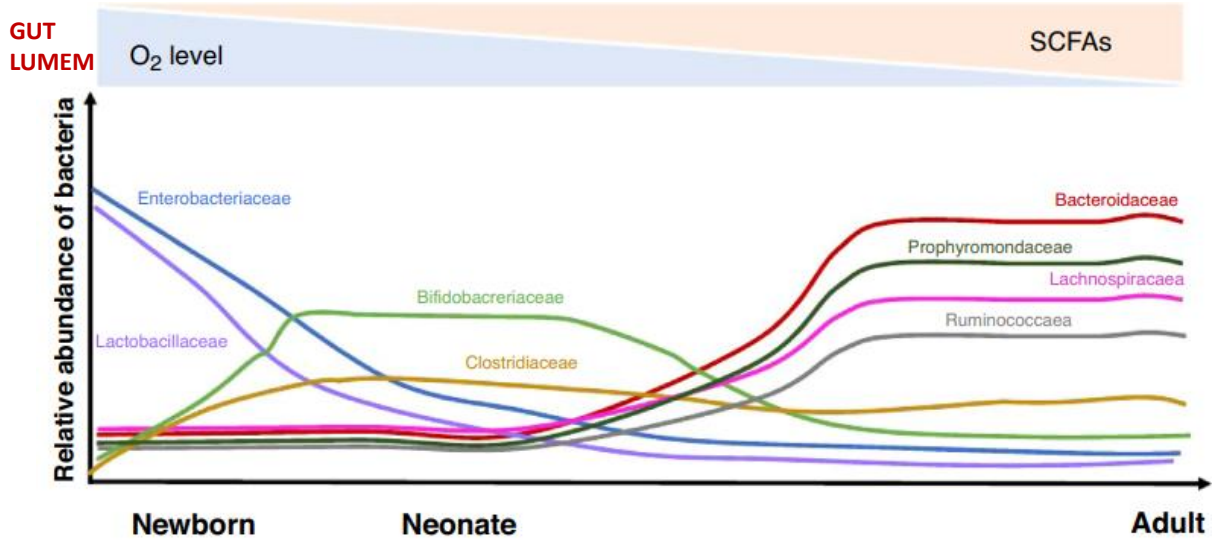


Figure 12: Human fecal microbiota composition from early life to adulthood.
Facultative anaerobes *Enterobacteriaceae* and *Lactobacillaceae* are the first colonizers of the gut after birth. During the following 6 months, obligate anaerobes, including *Bifidobacteriaceae* and *Clostridiaceae*, begin to accumulate in the gut. Butyrate-producing *Clostridia* decrease epithelial oxygenation in the colon and allow for the colonization of *Bacteroidaceae*, *Porphyromondaceae*, *Lachnospiraceae*, and *Ruminococcacea* in the adult gut (Sanidad and Zeng 2020).

The introduction of solid foods at weaning initiates the fermentation of indigestible dietary fibers in the gut, which leads to the production of short-chain fatty acids (SCFAs) like butyrate. Butyrate serves as the main carbon source of colonocytes and its metabolism has a direct bearing on the bioavailability of oxygen in the gut lumen. Indeed, butyrate binds to the intracellular receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) which is synthesized at high levels in differentiated colonocytes at the tip of the villi. Butyrate-induced PPAR-γ signalling drives the energy metabolism of colonocytes towards mitochondrial fatty acids β-oxidation. In this process, high amounts of oxygen are consumed through oxidative phosphorylation, making the top of the intestinal crypts hypoxic. This ensures limited oxygen diffusion from the mucosal surface, and creates an environment suitable for obligate anaerobes (Fig. 12) (Litvak, Byndloss, and Bäumler 2018).

Over time, the decrease in the luminal oxygenation allows obligate anaerobic species to expand and outnumber facultative bacteria. Within 3 years after birth, infant fecal microbiota reaches a mature adult-like profile (Yatsunenko et al. 2012), characterized by preponderance of the strict anaerobes *Lachnospiraceae* and *Ruminococcacea* (from the phyla *Firmicutes*), and *Bacteroidaceae*, *Prevotellaceae* and *Rikenellaceae* (from the phyla *Bacteroidetes*), with low abundance of *Actinobacteria*, *Proteobacteria* and *Verrumicrobia* (Tap et al. 2009; Sanidad and Zeng 2020) (Fig. 11).

Thus, the regulation of oxygen availability enables the host to control the ecological niche of the colon. The establishment of hypoxic conditions selects for anaerobic bacteria equipped with metabolic pathways for the fermentation of fibers, a feature beneficial to the host. By contrast, aerobic *Proteobacteria* can represent opportunistic pathogens, because they can strive in oxygen-rich host tissues in the gut and systemically.

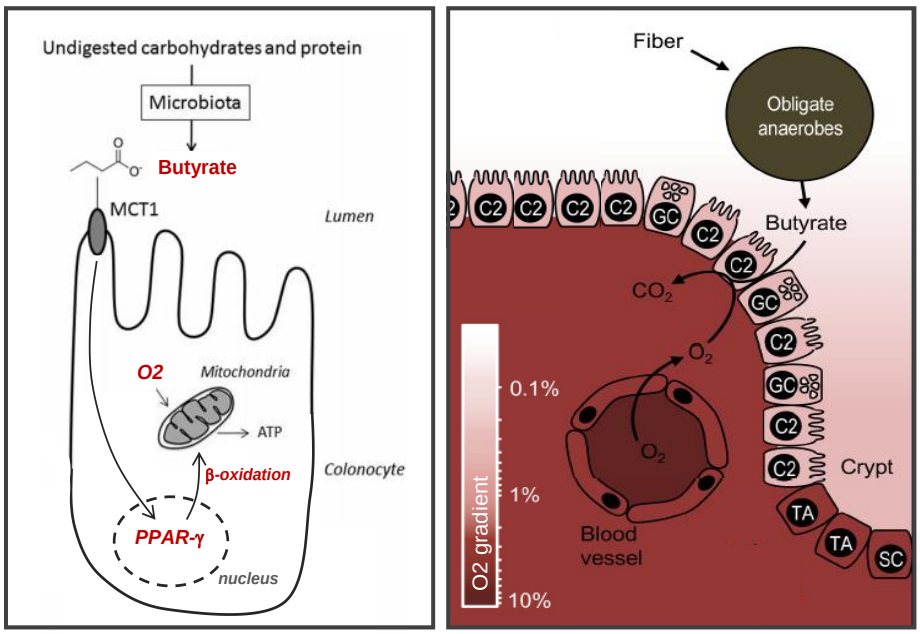


Figure 13: Microbiota-derived butyrate metabolism maintains anaerobiosis in a virtuous circle. Butyrate, which is produced from fermentation of undigested carbohydrates and protein, enters the differentiated colonocytes via the monocarboxylate transporter 1 (MCT1). Butyrate induces PPAR-γ–dependent activation of mitochondrial β-oxidation. By analogy with the metabolic programming of M2 macrophages, coloncytes having increased fatty acid oxidation are in a C2-metabolic state. C2-colonocytes consume oxygen and therefore maintains anaerobiosis and the dominance of obligate anaerobic bacteria in the microbial community. SC, stem cell; TA, undifferentiated transit-amplifying cell; C2, terminally differentiated C2-skewed colonocyte; GC, goblet cells (Litvak, Byndloss, and Bäumler 2018).

2.2. Along the longitudinal axis of the adult gut

The small intestine is subject to pronounced fluctuations of the partial pressure of oxygen (pO2) associated with ingestion of nutrients. However, in contrast to the colonic epithelium, which favors oxidative metabolism of butyrate, small intestinal epithelial cells are more flexible and use glucose and glutamine as metabolic substrates (L. Zheng, Kelly, and Colgan 2015). Accordingly, Guanglong He *et al.*, by Electron Paramagnetic Resonance (EPR) imaging, measured a steep oxygen gradient within the murine intestinal tract going from 32 mmHg in the duodenum to deeply anaerobic conditions (3 mmHg) in the distal colon (He et al. 1999). The hypoxia-inducible factor (HIF)-luciferase reporter mouse has also enabled the study of oxygen availability along the gut (Safran et al. 2006). In fact, the transcription factor HIF is the major cellular mechanism coordinating the transcriptional response to hypoxic conditions (Papandreou et al. 2006). This oxygen-sensitive transcription factor is rapidly degraded in the presence of oxygen. Fusing luciferase to HIF enabled visualization of the proximal-to-distal gradient of oxygen along the length of the gut (L. Zheng, Kelly, and Colgan 2015) (Fig. 13).

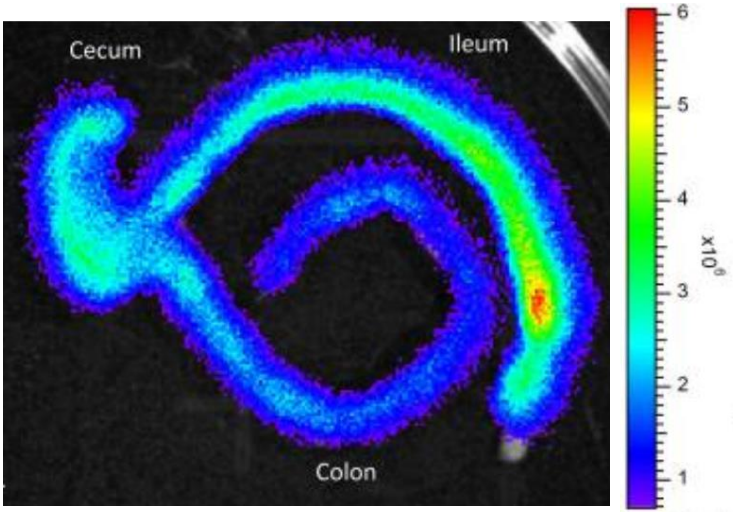


Figure 14: Oxygen gradient along the length of the gut.
Intestine from hypoxia-inducible factor-luciferase reporter mouse (L. Zheng, Kelly, and Colgan 2015).

The high oxygen availability of small intestine limits bacterial load and selects for the facultative anaerobes. Indeed, the small intestine is enriched in *Proteobacteria* (mainly *E. coli*), *Streptococcus spp.* and *Lactobacillaceae* as well as *Bacteroidetes* and *Lachnospiraceae* in human (Zoetendal et al. 2012; Pereira and Berry 2017) and *Lactobacillaceae*, *Bacteroidales* and *Desulfovibrionaceae* in mice (Gu et al. 2013; Pereira and Berry 2017). In contrast, in the large intestine, where less oxygen is available, microbiota is dominated by the strict anaerobes and is almost similar to fecal microbiota (Gu et al. 2013; Yasuda et al. 2015) (Fig. 14).

2.3. Along the cross-sectional axis of the adult gut

Beside the gradient of oxygen along the longitudinal axis of the gut, oxygen drops along the radial axis from the highly vascular lamina propria to the lumen (L. Zheng, Kelly, and Colgan 2015). Indeed, using the phosphorescence quenching method, the pO2 of mouse cecal tissue was found to be ~40 mmHg, while in the cecal lumen pO2 was <1 mmHg (Albenberg et al. 2014). The higher oxygen concentration in the gut tissue compared to the lumen, enables oxygen-tolerant organism's adherence to the mucosal surface. Compared to the lumen community, the mucosa is enriched in *Proteobacteria* in humans (Albenberg et al. 2014) and macaques (Yasuda et al. 2015), and in *Lactobacillus* in mice (Hill et al. 2010) (Fig. 14). The consumption of host-derived oxygen by the facultative anaerobes close to the mucosa contributes to the steep gradient of oxygen that decreases in concentration from tissue to lumen (L. Zheng, Kelly, and Colgan 2015).

At the oxic–anoxic interface near the intestinal epithelium, oxygen influx from the epithelium reacts directly with hydrogen sulfide (H_2S) generated by luminal bacteria, producing H_2O_2 (Imlay 2019) (Fig. 15). Accordingly, in humans, the mucosally adherent microbiota is enriched in genes required for resistance to reactive oxygen species (ROS) relative to the microbiota in the feces (Albenberg et al. 2014). Moreover, the riboflavin biosynthesis pathway, which is required for the regeneration of reduced glutathione, were found enriched in the macaque mucosal community (Yasuda et al. 2015). This was identified using LEfSe analysis (Segata et al. 2011) on the community metabolic pathways inferred by PICRUSt. PICRUSt for "phylogenetic investigation of communities by reconstruction of unobserved states", is a computational approach that serves to predict the metabolic potential of the microbiota communities, based on 16S rRNA microbial composition and a database of reference genomes (Langille et al. 2013).

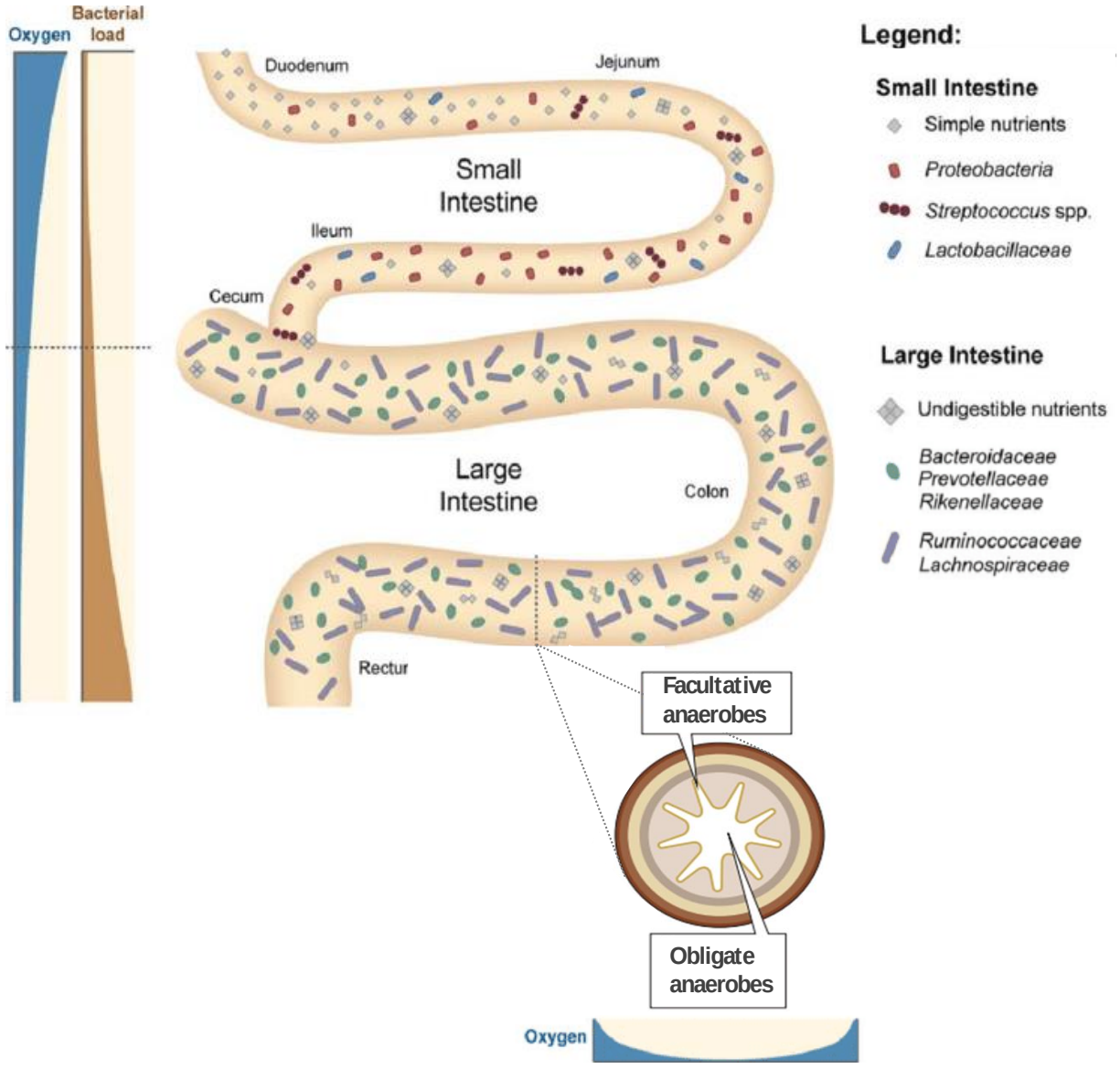


Figure 15: Spatial heterogeneity of the gut microbiota in the intestinal tract.
Oxygen availability is a major determinant of microbiota composition. The longitudinal gradient of oxygen limits bacterial load and diversity in the small intestine. In contrast to the large intestine, the small intestine is characterized by a greater relative abundance of facultative anaerobic bacteria. The cross-section of the colon shows the mucosa, which is colonized by facultative anaerobes, and the lumen dominated by obligates anaerobes (Figure adapted from Pereira and Berry 2017).

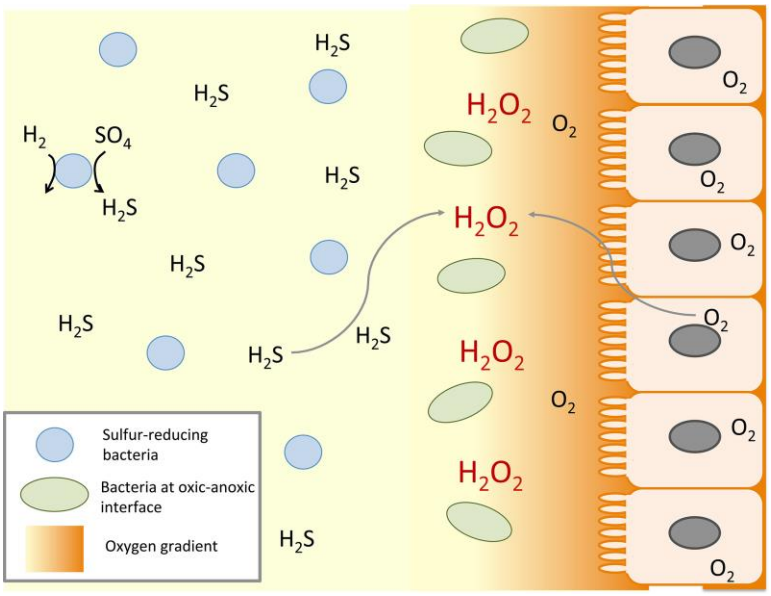


Figure 16: Plausible source of H2O2 in the oxic–anoxic interfaces near the intestinal epithelium (Imlay 2019).

2.4. Upon antibiotic treatment

Antibiotic treatment targeting fermenting microbes such as *Clostridia* or *Bacteroides* depletes butyrate and thus silences epithelial PPAR-γ signalling. As a result, antibiotic-mediated depletion of butyrate shifts the colonocytes metabolism toward anaerobic glycolysis (fermentation of glucose). This metabolic reprogramming of colonocytes conserves oxygen within the cell, resulting in the loss of epithelial hypoxia and in increased amounts of oxygen emanating from the mucosal surface. Because oxygen can freely diffuse across biological membranes, increased oxygenation of the epithelium is predicted to increase the luminal bioavailability of oxygen, and the expansion of facultative anaerobic bacteria (Litvak, Byndloss, and Bäumler 2018).

This scenario was supported by the decreased butyrate levels, increased epithelial oxygenation and increased abundance of endogenous *E. coli* in the colon of mice treated with streptomycin (Byndloss et al. 2017). Streptomycin is an aminoglycoside antibiotic, and causes a marked depletion of *Clostridia* which include abundant butyrate-producers, specifically *Lachnospiraceae* and *Ruminococcaceae* (Byndloss et al. 2017).

The expansion of *E. coli* upon streptomycin treatment required the *cydAB* gene, which encodes for cytochrome bd oxidase and is essential for *E. coli* aerobic respiration. Interestingly, hypoxia restoration through tributyrin (analogue of butyrate having delayed absorption in the colon) supplementation abrogated the fitness advantage conferred by aerobic respiration in streptomycin-treated mice (Byndloss et al. 2017).

2.5. Upon intestinal inflammation

Two phenomena contribute to increased luminal oxygen concentrations during chronic intestinal inflammation. First, epithelial injury leads to mucosal bleeding and to the direct release of haemoglobin carrying oxygen within the luminal environment in the inflamed tissue (Rigottier-Gois 2013). Second, oxygen consumption by the inflamed epithelium is reduced (Litvak, Byndloss, and Bäumler 2018). In fact, the epithelial repair responses to injury drive an accumulation of dividing transit-amplifying epithelial cells, thereby causing colonic crypt hyperplasia, a common feature of ulcerative colitis. Consequently, a reduction in the density of terminally differentiated epithelial cells, such as goblet cells, and a concomitant thinning of the adherent mucus layer are observed in ulcerative colitis patients (Strugala, Dettmar, and Pearson 2008). Because PPAR-γ is only expressed in terminally differentiated colonocytes, the accumulation of undifferentiated colonocytes in ulcerative colitis patients reduces mitochondrial β-oxidation of butyrate to CO_2. Dividing transit-amplifying cells convert glucose into lactate, a metabolism characterized by low oxygen consumption. This shift in colon epithelial metabolism reduces epithelial oxygen consumption, leading to increased amounts of oxygen emanating from the colonic surface (Litvak, Byndloss, and Bäumler 2018) (Fig. 16).

The dextran sodium sulfate (DSS)-induced colitis mouse model recapitulates several changes observed in intestinal tissues from patients with ulcerative colitis. DSS treatment triggers colitis in mice by inducing ER stress in colonocytes similar to that observed in IBD patients (CAO et al. 2013). Chronic ER stress impairs cell homeostasis and induce apoptosis leading to epithelial injuries, crypt elongation and increased epithelial oxygenation in DSS-treated mice (CAO et al. 2013; Chassaing et al. 2014; Cevallos et al. 2019). On the other hand, the destruction of mucosal barrier function upon DSS treatment, allows the translocation of microbes, leading to the induction of inflammatory responses (Kitajima, Takuma, and Morimoto 1999).

Studies in humans have shown that the gut microbiota of patients with UC or CD is characterized by a decrease in commensal bacteria diversity, an increased abundance of facultative anaerobic *Proteobacteria* (particularly *Enterobacteriaceae*), and a concomitant decreased proportion of *Firmicutes* compared with healthy subjects (Morgan et al. 2012). Within the phylum *Firmicutes*, the highly oxygen-sensitive *F. prausnitzii,* which is a major butyrate-producing bacteria, is decreased or even disappears in IBD patients (Sokol et al. 2009).

Likewise, a luminal expansion of the phylum *Proteobacteria* (in particular, members of the *Enterobacteriaceae* family) is observed in the cecal microbial communities upon DSS treatment (Hughes et al. 2017). Consistent enrichment in facultative anaerobes including *Pediococcus*, *Weissella*, *Lactococcus*, *Enterococcus* and *Streptococcus* also occurs in the bacterial-induced model of colitis involving infection of mice with *Helicobacter hepaticus* and treatment with anti-IL10 receptor antibody (Ilott et al. 2016a). This model is highly relevant to human IBD as loss of function mutations in IL-10 and IL-10-R are associated with severe infantile IBD (Kotlarz et al. 2012).

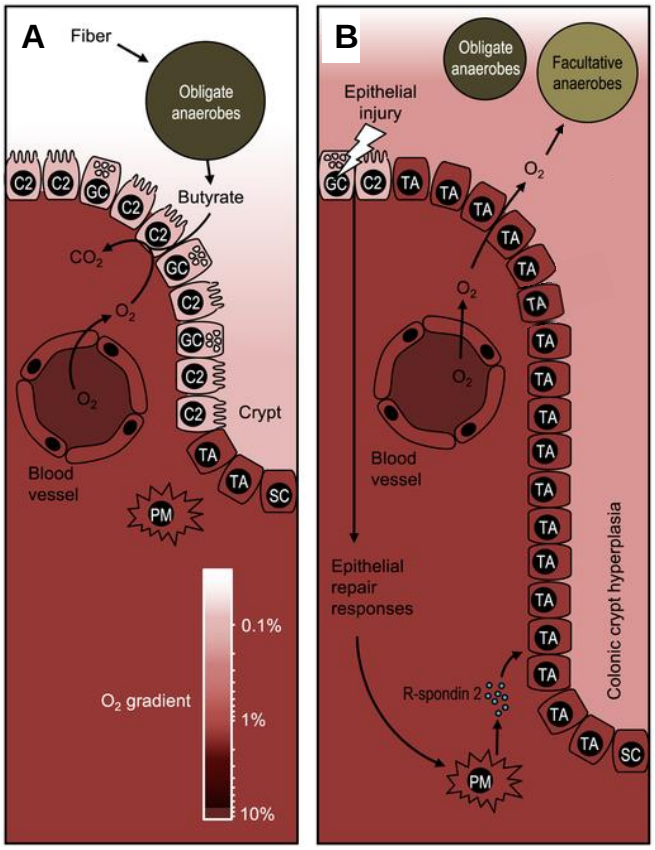


Figure 17: Increased epithelial oxygenation in inflamed tissues. To repair colon epithelial injury, pericryptal myofibroblasts release R-spondin 2, which stimulates division of undifferentiated transit-amplifying (TA) cells. Accumulation of dividing TA cells leads to crypt elongation, increased epithelial oxygenation, and expansion of facultative anaerobic bacteria (Litvak, Byndloss, and Bäumler 2018).

In the inflamed intestine, the disruption in anaerobiosis is suggested to induce this shift in the microbiota composition towards greater abundance of facultative anaerobes (Rigottier-Gois 2013) that are also equipped to confront ROS (Morgan et al. 2012). Several ROS are continuously produced in bacteria as byproducts of aerobic metabolism (Imlay 2019). Moreover, in addition to endogenous ROS, host immune responses under inflammatory conditions favor oxidative stress. For example, neutrophils transmigrate into the lumen and release ROS upon intestinal inflammation (Chin and Parkos 2006). Moreover, proinflammatory cytokine signalling induces the expression of dual oxidase 2 (*DUOX2*), which is a predominant source of intestinal epithelial ROS along the gut (Harper et al. 2005). Accordingly, *DUOX2* expression is elevated across all forms of IBD in association with an expansion of *Proteobacteria* (Haberman et al. 2014). Moreover, the anti-inflammatory/antioxidant *APOA1* gene, which is expressed in the unaffected ileum, is suppressed in CD patients (Haberman et al. 2014).

Associated with the release of ROS, genes of the riboflavin pathway are enriched (at the DNA level) in the microbiota of patients with ileal CD. Furthermore, the pentose phosphate pathway, which feeds the riboflavin pathway and produces the NADPH required for glutathione reduction, is overrepresented as well in ileal CD. Genes involved in the biosynthesis of cysteine (a precursor to glutathione) and glutathione transport genes are also increased in abundance in all forms of IBD (Morgan et al. 2012). Remarkably, in this study, patients with ileal CD were the only ones to have a significant increase in the abundance of *Enterobacteriaceae*. This bacterial family can exacerbate intestinal inflammation by stimulating the production of pro-inflammatory cytokines by intestinal dendritic cells (Y.-L. Huang et al. 2015). Altogether, these findings suggest a shift in the microbiota composition towards greater abundance of microbes able to grow in the inflamed intestine.

3. Role of intestinal epithelial barrier in shaping the microbiota

Under normal circumstances, the intestinal epithelium is composed of a single layer of absorptive epithelial cells that are interspersed by specialized cells called Paneth cells and goblet cells. Paneth cells are enriched in the bases of the small intestinal crypts and secrete an array of AMPs including defensins and C-type lectins, whereas goblet cells are highly abundant in the large intestine and continuously produce the gel-forming mucin to maintain a functional mucus layer. The mucus layer in the large intestine is composed of two distinct layers: an inner

mucus layer that contains large concentrations of antimicrobial factors, and an unattached outer mucus layer. However, the small intestine harbors a thinner and discontinuous mucus layer.

The production of AMPs is induced by the activation of pattern recognition receptors (TLRs and NLRs) by microbial products, and in response to cytokines (IL-22, IL-17) secreted by immune cells in the surrounding milieu (Levy et al. 2017). The release of mucin by goblet cells is triggered by immune mediators such as IFN-γ (Klose et al. 2013a). Through the secretion of antimicrobial peptides and mucus the host maintains bacteria away from the epithelium (De Weirdt and Van de Wiele 2015; Levy et al. 2017). Indeed, the mucus layer is characterized by low bacterial density compared to the lumen, and is restricted to bacterial species that can survive host antimicrobial strategies and can resist to ROS produced by aerobic metabolism. Moreover, as a result of exposure to intestinal microbes, B cells residing in the intestinal mucosa induce secretory IgA antibody which decreases microbial motility, accelerates *in vivo* intestinal transit, and limits exposure of the mucosa to microbial products (Uchimura et al. 2018).

This intestinal barrier allows for physical segregation of antigenic peptides from T cells. However, there are still communication channels between the microbiota and their mammalian host, in particular for the monitoring of bacterial activity, ie through the detection of microbial metabolites. Only a few have been identified to date, but there are probably many more conserved molecular interactions between the 2 symbionts. Among the metabolites generated by microbial metabolism are SCFAs, tryptophan-derived microbial metabolites, and secondary bile acids (BAs). These metabolites are small molecules (<1500Da) that have been shown to penetrate distant host tissues (Uchimura et al. 2018), and to impact both locally and systemically host metabolism and immune functions.

4. Influence of microbial metabolites on the host

4.1. Short-chain fatty acids

SCFAs such as butyrate, acetate and propionate, are the end-products of microbial anaerobic fermentation of dietary fibers, which is the dominant metabolism in the colon. Members of *Firmicute*s are the main producers of butyrate, whereas most acetate and propionate producers belong to *Bacteroidetes* (G. Wang et al. 2019; Koh et al. 2016).

Among the SCFAs, butyrate has been investigated most extensively. As described earlier, butyrate is the preferred energy substrate for colonocytes and the primary determinant of physiologic hypoxia in the colon by promoting intestinal epithelial O2 consumption. Consequently, butyrate stabilize HIF (Caleb J. Kelly et al. 2015) which in turn induce the expression of barrier-protective HIF target genes, including genes critical for defensin expression (C. J. Kelly et al. 2013) and mucin production (Louis et al. 2006). By this mechanism, SCFAs fortify IEC barrier function.

Additionally, butyrate and to a lesser extent propionate function as inhibitors of histone deacetylases (HDACs) and therefore act as an epigenetic regulator of host physiology (Koh et al. 2016). Via HDACs inhibition, butyrate and propionate make the immune system hyporesponsive to commensal bacteria. Indeed, butyrate and propionate block the differentiation of dendritic cells (DC) from bone marrow stem cells by suppressing the transcription factors necessary for DC development (Singh et al. 2010). Moreover, butyrate promotes tolerance towards beneficial microorganisms by inducing functional colonic Treg cells among $CD4^+$ T cell subsets, through acetylation at the *FoxP3* gene locus (Furusawa et al. 2013).

Beside their ability to inhibit HDACs, SCFAs can signal through the G-protein-coupled-receptors (GPR41, GPR43 and GPR109A) which are expressed on the surface of numerous cell types, including IECs and immune cells (Koh et al. 2016). Butyrate inducing Gpr109A signalling promotes anti-inflammatory properties in colonic antigen-presenting cells, which in turn induce differentiation of naïve T cells into Treg cells and IL-10-producing T cells (Singh et al. 2014). Accordingly, GPR109A deficient mice harbor significantly less frequency and number of Treg cells in the colon and show enhanced susceptibility to colitis (Singh et al. 2014).

Interestingly, metabolic studies have demonstrated a reduction in fecal SCFAs levels in IBD patients (Aonghus Lavelle and Sokol 2020; Marchesi et al. 2007), which is consistent with the loss of butyrate-producing bacteria and impaired butyrate oxidation in the colonic mucosa of UC patients (Roediger 1980). Given their role in maintaining homeostasis, SCFAs are a promising therapeutic target in IBD. Indeed, SCFAs administration attenuates colonic inflammation in mice (P. M. Smith et al. 2013; Furusawa et al. 2013; Singh et al. 2014), and butyrate enemas reduce clinical symptoms in IBD patients (Scheppach et al. 1992; Sabatino et al. 2005).

To note, metabolically beneficial effects of SCFAs (mostly acetate and possibly propionate) extend beyond the gut to the adipose tissue, brain, and liver (Koh et al. 2016).

4.2. Tryptophan-derived microbial metabolites

Tryptophan is an essential amino acid that can only be acquired through dietary proteins. Intestinal bacteria can catabolize dietary tryptophan as a precursor for synthesis of immunologically important metabolites, with indole and indole derivatives being the main tryptophan-derived microbial metabolites in the gut. These metabolites modulate the activity of aryl hydrocarbon receptor (Ahr), which is a ligand-dependent transcription factor expressed by epithelial cells and immune cells (G. Wang et al. 2019; Rooks and Garrett 2016).

In an Ahr-dependent manner, indole derivatives improve the barrier functions of epithelial cells *in vitro* through up-regulation of genes encoding for actin cytoskeleton, tight junction proteins, mucin production, and IL-10R (Bansal et al. 2010; Alexeev et al. 2018). Such activity is associated with protection in colitis models. Moreover, via Ahr stimulation, indole derivatives promotes intestinal homeostasis through IL-22 production by type 3 innate lymphoid cells (ILC3s) in the colon (Zelante et al. 2013).

Importantly, tryptophan-indole derivative metabolites were found decreased in both serum and colons of colitic mice, and serum samples from UC patients as compared to controls (Alexeev et al. 2018). Administration of indole derivatives to DSS-treated mice reduced the severity of colitis (Zelante et al. 2013; Alexeev et al. 2018), highlighting the crucial role of the microbial metabolism of dietary tryptophan for achieving and maintaining homeostasis.

4.3. Secondary bile acids

Primary BAs are the end products of cholesterol metabolism in the liver and are further secreted into the small intestine to facilitate absorption of lipids or fat-soluble vitamins. In the colon, BAs that escape active transport in the distal ileum are transformed by bacteria to secondary BAs (G. Wang et al. 2019). Song *et al.* observed that mice fed with minimal-diet had lower levels of fecal primary and secondary BAs with lower number of colonic RORγt^{+} Treg than did rich-diet

SPF mice. A combination of secondary BAs restored the number of colonic RORγt$^+$ Treg cells in minimal-diet mice, indicating that certain BAs biotransformed by bacteria are essential for the maintenance of colonic RORγt$^+$ Treg cells (Song et al. 2020).

Another study revealed additional anti-inflammatory roles of secondary BA metabolites by inhibiting Th17 and enhancing Treg cell differentiation, through respectively, a direct binding and inhibition of RORγt protein, and by increasing the expression of FOXP3 (Hang et al. 2019). Thus gut-residing bacteria may modulate the balance of Th17 and Treg cells through BA metabolites. Secondary BA supplementation alleviated colitis in minimal-diet mice by increasing the number of RORγt$^+$ Treg cells (Song et al. 2020).

Microbially transformed bile acids have also been potentially linked with IBD. Indeed, altered BA transformation in the gut lumen has been reported in IBD, involving increased fecal primary BAs and decreased serum and fecal secondary BAs (Duboc et al. 2013). In this study, the imbalance in BAs towards primary BAs has been proposed as a mediator of the pathogenesis of human IBDs by erasing the anti-inflammatory effects of secondary BA on gut epithelial cells. Indeed, secondary and not primary BAs inhibited anti-inflammatory effect of IL-1β on Caco-2 intestinal epithelial cells (Duboc et al. 2013).

Aims of the thesis

MAIT cells preferentially localize in barrier tissues and recognize microbial metabolites derived from the riboflavin biosynthesis pathway. Intestinal bacteria can produce riboflavin pathway-derived MAIT antigens (Tastan et al. 2018), suggesting a direct interaction between MAIT cells and the microbiota. Nevertheless, the concentration of MAIT antigens produced in the gut, and the mechanisms controlling MAIT antigen production by the microbiota have not been determined. Moreover, how MAIT cells monitor disturbances of the composition and function of the microbiota remains unknown.

Several studies showed activation of MAIT cells in inflammatory bowel diseases (Serriari et al. 2014; Haga et al. 2016; Tominaga et al. 2017) in which high oxygen levels and oxidative stress have been reported in the gut (Rigottier-Gois 2013). Riboflavin contributes to bacterial respiration and oxidative stress resistance, and bacteria harboring the riboflavin biosynthetic pathway are enriched in the microbiota of IBD patients (Morgan et al. 2012). Accordingly, we suggest that MAIT cell activation in IBD patients results from the overproduction of riboflavin precursor derivatives by the microbiota.

In this thesis, we explored the hypothesis that dysbiosis drives increased concentrations of riboflavin precursor derivatives which are sensed by MAIT cells.

The overall objective of my thesis studies was to decipher MAIT cell interactions with the microbiota which helps understanding why the mammalian immune system has evolved recognition of riboflavin-derived metabolites.

The study has the two following specific aims:

I. Identify the mechanisms of MAIT antigen production by the microbiota.
II. Define the function of MAIT cells in the gut.

Results

Part I: MAIT cell surveillance of colitis-associated bacterial metabolites

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Summary

Changes in the composition and metabolic activity of the gut microbiota are associated with many pathological processes, but how the host monitors such changes remains poorly understood. Mucosal associated invariant T (MAIT) cells recognize metabolites from the microbial riboflavin biosynthesis pathway presented by the MHC-related molecule MR1[1,2]. Riboflavin contributes to bacterial respiration and oxidative stress resistance, but why T cells recognize riboflavin precursor derivatives is unclear. Here we show that hypoxia disruption in the colon, upon antibiotic treatment or colitis is associated with increased production of MAIT antigen by the microbiota and triggers a protective MAIT cell response. The bacterial genes *ribA*, *ribB* and *ribD*, which control MAIT antigen production, were over-expressed during colitis. Riboflavin production provided a fitness advantage to *Escherichia coli* in the inflamed intestine. Riboflavin precursor adducts rapidly crossed the intestinal barrier and induced T cell receptor (TCR) signalling in MAIT cells, which produced the tissue-repair mediator amphiregulin and reduced colitis severity. Thus, MAIT cells directly monitor a bacterial metabolism associated with colitis and provide host protection in return. The study helps understand the role of T cell recognition of riboflavin precursor derivatives. Manipulation of this new host-microbiota interaction may provide clinical benefits in inflammatory bowel diseases.

Main text

MAIT cells are evolutionarily conserved T cells that recognize metabolic adducts derived from the riboflavin biosynthetic pathway. MAIT cells preferentially localise in barrier tissues and have been implicated in pathologies associated with intestinal dysbiosis such as inflammatory bowel diseases[3,4] and colorectal cancer[5-7]. Intestinal bacteria from the *Bacteroidetes* and *Proteobacteria* phyla can produce large amounts of riboflavin pathway-derived MAIT antigens[8], suggesting a direct interaction between MAIT cells and the microbiota. Bacteria harbouring the riboflavin biosynthetic pathway are enriched in the microbiota of patients with inflammatory bowel diseases[9-11], raising the possibility that MAIT cell activation in these patients results from the overproduction of riboflavin adduct antigens by the microbiota. The amounts of MAIT antigens produced in the gut by the microbiota, and the mechanisms controlling this process remain unknown. The role of MAIT cells during colitis is also unknown.

MAIT cells represented 1-10% of T cells in the colonic lamina propria of B6-MAIT^{Cast} mice[12] (Fig. 1a). Parabiosis experiments indicated that the population of colonic MAIT cells, like invariant natural killer T (iNKT) cells and unlike conventional T cells, was not replaced by blood-derived cells during a 5-week time period (Fig. 1b), indicating residency in the colon. Colonic MAIT cells expressed the activation marker CD25 at high levels as compared to iNKT cells (Fig. 1c). Given the shared functional programs but distinct specificities of MAIT and iNKT cells[13], CD25 expression by MAIT cells suggested MAIT antigen availability in the colon. To assess TCR signalling in MAIT cells, we used the *Nr4a1*-GFP reporter mouse[14], in which TCR but not cytokine stimulation drives expression of GFP in MAIT cells (Fig. S1a). iNKT cells expressed GFP in the mesenteric lymph nodes (mLN), but not in the colon, consistent with iNKT cell priming in the mLN[15]. By contrast, MAIT cells expressed high levels of GFP in the colon (Fig. 1d). Lower but significant levels of GFP were also detected in MAIT cells from the mLN and the liver, which drain intestinal lymph and blood, respectively, further suggesting MAIT antigen production in the colon.

To quantify MAIT antigens in the large intestine, we used an in vitro biological assay using MAIT ligand-specific reporter T cells and MR1-expressing antigen-presenting cells[16] loaded with intestinal contents (Fig. 1e). The assay enables the detection of the canonical MAIT ligand 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) at a sensitivity of 0.1 nM (Fig. S1b). The reporter T cells were activated in the presence of cecum, colon and feces contents, revealing the presence of MAIT antigens throughout the large intestine (Fig. 1e and S1c).

Both fungi and bacteria can produce riboflavin-derived MAIT antigens[1,17]. MAIT antigens were depleted from the intestinal contents when mice were treated with broad-spectrum antibiotics (Fig. 1e), but not with the anti-fungi drug fluconazole (Fig. S1d), indicating that MAIT antigens were mostly produced by the commensal bacteria. The colon presents ecological niches populated by microbial communities with distinct metabolic requirements[17], which could affect MAIT antigen production. In particular, the oxygen concentration is higher in the mucosa as compared to the lumen and shapes the composition of bacterial communities[18]. Mucosal scraps contained fewer bacteria (Fig. S1e), but were more potent than luminal content at activating reporter T cells (Fig. 1f), indicating higher MAIT antigen concentration in the mucus layer than in the lumen. Thus, colonic bacteria residing close to the epithelium produce MAIT antigens, which are sensed by MAIT cells in the underlying lamina propria.

To explore the mechanisms underlying increased MAIT antigen production in the mucosa compared to the lumen, we sequenced the 16S rRNA genes from the two compartments. In both cecum and colon, the mucosal niche was enriched in *Proteobacteria* (Fig. 2a and S2a), in particular in the facultative anaerobes *Enterobacteriaceae* (Fig. 2b and S2b), consistent with higher oxygen availability in the mucus as compared to the lumen[18]. The higher production of MAIT antigens by the microbiota of the mucus may be a consequence of higher oxygen availability in that location, as riboflavin contributes to oxidative stress reduction in bacteria. For example, riboflavin accumulates and reduces hydrogen peroxide formation in the gut bacterium *Enterococcus faecalis* under aerobic conditions[19]. Riboflavin is also used by the anaerobe *Faecalibacterium prausnitzii* to reduce oxygen and grow in contact with air[20]. Consistent with a role for riboflavin in the oxidative stress response, the *ribD* and *ribH* genes of the *riboflavin* biosynthesis operon were expressed upon oxidative stress in *Clostridioides difficile* (Fig 2c and S2c), consistent with a previous report[21]. *Clostridium perfringens* and *Clostridium butyricum* also expressed riboflavin biosynthesis genes upon air exposure (Fig. 2c and S2c), suggesting that riboflavin production is a conserved oxidative stress response in *Clostridia*. *Escherichia coli* and *Bacteroides thetaiotaomicron* constantly produce high amounts of riboflavin as compared to *Clostridia*[8], which may explain that *ribD* or *ribH* were not overexpressed in these bacteria upon exposure to oxygen (Fig. 2c and S2c).

Intestinal hypoxia is established at weaning and is accompanied with a reduction in the abundance of *Proteobacteria*[22,23]. Compatible with a link between intestinal oxygen levels and MAIT antigen production, the colonic microbiota produced more MAIT antigens before weaning than in adults (Fig. S2d). Colonic hypoxia in adults is maintained by epithelial cells through the β-oxidation of microbial fermentation products such as the short-chain fatty acid butyrate, and can be disrupted upon antibiotic-mediated depletion of butyrate producers[24,25]. To increase oxygen availability in the gut, we treated mice with antibiotics and quantified short-chain fatty acids. Ampicillin and vancomycin, but not neomycin treatment, depleted short-chain fatty acids (Fig. S2e) and concomitantly induced the production of MAIT antigens in the large intestine (Fig. 2d). The loss of hypoxia was evidenced by the expansion of facultative anaerobes such as *Enterobacteriaceae* (Fig. 2f) upon vancomycin treatment, and by the loss of the exogenous hypoxic marker pimonidazole[26] at the surface of colonic epithelial cells (fig 2g and S2f). Additional treatment with fluconazole had no effect on MAIT antigen concentrations in the cecum (not shown), which indicates that bacteria, not fungi, produced most MAIT antigens in this setting. Thus, increased oxygen availability in the colon is associated with overproduction of MAIT antigen by the microbiota.

At steady-state, the intestinal barrier separates microbes from the host immune system, preventing T cell responses to commensal bacteria antigens[27]. We used fluorescently labelled dextran administered by oral gavage to assess the integrity of the epithelial barrier upon vancomycin treatment. By contrast with dextran sodium sulfate (DSS) treatment, which increased gut permeability, the intestinal barrier was not affected by vancomycin treatment (Fig. S2g). Since MAIT antigens can rapidly cross the mouse skin[28], we asked whether intact intestinal epithelium was permeable to 5-OP-RU. A mixture of antigenic peptide and 5-OP-RU metabolite was deposited into the lumen of intestinal explants and incubated for 10 minutes on top of antigen-presenting cells (Fig. 2h). Reporter T cells detected the presence of 5-OP-RU antigen, but not peptide, in the supernatant (Fig.2h and S2h), which indicated efficient transfer of 5-OP-RU across the intestinal barrier ex vivo. Given the hydrophilic nature of 5-OP-RU, this result suggests the presence of a transporter expressed by intestinal epithelial cells.

In vivo, vancomycin treatment resulted in increased *Nr4a1*-GFP expression in colonic MAIT cells (Fig. 2i), indicating that overproduction of MAIT antigens by the microbiota was efficiently detected by MAIT cells through the TCR. MAIT cells also received more TCR signals in the mLN, liver and spleen upon vancomycin treatment (Fig. 2i), consistent with a transfer of MAIT

antigens through the lymph and the blood to these tissues. By contrast, vancomycin treatment had no effect on *Nr4a1*-GFP expression in iNKT cells (Fig. S2i). Collectively, MAIT cells detect microbial MAIT antigens produced upon antibiotic-mediated dysbiosis.

Loss of intestinal hypoxia is a hallmark of gut inflammation[29] and bacteria having the riboflavin biosynthesis pathway are enriched in patients with inflammatory bowel diseases[9-11]. To investigate the potential role of MAIT cells during intestinal inflammation, we analyzed expression of the riboflavin pathway genes in the *Helicobacter hepaticus* model of colitis using a published metatranscriptomic dataset[31]. Principal component analysis (PCA) of riboflavin biosynthesis gene expression readily distinguished mice before and after colitis induction (Fig 3a), which indicates that the riboflavin biosynthesis pathway can be used as a biomarker of colitis. All the genes controlling production of the MAIT ligand precursor 5-amino-ribityl uracil (5-A-RU), including *ribD* coding for the 5-A-RU-producing protein RibD, were over-expressed during colitis (Fig. 3b). The bacteria driving *ribD* over-expression belonged to the *Bacteroidaceae*, *Clostridiaceae* and *Enterobacteriaceae* families (Fig. 3c). In particular, *Bacteroidaceae* contributed up to 70% of the expressed *ribD* upon colitis induction. Total gene expression by *Bacteroidaceae* was also increased upon colitis (Fig. S3a), suggesting their expansion in the inflamed gut.

We then assessed whether changes in microbiota composition would be associated with MAIT antigen production in another colitis model. In the DSS model of intestinal inflammation (Fig. S3b), colitis was associated with reduced hypoxia (Fig. 3d) and with a bloom of cecal *Bacteroidaceae* and *Enterobacteriaceae* from the *Escherichia/Shigella* genera (Fig. 3e and S3c). The dysbiotic expansion of *Escherichia coli* relies on microaerobic respiration[32]**.** To assess the role of riboflavin production in this process, wild-type (WT) or isogenic Δ*ribC E. coli*, which lack riboflavin production[16], were administered to mock- or DSS-treated mice by oral gavage and were quantified in the cecum a day later. Δ*ribC* bacteria were recovered in smaller numbers than WT bacteria (Fig. 3f), indicating that riboflavin production contributes to the fitness advantage of *E. coli* during intestinal inflammation. The mechanisms underlying *Bacteroidaceae* expansion during colitis are unclear, but may also involve respiration given the ability of some representatives from the *Bacteroidaceae* family such as *B. thetaiotaomicron,* which expanded during colitis (Fig. 3g), to respire under nanomolar concentrations of oxygen[33]. Consistent with the strong MAIT stimulatory capacities of *Bacteroides* species[8], *B. thetaiotaomicron* secreted large amounts of MAIT antigens in vitro (Fig. 3h).

As a whole, the microbiota produced high concentrations of MAIT antigens during DSS-induced colitis (Fig. 3i), in agreement with the increased expression of riboflavin pathway genes in the *H. hepaticus* model of intestinal inflammation (Fig. 3b). MAIT cells from the colon, but also from the mLN, liver, inguinal and brachial (I+B) LN and spleen up-regulated *Nr4a1*-GFP upon DSS treatment (Fig. 3j), indicating TCR-mediated activation. By contrast, intestinal inflammation had no effect on *Nr4a1*-GFP expression in iNKT cells (Fig. S3d). Thus, colitis drives expression of the riboflavin pathway, resulting in MAIT antigen production and in MAIT cell activation.

To assess the effect of TCR signalling on intestinal MAIT cells without affecting the microbiota, we generated a conditional MR1 transgenic mouse (LSL-MR1) for Cre-mediated over-expression of MR1 (Fig. 4a). The LSL-MR1 mouse was crossed to the Villin-CreER mouse for inducible expression in intestinal epithelial cells[34]. Transgenic MR1 expression in intestinal epithelial cells elicited strong TCR signalling in MAIT cells from the colon, but not spleen and liver, as expected (Fig. 4b). Robust TCR signalling in MAIT cells from the colon induced the production of the epidermal growth factor amphiregulin (Areg) (Fig. 4c), suggesting that MAIT cells may promote epithelial function during colitis.

MAIT cells are activated and recruited to inflammatory lesions in patients with ulcerative colitis and Crohn's disease[3,4], but whether MAIT cells worsen or ameliorate intestinal pathology is unclear. We used a chronic DSS model (Fig. S4a) to investigate the role of MAIT cells in colitis. The proportion of colonic MAIT cells expressing RORγt increased during colitis, while the proportion of MAIT cells co-expressing RORγt and Tbet was reduced (Fig. 4d). Consistent with an activated phenotype, MAIT cells from colitic mice spontaneously produced interferon-gamma (IFN-γ), interleukin (IL)-17, IL-22 and Areg in the colon (Fig. 4e). IFN-γ promotes mucus secretion by goblet cells[35], while IL-17 and IL-22 promote the secretion of anti-microbial peptides and the expression of tight-junction proteins in epithelial cells[36], thus reinforcing the epithelial barrier. Accordingly, $B6^{Cast}$ $Mr1^{-/-}$ mice, which lack MAIT cells[12], presented higher pathophysiology than $Mr1^{+}$ counterparts upon chronic colitis (Fig. 4f and S4b). The presence of MAIT cells resulted in reduced epithelial damage, inflammatory cell infiltrates, splenomegaly and weight loss (Fig. 4f and S4b). Thus, MAIT cells protect against chronic intestinal inflammation.

Collectively, intestinal inflammation drives riboflavin production and the accumulation of metabolite antigens captured by MR1 and presented to MAIT cells in the lamina propria, which provide tissue-repair mediators in return. Overproduction of riboflavin by the microbiota may result from increased oxygen concentration in the colon. The study helps understand how the host immune system monitors and responds to altered microbial metabolism during intestinal inflammation.

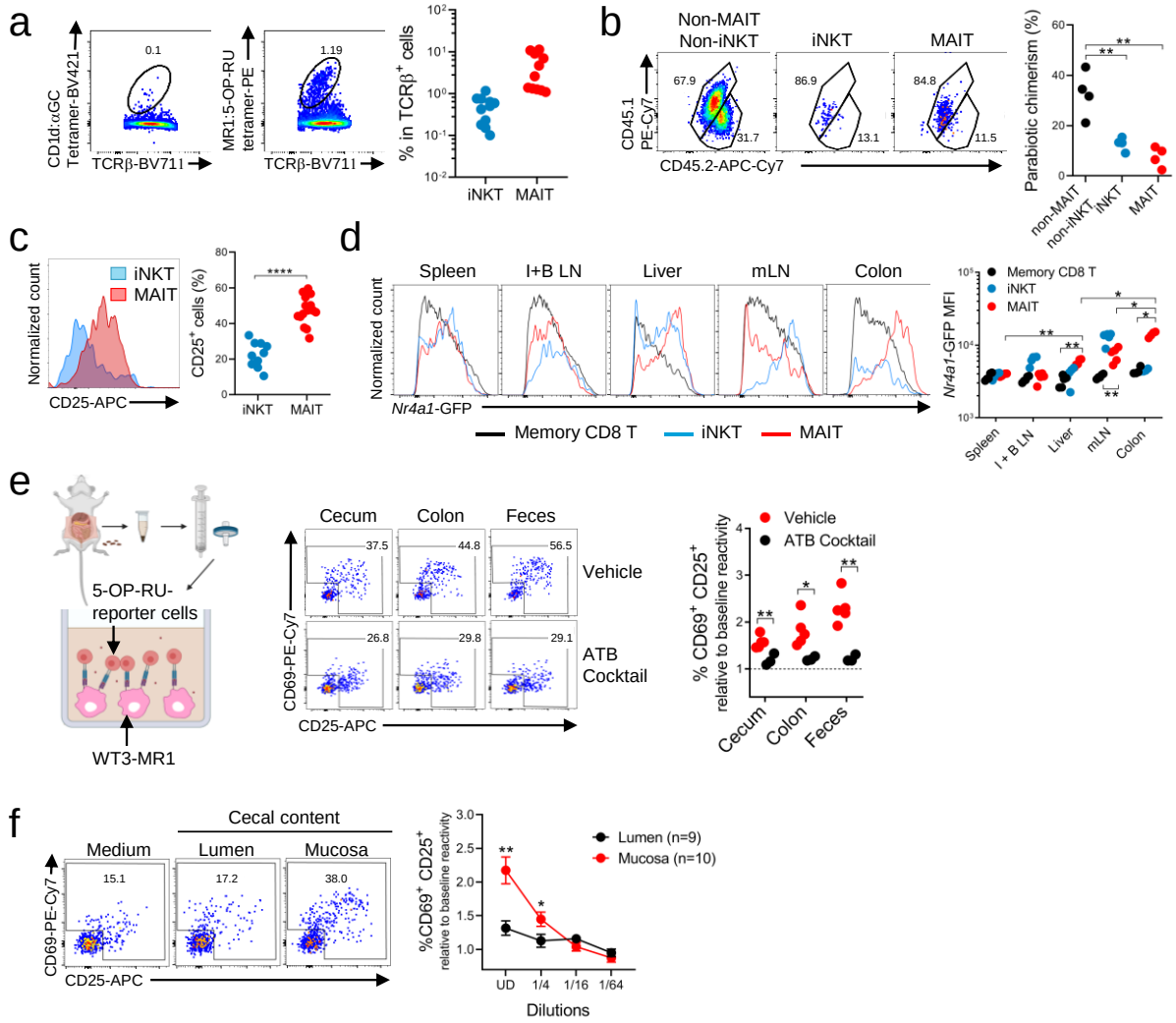


Figure 1: MAIT cells are resident in the colon and sense mucosal bacterial antigens. a. Left: CD1d:αGC tetramer (iNKT) and MR1:5-OP-RU tetramer staining of B6-MAIT^{Cast} colon lamina propria T cells. Right: Pooled data from five independent experiments. **b.** Parabiotic CD45.1/2 and CD45.2/2 congenic B6-MAIT^{Cast} mice were examined 5 weeks after establishment. Left: Example of CD45.1 and CD45.2 staining of the indicated T cell subsets in the colon of a CD45.1/2 parabiont. Right: Chimerism of the indicated T cell subsets from n = 4 mice corresponding to two pairs. **c.** Left: CD25 expression by iNKT and MAIT cells in the colon lamina propria. Right: Pooled data from 4 independent experiments. **d.** Left: *Nr4a1*-GFP expression in the indicated cell subsets and tissues; representative of at least three independent experiments. I+B LN: Inguinal and brachial lymph nodes. mLN: mesenteric lymph nodes. Right: Mean fluorescence intensity (MFI) in the indicated cell subsets and tissues (n= 4 mice; representative of at least three independent experiments). **e.** Left: Scheme of the in vitro assay used to quantify MAIT antigens in intestinal contents. Middle: Activation of MR1:5-OP-RU-specific reporter T cells after overnight incubation with WT3-MR1 cells pulsed with 100 mg of luminal contents from the indicated segment of the large intestine of control mice (vehicle) or of mice treated with an antibiotic (ATB) cocktail for 7d. Right: Relative expression of CD25 and CD69 by reporter T cells in the indicated conditions (n = 3-5 mice per group; representative of two independent experiments). **f.** Left: Activation of reporter T cells after overnight incubation with WT3-MR1 cells left untreated or pulsed with 50 mg of lumen or mucosal cecal contents. Right: Relative expression of CD25 and CD69 by reporter T cells in the indicated conditions (means ± SEMs, n = 10 mice; data pooled from two independent experiments), (UD: undiluted). *p<0.05, ** p<0.01 and ****p<0.0001 by Student's *t* (**b, c, e** and **f**) and by Mann-Whitney *U* test (**d**).

El Morr et al, Figure S1

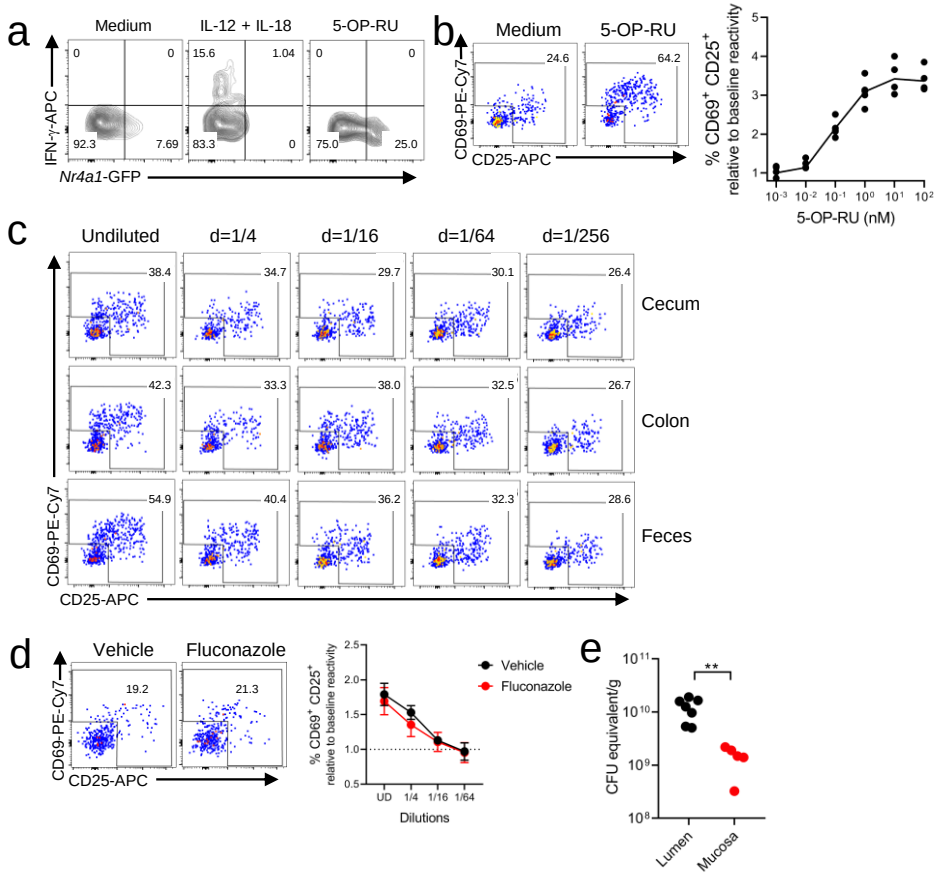


Figure S1: MAIT cells are resident in the colon and sense mucosal bacterial antigens. a. Interferon (IFN)-γ and *Nr4a1*-GFP expression in splenic MAIT cells untreated or incubated overnight with interleukin (IL)-12 and -18 or with 5-OP-RU-pulsed WT3-MR1 cells. **b.** Left: Activation of MR1:5-OP-RU-specific reporter T cells after overnight incubation with WT3-MR1 cells pulsed with 5-OP-RU (100 nM). Right: Relative expression of CD25 and CD69 by reporter T cells in the indicated conditions (data pooled from three independent experiments). **c.** Staining of reporter T cells after overnight incubation with WT3-MR1 cells pulsed with luminal contents at the indicated dilution. **d.** Left: Activation of reporter T cells after overnight incubation with WT3-MR1 cells pulsed with 100 mg of luminal contents from the cecum of control (vehicle) or of mice treated for 7d with fluconazole. Right: Relative expression of CD25 and CD69 by reporter T cells in the indicated conditions (means ± SEMs, n = 3 mice per group). **e.** Quantification of bacterial numbers in the cecal lumen and mucosa by qPCR amplification of the 16S rRNA gene. Colony-forming unit (CFU) equivalents were estimated using a standard curve with *Escherichia coli* (data pooled from 2 independent experiments). ** p<0.01 by Mann-Whitney *U* test.

El Morr et al, Figure 2

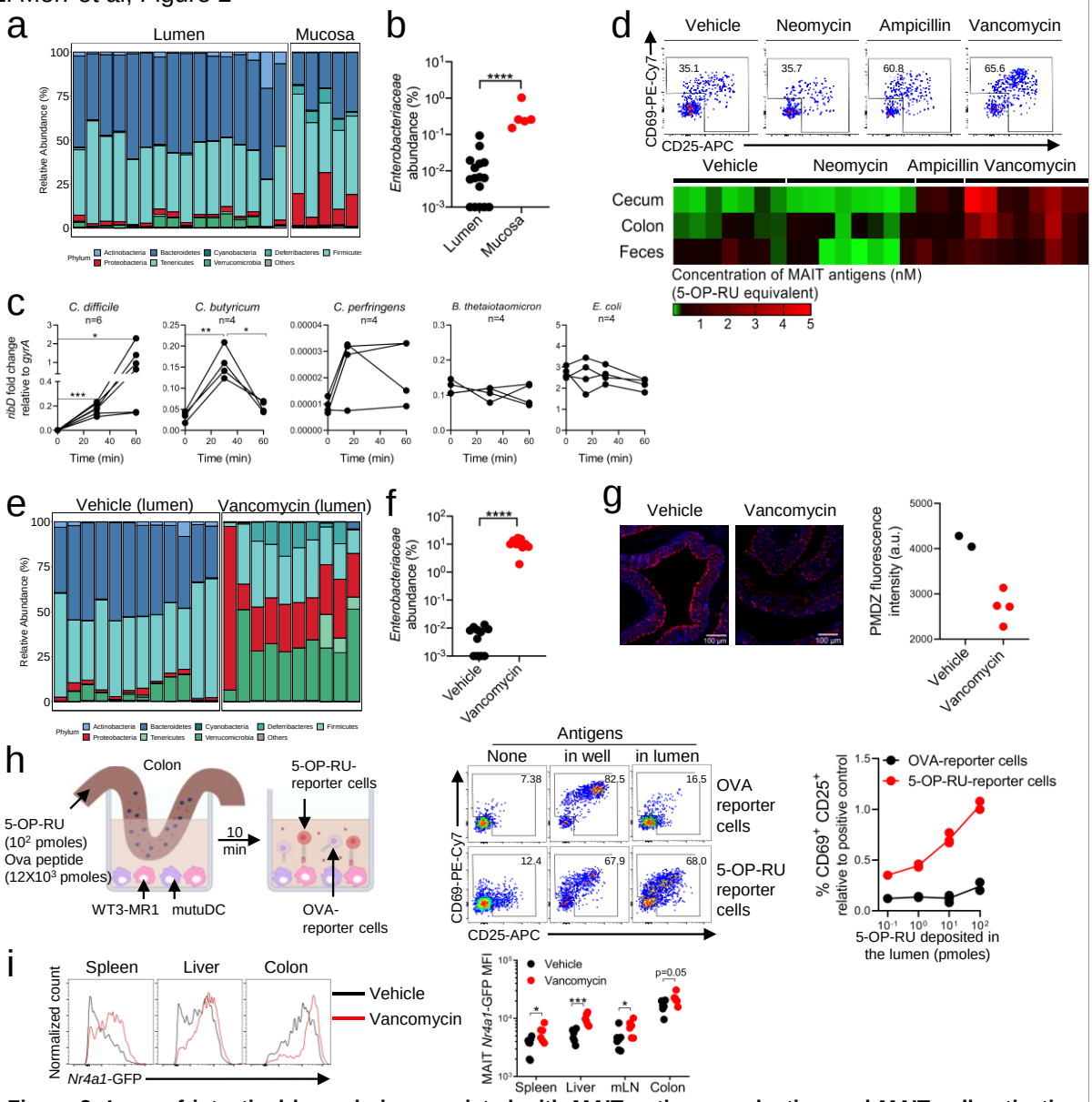


Figure 2: Loss of intestinal hypoxia is associated with MAIT antigen production and MAIT cell activation. **a,b.** Abundance of the indicated bacterial phyla (**a**) and of the *Enterobacteriaceae* family (**b**) in the cecal lumen and mucosa based on 16S rRNA gene sequencing (columns and dots indicate individual mice; data pooled from two independent experiments, except for mucosa). **c.** Relative *ribD* expression in the indicated bacterial strains grown anaerobically and placed in aerobiosis for the indicated period of time (data from 4-6 biological replicates). **d.** Top: Activation of MR1:5-OP-RU-specific reporter T cells after overnight incubation with WT3-MR1 cells pulsed with luminal contents from the cecum of indicated mice. Bottom: MAIT antigen concentration in the indicated conditions as estimated using synthetic 5-OP-RU (columns indicate individual mice; data pooled from two independent experiments). **e,f.** Abundance of the indicated bacterial phyla (**e**) and of the *Enterobacteriaceae* family (**f**) in the cecal lumen of indicated mice (n= 10-12 mice per group; data pooled from two independent experiments). **g.** Left: Representative images showing pimonidazole (PMDZ) adduct (red) and DNA (blue) staining of the colon of indicated mice. Right: Quantification of PMDZ adduct staining at the epithelium surface of the colon (each dot is one mouse). **h.** Left: Scheme of the assay used to assess intestinal permeability to antigens. Middle: Activation of the indicated reporter splenocytes after incubation in the indicated condition. Right: Summary of the results after luminal administration of 5-OP-RU at the indicated concentration (data pooled from two independent experiments). **i.** Left: *Nr4a1*-GFP expression by MAIT cells from the indicated tissues of control (vehicle) or mice treated with vancomycin for 4d. Right: Pooled data from two independent experiments (n= 6-7 mice per group). *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 by Mann-Whitney *U* tests (**b** and **f**) or by Student's *t* tests (**c** and **i**).

El Morr et al, Figure S2

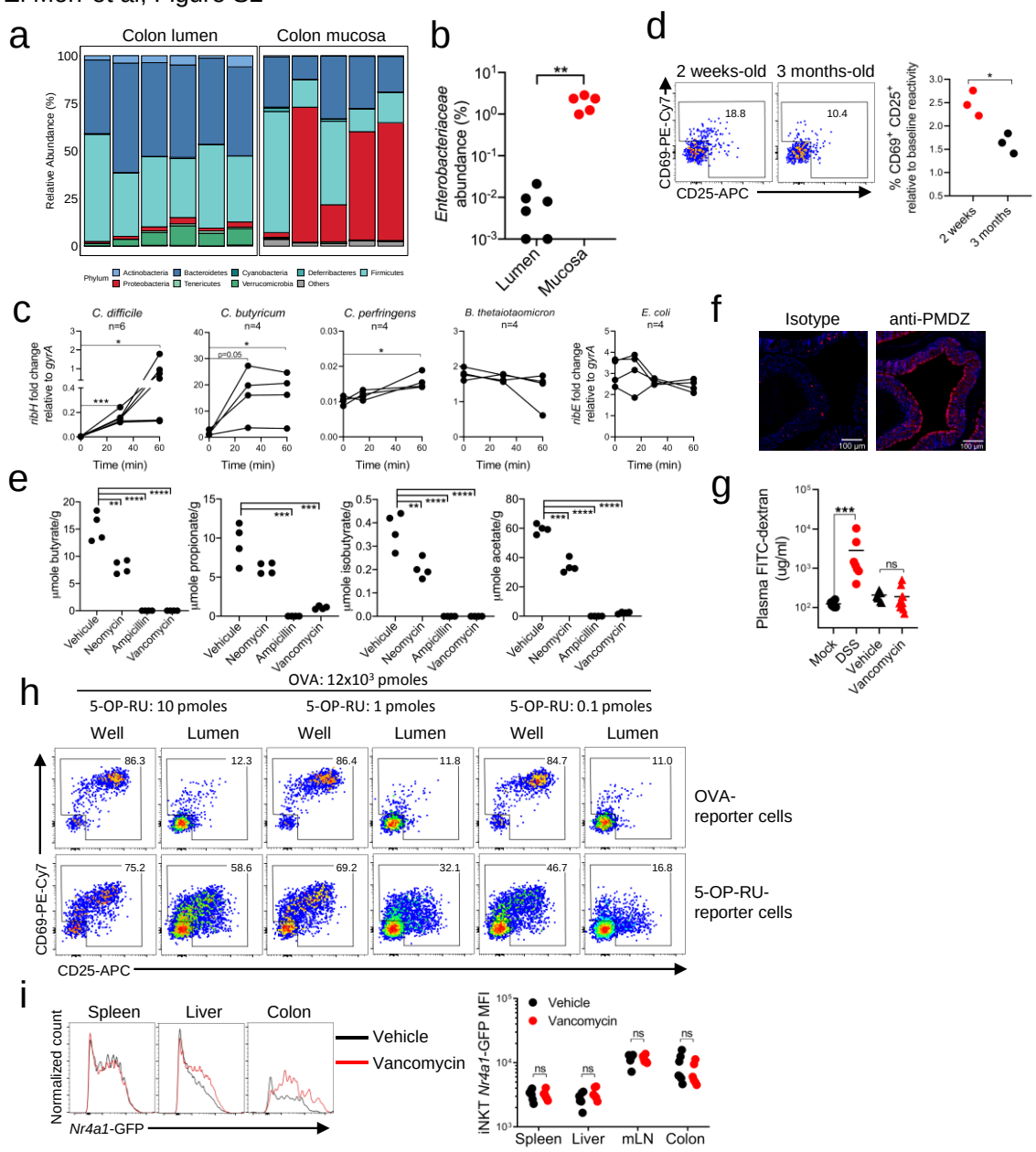


Figure S2: Loss of intestinal hypoxia is associated with MAIT antigen production and MAIT cell activation. **a,b.** Abundance of the indicated bacterial phyla (**a**) and of the *Enterobacteriaceae* family (**b**) in the colon lumen and mucosa based on 16S rRNA gene sequencing (columns and dots indicate individual mice; data pooled from two independent experiments, except for mucosa). **c.** Relative *ribH/E* expression in the indicated bacterial strains grown anaerobically and placed in aerobiosis for the indicated period of time (data from 4-6 biological replicates). **d.** Left: Activation of MR1:5-OP-RU-specific reporter T cells after overnight incubation with WT3-MR1 cells pulsed with luminal contents from the colon of mice at the indicated age. Right: Summary of the results (n =3 mice per group). **e.** Amounts of the indicated short chain fatty acids in the cecum of control (vehicle) or antibiotic-treated mice (n = 4 mice per group). **f.** Representative images showing staining of colon from a control (vehicle) mouse with control or anti-PMDZ antibody (red). DNA staining with DAPI is in blue. **g.** FITC-dextran concentration in the plasma of the indicated mice following oral administration, (n = 7-10 mice per group; data pooled from 2 independent experiments). **h.** Activation of the indicated reporter splenocytes in the assay described in Fig. 2f after deposition of the indicated doses in the colon lumen or directly on antigen-presenting cells. **i.** Left: *Nr4a1*-GFP expression by iNKT cells from the indicated tissues. Right: Pooled data from 2 independent experiments (n= 6-7 mice per group). ns p>0.05, *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 by Mann-Whitney *U* (**b** and **g**) or by Student's *t* tests (**c**, **d**, **e,** and **i**).

El Morr et al, Figure 3

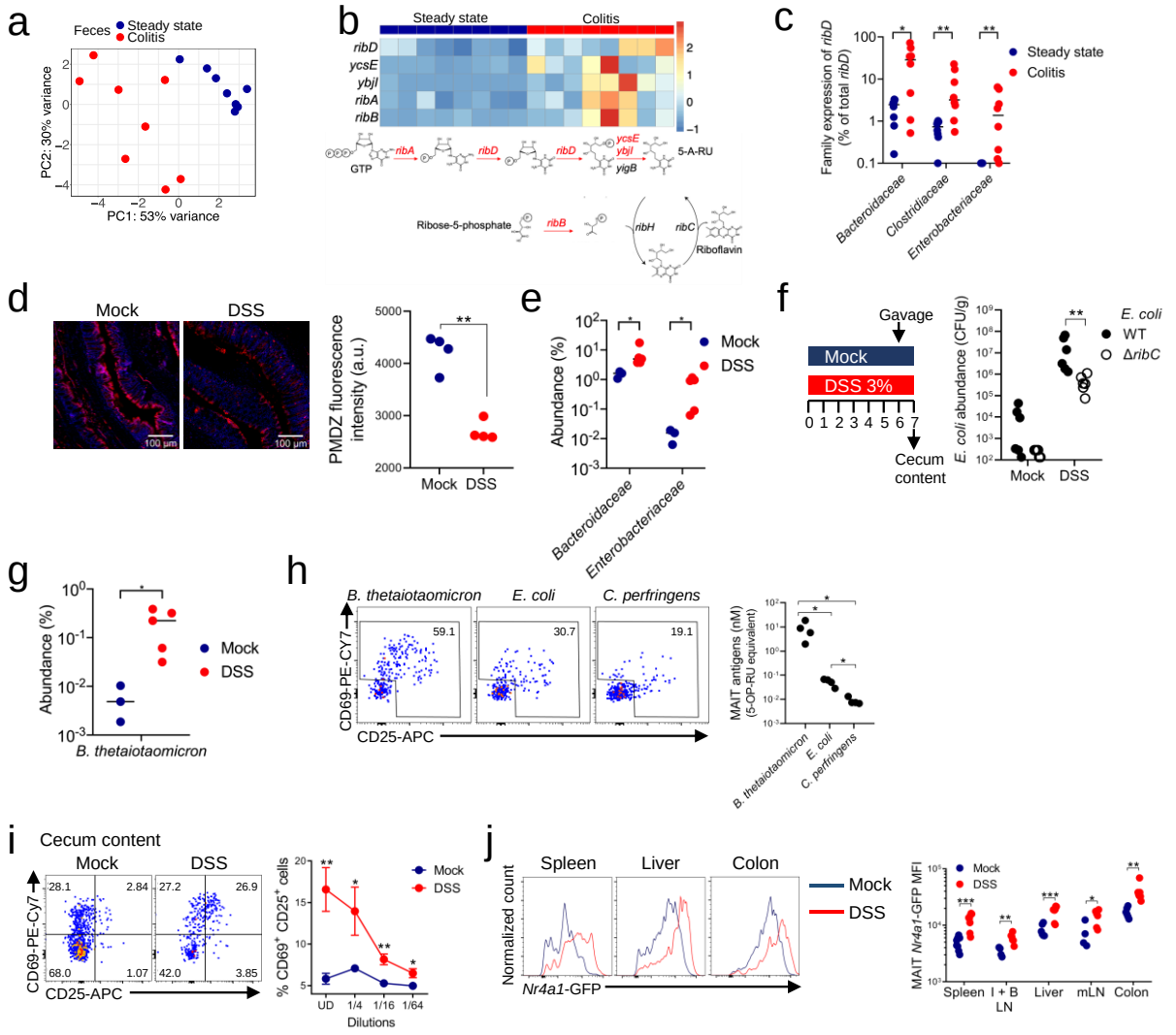


Figure 3: Up-regulation of the riboflavin biosynthetic pathway during colitis a. Principal component analysis (PCA) of the riboflavin biosynthesis pathway genes expressed in the feces before (steady state, blue) or after helicobacter-induced colitis (red) (n= 8 mice); in **a-c**, original data are from ref 31. **b.** Top: Heatmap showing the genes of the riboflavin biosynthetic pathway found up-regulated in the feces upon colitis induction (p<0.01 by Mann-Whitney *U* test). Bottom: Scheme of the riboflavin biosynthesis pathway. The genes found up-regulated during colitis are in red. **c.** Proportion of *ribD* expression contributed by the indicated bacterial families during colitis as compared with steady-state. Only families contributing 2% or more of total *ribD* expression are shown. **d.** Left: Representative images showing PMDZ adduct (red) and DNA (blue) staining of the colon from control (Mock) and DSS-treated mice. Right: Quantification of PMDZ adduct staining at the epithelium surface of the colon (each dot represents one mouse). **e.** Abundance of the *Bacteroidaceae* and *Enterobacteriaceae* families in the cecum of the indicated mice as determined by 16S rRNA gene sequencing. **f.** Left: Experimental strategy. Right: Numeration of the indicated isogenic WT or Δ*ribC E. coli* strains after administration to control (Mock) or DSS-treated mice (n= 6 mice per group; data pooled from two independent experiments). **g.** Abundance of *B. thetaiotaomicron* in the cecum of the indicated mice, as determined by 16S rRNA gene sequencing. **h.** Left: Activation of MR1:5-OP-RU-specific reporter T cells after overnight incubation with WT3-MR1 cells pulsed with supernatant from the indicated bacterial cultures. Right: MAIT antigen concentration in the supernatant of the indicated bacterial cultures as estimated using 5-OP-RU (data from 4 biological replicates). **i.** Left: Activation of reporter T cells after incubation with WT3-MR1 cells pulsed with 50 mg of cecal contents from control (Mock) or DSS-treated mice. Right: Pooled data from two independent experiments (means ± SEMs, n= 6 mice per group). **j.** Left: *Nr4a1*-GFP expression in MAIT cells from the indicated tissues. Right: Data pooled from three independent experiments. *p<0.05, **p<0.01 and ***p<0.001 by Mann-Whitney *U* (**c, d, e, f, g, h,** and **j**) or by Student's *t* tests (**i**).

El Morr et al, Figure S3

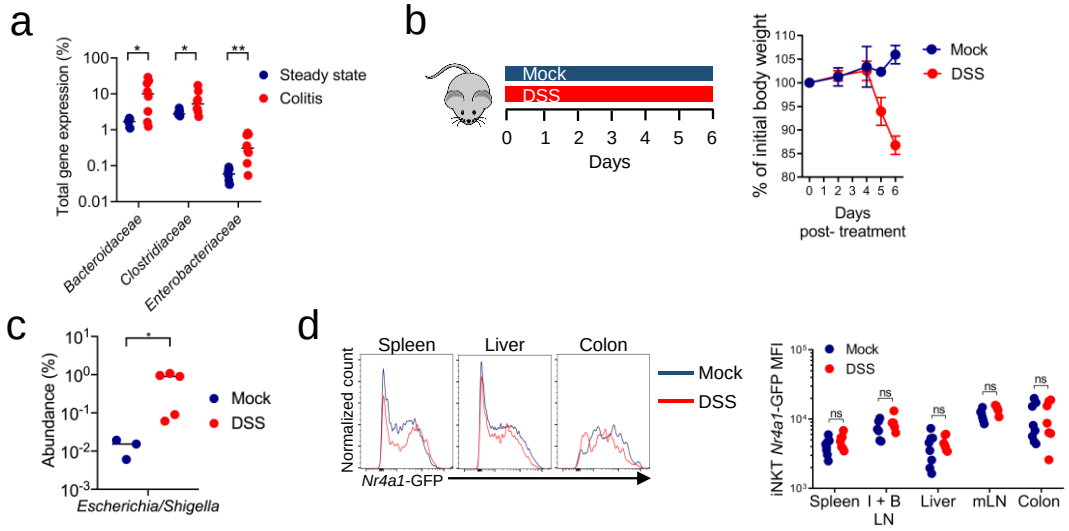


Figure S3: Up-regulation of the riboflavin biosynthetic pathway during colitis **a.** Contribution (in %) of the indicated bacterial families to total gene expression in the feces of mice before or after helicobacter-induced colitis. Original data are from ref 31. **b.** Scheme of the acute DSS model of colitis. Mouse weight as percentage of initial body weight (means ± SEMs, n=6 mice per group). **c.** Abundance of the *Escherichia and Shigella genera* in the cecum of the indicated mice as determined by 16S rRNA gene sequencing. **d.** Left: *Nr4a1*-GFP expression of iNKT cells from the indicated tissues. Right: Pooled data from three independent experiments. ns $p>0.05$, *$p<0.05$ and **$p<0.01$ by Mann-Whitney *U* tests.

El Morr et al, Figure 4

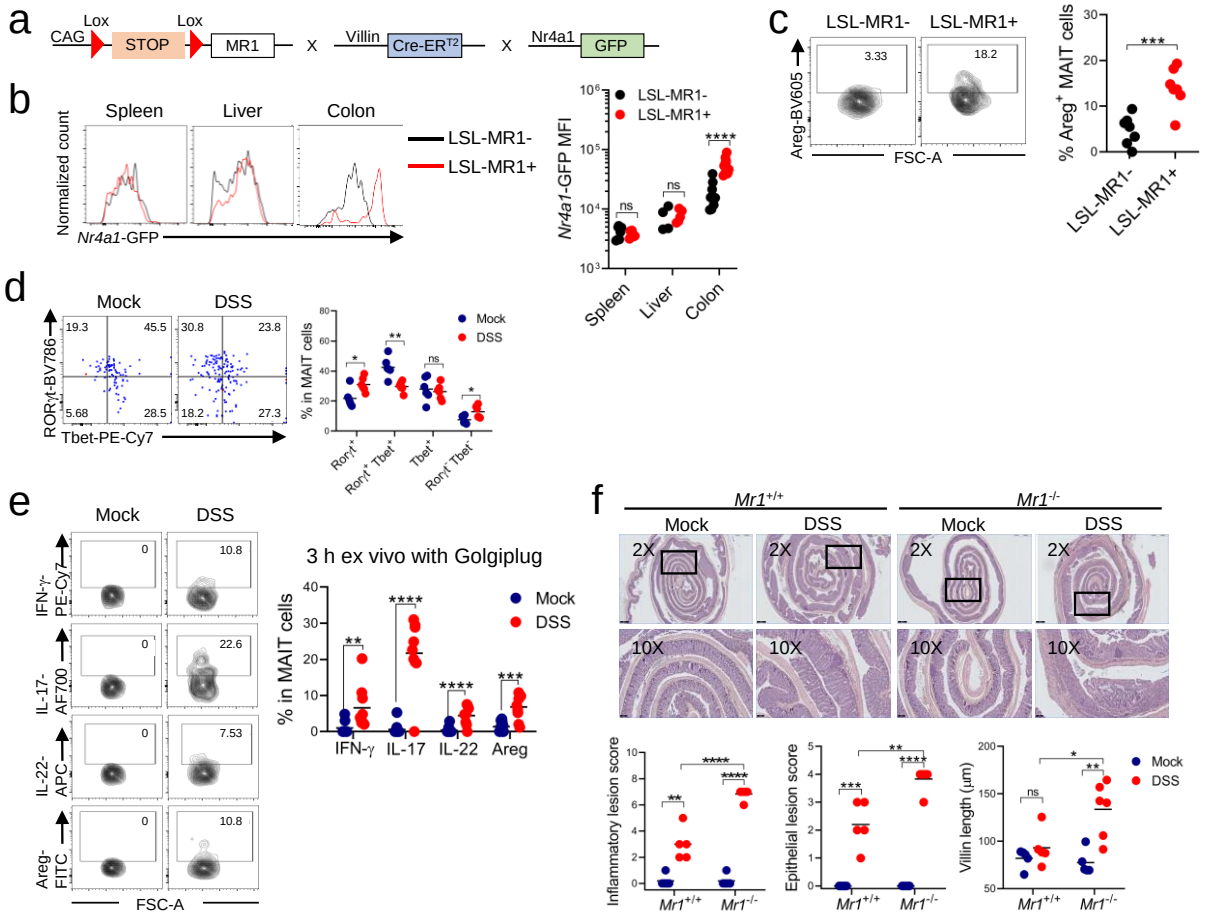


Figure 4: MAIT cells protect against colitis. a. Scheme of the lox-stop-lox-MR1 transgenic construct. **b.** Left: *Nr4a1*-GFP expression by MAIT cells from the indicated tissues. Both LSL-MR1$^+$ and LSL-MR1$^-$ mice received tamoxifen injections. Right: Pooled data from four independent experiments. **c.** Left: Areg expression by colonic MAIT cells from the indicated mice. MAIT cells were stained for Areg without *in vitro* re-stimulation. Right: Pooled data from two independent experiments (n= 7 mice per group). **d** and **e.** Left: Representative staining for the indicated proteins of colonic MAIT cells from control (Mock) mice and from mice with chronic colitis. Right: Percentages of colonic MAIT cells expressing the indicated transcription factors **(d,** n=6 mice per group; representative of two independent experiment**)** and cytokines **(e,** n=9-11 mice per group; pooled data from two independent experiments**)** without *in vitro* re-stimulation. **f.** Top: Hematoxylin and eosin staining of the colon of the indicated control (Mock) mice and mice with chronic colitis. Representative 2X and 10X magnifications are shown. Bottom: Summary of the investigator-blinded inflammatory and epithelial lesion scores assessments in the indicated conditions. Summary of villi length measured in the indicated conditions (n=5-7 mice per group; data representative of three independent experiments). *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 by Mann-Whitney *U* (**b**) or by Student's *t* tests (**c, d, e** and **f**).

El Morr et al, Figure S4

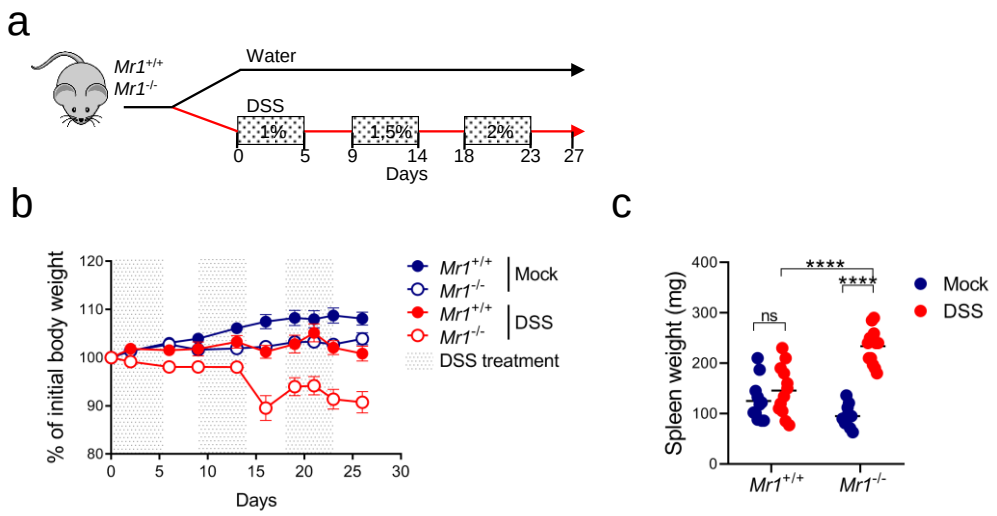


Figure S4: MAIT cells protect against colitis. a. Scheme of the chronic DSS-induced colitis model. **b.** Weight of the indicated mice following mock or DSS treatment. **c.** Summary of spleen weight in *Mr1*$^{+/+}$ and *Mr1*$^{-/-}$ mice in the indicated conditions; in **b** and **c,** n=12-13 mice per group; data are representative of three independent experiments. *p<0.05, ns p>0.05, ****p<0.0001 by Student's *t* tests.

Methods

Mice

B6 and CD45.1 mice were purchased from Charles River Laboratories. iVα19 and iVα19 Vβ8 TCR transgenic Cα$^{-/-}$ B6 mice, B6-MAIT^{Cast} mice have been described[12,38]. *Nr4a1*-GFP mice[14] were imported from the MMRRC (UC Davis). OTII mice were maintained on a RAG$^{-/-}$ background. All mice were bred and maintained on a standard chow diet in SPF conditions at Institut Curie. Prior to inclusion in experimental groups, mice from different litters were co-housed, or cage contents were mixed for at least two weeks in order to normalize microbiota. All experiments were performed according to national and international guidelines and were approved by the Institut Curie Ethic committee (APAFIS#25203-2020042318109119, #24245-2020021921558370 and #IOI68-2017060909448377).

To generate Lox-stop-Lox-MR1 (LSL-MR1) transgenic mouse, a DNA segment containing a stop cassette flanked with loxP sites upstream of the murine *Mr1* gene was prepared by digestion of the pOD2G-LS CAGGS (7279 bp) plasmid[39] (a gift from J. J. Moon, Massachusetts General Hospital) with the enzymes HindIII and SpeI. Efficient induction of MR1 protein expression under the control of the chicken actin promoter was validated in HEK293 cells co-transfected with a Cre expression vector (data not shown). The construct was then injected into pronuclei of fertilized oocytes. Founders were crossed to transgenic mice bearing a tamoxifen-dependent Cre recombinase expressed under the control of the villin promoter[35].

Antibiotic treatments, colitis induction, and tamoxifen administration

Female mice aged 6-10 weeks at the start of the experiments were used throughout the study. For microbiota depletion, mice were given four antibiotics (vancomycin (0.5 g/l, Mylan), ampicillin (10 g/l, Coophavet), metronidazole (1 g/l, B Braun) and neomycin (1g/l, Coophavet) in the drinking water supplemented with 2% sucrose for 7 days. Single antibiotics were also provided for 7 days or for the indicated period of time. Fresh antibiotic solutions were provided after 3 days of treatment. For fungi depletion, fluconazol (0.5 mg/ml, Sigma-Aldrich) was provided in the drinking water for 7 days.

Acute colitis was induced in mice by adding 2% (wt/vol) DSS (molecular weight: 36,000–50,000 Da; MP Biomedicals) to the drinking water for 7 days or until mice had lost 15% of initial weight. For bacterial gene amplification and sequencing, DSS-treated mice were switched to normal drinking water 1 day prior to the end of the experiment to reduce DSS concentration from samples and avoid PCR inhibition. For modeling chronic colitis, mice received 3 to 5 5-day

treatments with DSS. Each DSS treatment was followed by 4 days with water only. Control groups (Mock) received only water.

For inducible MR1 over-expression, transgenic mice were injected intraperitoneally with 2 mg tamoxifene (T5648, Sigma-Aldrich) in a corn oil-ethanol mixture once per day for 3 consecutive days. Tissues were collected 24 h after the last injection.

Parabiosis

Parabiosis surgery was performed as described previously[40]. Briefly, age- and weight-matched CD45.1/2 and CD45.2/2 B6-MAIT^{Cast} female mice were surgically joined through the abdominal skin, olecranon and knee joints. Parabiotic pairs were euthanized 5 weeks later.

Cell isolation

Spleens were mashed over 40 µm cell strainer to create single cell suspensions followed by red blood cell lysis. Livers were perfused with PBS through the portal vein, mashed over 100 µm cell strainer, and centrifuged over Histopaque 1077 (Sigma) layers to isolate the lymphocyte-containing fraction. Lymph nodes were incubated with collagenase D (1 mg/ml; Roche) and DNAse I (100 ug/ml; Roche) for 30 min at 37°C, and mashed over 30 µm filters (Miltenyi).

Colons were opened longitudinally and cut into approximately 0.5 cm pieces. Dissociation of epithelial cells was performed by incubation with constant stirring in HBSS w/o Ca/Mg (Life Technologies) containing 5 mM EDTA (5mM; Thermo Fisher Scientific), DTT (1mM; Euromedex), and 5% FCS twice for 20 min at 37 °C. After each step, samples were vortexed and the epithelial fraction discarded. Tissue fragments were then washed in HBSS and enzymatic digestion was performed in CO_2-independent medium (Life Technologies) containing collagenase D (1 mg/ml; Roche), liberase TM (0.17 U/ml; Roche) and DNase I (100 ug/ml; Roche) on a shaker for 30 min at 37 °C. Colon fragments were then dissociated with a gentleMACS dissociator according to the manufacturer's instructions (Miltenyi). Lymphocytes were collected at the interface of a 40%/80% Percoll gradient (GE Healthcare).

Tetramers and flow cytometry

CD1d tetramers conjugated to BV421, and MR1 tetramers loaded with 5-OP-RU and conjugated to PE were provided by the National Institutes of Health (NIH) tetramer core facility (Emory University). Cell suspensions prepared from various tissues were stained in PBS, 2% FCS, 2 mM EDTA complemented with rat anti-mouse CD16/CD32 antibody (clone 2.4G2 produced in-house) to block non-specific binding to Fcγ receptor.

Tetramer staining was performed at room temperature for 30 min. Surface staining was performed at 4 °C with fluorescently labeled antibodies and fixable viability dye eFluor780 (eBioscience, Hatfield, UK).

Antibodies were purchased from Biolegend, eBioscience, BD, Invitrogen or R&D Systems. The following antibodies were used for surface staining: anti-CD19 (1D3), -B220 (RA3-6B2), -CD11c (N418), -CD5 (53-7.3), -TCRβ (H57-597), -CD24 (M1/69), -CD44 (IM7), -CD45.1 (A20), -CD45.2 (104), -CD4 (RM4-5), -CD8 (53-6.7), -CD69 (H1.2F3), -CD25 (PC61), -Vα2 (B20.1).

Fixation and permeabilization were performed for intranuclear staining (FoxP3 staining kit; eBioscience) and intra-cytoplasmic staining (Cytofix/Cytoperm kit; BD Biosciences) according to the manufacturer's instructions. For intranuclear staining, cells were stained with anti-RORγt (Q31-378) and -Tbet (4B10) for 20 min at 4 °C. For intra-cytoplasmic staining, cells were cultured for 3 h at 37 °C in complete RPMI in the presence of GolgiPlug (1:1000; BD). Cultured cells were subsequently stained for surface antigens as above. Cells were fixed and permeabilized, washed, and incubated for 30 min at 4 °C with anti-IL17 (TC11-18H), -IL22 (IL22JOP) and -IFN-γ (XMG1.2). Cells were then stained overnight with anti-amphiregulin-biotin (BAF989) followed by FITC- or BV605-conjugated streptavidin.

In some experiments, non-fixed dead cells were stained with the DAPI staining solution and directly analyzed by flow cytometry. Cells were acquired either on Cytoflex (Beckman), LSRII (BD Biosciences) or Fortessa (Becton Dickinson), and the results were analyzed with FlowJo V10.2 (Treestar).

5-OP-RU preparation

5-A-RU was synthesized as described[16]. For preparation of 5-OP-RU, 5-A-RU was incubated with equimolar amounts of methylglyoxal (Sigma) for 10 min at room temperature.

***In vitro* validation of the *Nr4a1*-GFP mouse**

Splenic T cells from a transgenic *Nr4a1*-GFP mouse were isolated by negative cell enrichment using the Magnisort mouse T cell enrichment kit (Thermo Fisher Scientific). Isolated cells were cultured at a density of $20x10^6$ /ml in complete RPMI and stimulated with either recombinant mouse IL-12 (10 ng/ml) and IL-18 (12.5 ng/ml) or with 10 nM 5-OP-RU in the presence of murine MR1-overexpressing WT3 (WT3-MR1 cells, $5x10^6$/ml)[41] for 16h at 37°C. GolgiPlug was added during the final 6h of culture. MAIT cells were then analysed for *Nr4a1*-GFP expression and IFN-γ production.

In vitro detection of MAIT antigens

To detect intestinal MAIT antigens, 50 mg or 100 mg of luminal contents were collected from the cecum or colon. Mucosal scrapings were sampled by scraping biomass from intestinal walls with a sterile scalpel. Feces were collected from cage floors immediately after defecation. Samples were weighted and dissolved in complete RPMI medium (RMPI without folic acid supplemented with 10% FCS, penicillin and streptomycin), and kept on ice. Tubes were vortexed to homogenize the mixture and centrifuged at maximum speed for 1 min at 4°C. Supernatants were filtered through a 0.22-µm-pore-size filter and loaded at different dilutions on WT3-MR1 cells for 2h30 min at 37°C.

To detect MAIT antigens in bacterial culture supernatants, *Bacteroides thetaiotaomicron, Escherichia coli* and *Clostridium perfringens* grown to late exponential phase in an anaerobic chamber were centrifuged at 14000 g for 30 s. The supernatant of each sample was collected and flash-frozen in liquid nitrogen. Supernatants were thawed and incubated with WT3-MR1 cells at 37°C for 2h 30 min.

WT3-MR1 cells were then washed and co-cultured overnight at a 1:1 ratio with splenocytes from a Vα19 TCR Tg $Cα^{-/-}$ or Vα19Vβ8 TCR Tg $Cα^{-/-}$ mouse. Cells were then harvested and stained for CD25 and CD69 expression on 5-OP-RU:MR1-specific T cells. MAIT antigen concentrations were estimated using a standard curve prepared with serial dilutions of synthetic 5-OP-RU.

Intestinal permeability to 5-OP-RU

75 µl of PBS containing the indicated amounts of mixed OVA 323-339 peptide (Invivogen) and 5-OP-RU were added into the lumen of freshly isolated colons from healthy mice. Colon segments were then immersed in complete RPMI medium at 37°C in a well on top of mixed MutuDC and WT3-MR1 cells, for OVA and 5-OP-RU presentation, respectively. As positive control, antigen solutions were also added directly on top of mixed antigen presenting cells. Colon segments were removed from the wells after 10 min of incubation. For the measurement of antigen-presentation, splenocytes from a Vα19 TCR Tg $Cα^{-/-}$ mouse and from a OTII-$Rag1^{-/-}$ mouse were cultured overnight with antigen presenting cells at respectively 1:1 and 1:10 ratios. Reporter splenocytes were then harvested and stained for activation markers.

Intestinal sample collection for nucleic acids extraction

After sacrifice, the intestinal tract was quickly removed and splayed open through a longitudinal incision. Luminal contents and mucosal scrapings were collected from the cecum and the colon.

Intestinal contents were resuspended in ATL lysis buffer (Qiagen) and bacteria were lysed using a Fast Prep Bead beater (MP Biomedicals) for 3 times 1 min at 6.5 m/s. Genomic DNA was purified using the Qiamp DNA Mini Kit (Qiagen).

16S rRNA gene PCR amplification and sequencing

16S rRNA gene sequencing was performed in-house or at the University of Minnesota Genomics center with similar results. In-house library preparation was performed as described[42]. Briefly, a single PCR step was used for amplification of the V3-V4 region of the 16S rRNA gene and for dual-index labelling of each sample. PCR amplification was performed using the Accuprime Pfx DNA polymerase (ThermoFisher). PCR products were purified (AMPure XP beads, Beckman Coulter) and sequenced on MiSeq (Illumina, USA) to generate paired-end 300-bp reads.

Bioinformatic analysis of 16S rRNA sequencing data

Adapter sequences were trimmed from the raw fastq files using Cutadapt v 2.10[43]. Subsequent analyses were performed using a modified DADA2 microbiome pipeline v 1.12.11[44] (https://benjjneb.github.io/dada2). In brief, raw sequence reads were first filtered using DADA2 default parameters. Filtered reads were then dereplicated and denoised using DADA2 default parameters. The DADA2 pipeline produced the non-chimeric ASV table containing the frequency with which each ASV was present in each sample. Taxonomic assignment of selected ASVs was performed against the SILVA reference database (v 132 - https://www.arb-silva.de)[45].

Downstream analyses, statistical tests, and visualization were performed using different packages in the R environment v 4.0.4 (R Core Team 2019). To search for differentially abundant taxa and differentially expressed genes among different treatments, locations, and layers, DESeq2 (v 1.24.0)[46] was used by performing the Wald significance test.

Metatranscriptomic data analysis

Metatranscriptomic datasets[32] stored under the accession number E-MTAB-4082 were downloaded from EBI ENA. Paired end reads were quality checked and trimmed using TrimGalore v0.6.6 (https://github.com/FelixKrueger/TrimGalore). Host reads were removed by aligning metatranscriptomes against mouse genome (http://ftp.ensembl.org/pub/release-102/fasta/mus_musculus/dna/ Mus_musculus.GRCm38.dna.primary_assembly.fa.gz) using STAR v2.7.7[47]. Ribosomal reads were removed from metatranscriptomic datasets using

sortmerna v4.2.0[48]. Finally, remaining non ribosomal reads were mapped against the iMGMC mouse gene catalogue[49] using bwa v0.7.17-r1188 with default parameters. Read counts aligned to the iMGMC gene catalogue were obtained from bam files processed with featureCounts v2.0.1[50]. Read counts table were parsed using in-house custom scripts to extract rib gene expression data used as input for DESeq2 analysis. Heatmaps were generated using the pheatmap R package v 1.0.12.

Estimation of bacterial number in intestinal samples

Total bacterial numbers from intestinal samples were estimated by TaqMan qPCR quantification of bacterial 16S rRNA (LightCycler 480 Instrument II, Roche). The universal 16S rRNA gene primers and probe sequences used in this study are listed in table S1. A standard curve was built using *Escherichia coli* DNA to convert the threshold cycle (Ct) values into colony-forming unit (CFU) equivalents.

Bacterial cultures

All bacterial strains were first grown anaerobically (5% H_2, 5% CO_2, 90% N_2). *Clostridium butyricum* VPI13266 (Virginia Polytechnic Institute Culture Collection, Blackburn, Va.), *C. difficile* 630Δ*erm*[51]*, C. perfringens* SM101[52]*,* and *E. coli* MG1655[53] in Brain Heart Infusion (BHI, Difco) and *B. thetaiotaomicron* VPI-5482[54] (collection of S. Rabot, INRAE, Jouy en Josas, France) in M17 (Difco) supplemented with glucose 0.5 % and hemin 5 µM (Sigma-Aldrich). An overnight liquid preculture was diluted in 10 ml fresh medium 1/1000 for *E. coli* and *C. perfringens,* 1/50 for the other strains and grown anaerobically until late exponential growth phase. After respectively 3 h for *E. coli* and *C. perfringens,* 8 h for *B. thetaiotaomicron* and 6 h for the other strains, cultures were distributed into 24-well plates, 1 mL per well and placed in aerobiosis at 37°C. Bacteria were harvested after 0, 30 or 60 min air-exposure and fast frozen in liquid nitrogen. Bacterial RNAs were extracted, cDNAs synthesised and real-time quantitative PCRs performed as previously described[55,56] using the primers listed in table S1. The relative change in gene expression was given by the [ΔC_T] method[57] with cDNA quantity of the *rib* genes normalised to the *gyrA* gene.

***In vivo* expansion of *E. coli* during DSS treatment**

Six days after the start of DSS treatment (3%), B6 mice *were* intragastrically inoculated with 10^9 CFUs of *E. coli* wild-type strain carrying an ampicillin-resistance plasmid, or with the isogenic *ΔribC* strain resistant to chloramphenicol. The mice were euthanized after one day.

Cecum contents were collected in sterile phosphate buffered saline for bacterial counts. The bacterial loads of *E. coli* were determined by plating serial 10-fold dilutions on LB plates containing the appropriate antibiotic and supplemented with 20 µg/mL of riboflavin.

Short-chain fatty acid (SCFA) analysis

SCFA analysis was carried out as described previously[58], with slight modifications. Samples were water-extracted and proteins were precipitated with phosphotungstic acid. A volume of 0.3 µl of the supernatant fraction was injected onto a gas–liquid chromatograph (Agilent 6890; Les Ulis, France) equipped with a split-splitless injector, a flame-ionization detector and a capillary polyethylene glycol column (15 m x 0.53 mm, 0.5µm). Carrier gas (H_2) flow rate was 10 ml/min and inlet, column and detector temperatures were 200°C, 100°C and 240°C, respectively. Between each sample injection, the column was cleaned by increasing the temperature from 100°C to 180°C (20°C/min) followed by holding at 180°C for 2 min before returning to 100°C for the next analysis. 2-Ethylbutyrate was used as an internal standard. Samples were analyzed in duplicate. Data were collected and peaks integrated with the OpenLab CDS Chemstation Edition software (Agilent, Les Ulis, France).

Detection of intestinal hypoxia

Pimonidazole (PMDZ) (Sigma-Aldrich) was administered at 60 mg/kg by intraperitoneal injection 1h prior to sacrifice. Colon samples were fixed in formalin solution 10% neutral buffer (Sigma-Aldrich). Paraffin-embedded sections were blocked with protein block (Dako, X0909) and treated with avidin/biotin blocking reagents (Dako, X0590) followed by overnight incubation at 4°C with mouse monoclonal anti-pimonidazole IgG1 (Hypoxyprobe^{TM}-1) at 1.2 µg/ml of concentration according to the manufacturer's instructions. Negative control sections were treated identically except that primary antibody was substituted with control antibody (BD) for overnight incubation. All sections were then incubated for 30 min with *biotinylated* anti-mouse *IgG* secondary *antibody* (Vectastain) and then stained for 30 min with Alexa546-conjugated Streptavidin (ThermoFisher). Tissues were counterstained with DAPI. PMDZ staining was quantified using Fiji and plotted as fluorescence intensity per 400 µm ^{2}.

FITC-Dextran permeability assay

Mice were given an oral gavage of FITC-dextran (4 kDa; Sigma-Aldrich FD4) solution at 60 mg/100 g body weight. FITC fluorescence pre- and 4h post-gavage was measured in plasma, and FITC dextran concentrations were calculated with the help of a standard curve prepared with serial dilutions of FITC-dextran in PBS.

Assessment of intestinal pathophysiology

In chronic DSS experiments, the spleen and colon were dissected from each mouse. Individual spleen tissues were weighed. The entire colon was fixed in formalin solution 10% neutral buffer, embedded in paraffin and stained with hematoxylin and eosin (H&E). H&E-stained sections of the colon were analysed for colitis severity in an investigator-blinded manner. Epithelial damage (architectural lesion), inflammatory cell infiltration and villus length were scored using the histopathological scoring system described previously[59].

Statistical analyses

Statistical analyses and graphical representations related to the microbiome pipeline were conducted in R (v 4.0.4) (R Core Team 2019) with the R package ggplot2 v 3.2.0. Inkscape 0.92 was used to finalize the figures. All other statistical analyses were performed with Prism software (GraphPad). Two-tailed *P* values were determined using Wilcoxon's and Mann–Whitney's tests for paired and non-paired samples, as appropriate.

Data Availability

16S rRNA amplicon sequencing data generated in this study are available on the EBI ENA under the accession number PRJEB44022.

Table S1:
Primers and probe for real-time PCR used in this study.

Primers and probe	Sequence (5'– 3')
Universal 16S rRNA[60]	
Forward	TGGAGCATGTGGTTTAATTCGA
Reverse	TGCGGGACTTAACCCAACA
Probe	CACGAGCTGACGACA(AG)CCATGCA
Clostridioides difficile	
ribD Forward	GGAAAACGCCTCCTTGTGTA
ribD Reverse	TGCCTCATTTAAATCTCTTGCTT
ribH Forward	TTGGGTACCTGGAGCTTTTG
ribH Reverse	TTCCAGCTTTTGTACCTGCTC
gyrA Forward	TTTTGTTGTGTCTATGAACCTTTGT
gyrA Reverse	TCCTTTACCAGCTCTTATTTGACTT
Clostridium perfringens	
ribD Forward	TTGGAGGAAATCATGCTGAA
ribD Reverse	GGATTTTGGTCTAAGGTTCCA
ribH Forward	CTTGGGTTCCAGGAGCTTTT
ribH Reverse	CAGCTTTAGTTCCGGCTCTTT
gyrA Forward	CCAAACTTTGATGGTGAGGAA
gyrA Reverse	CCTGCTGTAGGGAAATCTGG
Clostridium butyricum	
ribD Forward	GCATGCTCATGCTGAAAGAA
ribD Reverse	CTGCCACCAAAGGATTAGGA
ribH Forward	TATAGCGTGGGTTCCAGGAG
ribH Reverse	TACGCTTGCAATTCCCTTTG
gyrA Forward	CATCGGGAATAGCTGTTGGT
gyrA Reverse	CACCTGTTGGGAAATCTGGT
Bacteroides thetaiotaomicron	
ribD Forward	CGGCAATCCCGTAGTACTGT
ribD Reverse	GGTGGGTACTTCTCCGTCAA
ribH Forward	TACCGGAGCCTTATTGAACG
ribH Reverse	TCCTTGAGTCGCTCCCATAC
gyrA Forward	GTACCATCCGCATGGAGACT
gyrA Reverse	AGGTCATCCATCATCGCTTC
Escherichia coli	
ribD Forward	AGGACGTTTTACCACGCATC
ribD Reverse	ATGGCTACAGGGTTCGAGTG
ribE Forward	GGGTACGGTTATTCGTGGTG
ribE Reverse	GGCCTTGATGGCTTTCAATA
gyrA Forward	ATGAACTGATGGCCGATCTC
gyrA Reverse	ATGTGTTCCATCAGCCCTTC

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Competing interests: The authors declare no competing interests.

Author contributions

Y.E.M. designed the project, performed experiments, analyzed data, and wrote the paper. A.V. performed the bioinformatic analysis. K.F., C.M., A.D.H., and S.R. designed and performed experiments. B.J., V.P., M.S., C.P. and A.D. performed experiments. M.M. analysed data. S.M. performed bioinformatic analysis and provided advice. O.L. supervised the project, designed experiments, analyzed data and edited the paper. F.L. designed and supervised the project, analyzed data and wrote the paper.

References

1. Corbett, A. J. *et al.* T-cell activation by transitory neo-antigens derived from distinct microbial pathways. *Nature* **509**, 361–365 (2014).
2. Legoux, F., Salou, M. & Lantz, O. MAIT Cell Development and Functions: the Microbial Connection. *Immunity* **53**, 710–723 (2020).
3. Serriari, N.-E. *et al.* Innate mucosal-associated invariant T (MAIT) cells are activated in inflammatory bowel diseases. *Clin. Exp. Immunol.* **176**, 266–274 (2014).
4. Haga, K. *et al.* MAIT cells are activated and accumulated in the inflamed mucosa of ulcerative colitis: MAIT cell reflects mucosal inflammation. *J. Gastroenterol. Hepatol.* **31**, 965–972 (2016)
5. Ling, L. *et al.* Circulating and tumor-infiltrating mucosal associated invariant T (MAIT) cells in colorectal cancer patients. *Sci. Rep.* **6**, 20358 (2016).
6. Zabijak, L. *et al.* Increased tumor infiltration by mucosal-associated invariant T cells correlates with poor survival in colorectal cancer patients. *Cancer Immunol. Immunother. CII* **64**, 1601–1608 (2015).
7. Li, S. *et al.* Human Tumor-Infiltrating MAIT Cells Display Hallmarks of Bacterial Antigen Recognition in Colorectal Cancer. *Cell Rep. Med.* **1**, 100039 (2020).
8. Tastan, C. *et al.* Tuning of human MAIT cell activation by commensal bacteria species and MR1-dependent T-cell presentation. *Mucosal Immunol.* **11**, 1591–1605 (2018).
9. Morgan, X. C. *et al.* Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol.* **13**, R79 (2012).
10. Gevers, D. *et al.* The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* **15**, 382–392 (2014).
11. Schirmer, M. *et al.* Compositional and Temporal Changes in the Gut Microbiome of Pediatric Ulcerative Colitis Patients Are Linked to Disease Course. *Cell Host Microbe* **24**, 600-610.e4 (2018).
12. Cui, Y. *et al.* Mucosal-associated invariant T cell-rich congenic mouse strain allows functional evaluation. *J. Clin. Invest.* **125**, 4171–4185 (2015).
13. Salou, M. *et al.* A common transcriptomic program acquired in the thymus defines tissue residency of MAIT and NKT subsets. *J. Exp. Med.* (2018) doi:10.1084/jem.20181483.
14. Zikherman, J., Parameswaran, R. & Weiss, A. Endogenous antigen tunes the responsiveness of naive B cells but not T cells. *Nature* **489**, 160–164 (2012).
15. Sáez de Guinoa, J. *et al.* CD1d-mediated lipid presentation by CD11c+ cells regulates intestinal homeostasis. *EMBO J.* **37**, (2018).
16. Soudais, C. *et al.* In Vitro and In Vivo Analysis of the Gram-Negative Bacteria-Derived Riboflavin Precursor Derivatives Activating Mouse MAIT Cells. *J. Immunol. Baltim. Md 1950* **194**, 4641–4649 (2015).
17. Le Bourhis, L. *et al.* Antimicrobial activity of mucosal-associated invariant T cells. *Nat. Immunol.* **11**, 701–708 (2010).
18. Donaldson, G. P., Lee, S. M. & Mazmanian, S. K. Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* **14**, 20–32 (2016).
19. Albenberg, L. *et al.* Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology* **147**, 1055-1063.e8 (2014).

20. Seeley, H. W. & Del Rio-Estrada, C. The role of riboflavin in the formation and disposal of hydrogen peroxide by streptococcus faecalis. *J. Bacteriol.* **62**, 649–656 (1951).
21. Khan, M. T. *et al.* The gut anaerobe Faecalibacterium prausnitzii uses an extracellular electron shuttle to grow at oxic-anoxic interphases. *ISME J.* **6**, 1578–1585 (2012).
22. Neumann-Schaal, M. *et al.* Tracking gene expression and oxidative damage of O2-stressed Clostridioides difficile by a multi-omics approach. *Anaerobe* **53**, 94–107 (2018).
23. Shin, N.-R., Whon, T. W. & Bae, J.-W. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol.* **33**, 496–503 (2015).
24. Constantinides, M. G. *et al.* MAIT cells are imprinted by the microbiota in early life and promote tissue repair. *Science* **366**, (2019).
25. Rivera-Chávez, F. *et al.* Depletion of Butyrate-Producing Clostridia from the Gut Microbiota Drives an Aerobic Luminal Expansion of Salmonella. *Cell Host Microbe* **19**, 443–454 (2016).
26. Litvak, Y., Byndloss, M. X. & Bäumler, A. J. Colonocyte metabolism shapes the gut microbiota. *Science* **362**, eaat9076 (2018).
27. Terada, N., Ohno, N., Saitoh, S. & Ohno, S. Immunohistochemical detection of hypoxia in mouse liver tissues treated with pimonidazole using 'in vivo cryotechnique'. *Histochem. Cell Biol.* **128**, 253–261 (2007).
28. Cong, Y., Feng, T., Fujihashi, K., Schoeb, T. R. & Elson, C. O. A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. *Proc. Natl. Acad. Sci.* **106**, 19256–19261 (2009).
29. Legoux, F. *et al.* Microbial metabolites control the thymic development of mucosal-associated invariant T cells. *Science* (2019) doi:10.1126/science.aaw2719.
30. Rigottier-Gois, L. Dysbiosis in inflammatory bowel diseases: the oxygen hypothesis. *ISME J.* **7**, 1256–1261 (2013).
31. Morgan, X. C. *et al.* Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol.* **13**, R79 (2012).
32. Ilott, N. E. *et al.* Defining the microbial transcriptional response to colitis through integrated host and microbiome profiling. *ISME J.* **10**, 2389–2404 (2016).
33. Byndloss, M. X. *et al.* Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science* **357**, 570–575 (2017).
34. Baughn, A. D. & Malamy, M. H. The strict anaerobe Bacteroides fragilis grows in and benefits from nanomolar concentrations of oxygen. *Nature* **427**, 441–444 (2004).
35. el Marjou, F. *et al.* Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. *Genes. N. Y. N 2000* **39**, 186–193 (2004)
36. Klose, C. S. N. *et al.* A T-bet gradient controls the fate and function of CCR6-RORγt+ innate lymphoid cells. *Nature* **494**, 261–265 (2013).
37. Liang, S. C. *et al.* Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J. Exp. Med.* **203**, 2271–2279 (2006).
38. Martin, E. *et al.* Stepwise development of MAIT cells in mouse and human. *PLoS Biol.* **7**, e54 (2009).

39. Zhang, Z., Legoux, F. P., Vaughan, S. W. & Moon, J. J. Opposing peripheral fates of tissue-restricted self antigen-specific conventional and regulatory CD4+ T cells. *Eur. J. Immunol.* **50**, 63–72 (2020).
40. Kamran, P. *et al.* Parabiosis in mice: a detailed protocol. *J. Vis. Exp. JoVE* (2013) doi:10.3791/50556.
41. Huang, S. *et al.* MR1 antigen presentation to mucosal-associated invariant T cells was highly conserved in evolution. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 8290–8295 (2009).
42. Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* **79**, 5112–5120 (2013).
43. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* **17**, 10 (2011).
44. Callahan, B. J. *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581–583 (2016).
45. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590-596 (2013).
46. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
47. Dobin, A. *et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinforma. Oxf. Engl.* **29**, 15–21 (2013).
48. Kopylova, E., Noé, L. & Touzet, H. SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinforma. Oxf. Engl.* **28**, 3211–3217 (2012).
49. Lesker, T. R. *et al.* An Integrated Metagenome Catalog Reveals New Insights into the Murine Gut Microbiome. *Cell Rep.* **30**, 2909-2922.e6 (2020).
50. Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinforma. Oxf. Engl.* **30**, 923–930 (2014).
51. Hussain, H. A., Roberts, A. P. & Mullany, P. Generation of an erythromycin-sensitive derivative of Clostridium difficile strain 630 (630Deltaerm) and demonstration that the conjugative transposon Tn916DeltaE enters the genome of this strain at multiple sites. *J. Med. Microbiol.* **54**, 137–141 (2005).
52. Zhao, Y. & Melville, S. B. Identification and characterization of sporulation-dependent promoters upstream of the enterotoxin gene (cpe) of Clostridium perfringens. *J. Bacteriol.* **180**, 136–142 (1998).
53. Blattner, F. R. *et al.* The complete genome sequence of Escherichia coli K-12. *Science* **277**, 1453–1462 (1997).
54. Cato, E. P. & Johnson, J. L. Reinstatement of Species Rank for Bacteroides fragilis, B. ovatus, B. distasonis, B. thetaiotaomicron, and B. vulgatus: Designation of Neotype Strains for Bacteroides fragilis (Veillon and Zuber) Castellani and Chalmers and Bacteroides thetaiotaomicron (Distaso) Castellani and Chalmers. *Int. J. Syst. Bacteriol.* **26**, 230–237 (1976).

55. Saujet, L., Monot, M., Dupuy, B., Soutourina, O. & Martin-Verstraete, I. The key sigma factor of transition phase, SigH, controls sporulation, metabolism, and virulence factor expression in Clostridium difficile. *J. Bacteriol.* **193**, 3186–3196 (2011).
56. Soutourina, O. A. *et al.* Genome-wide identification of regulatory RNAs in the human pathogen Clostridium difficile. *PLoS Genet.* **9**, e1003493 (2013).
57. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods San Diego Calif* **25**, 402–408 (2001).
58. Lan, A. *et al.* Survival and metabolic activity of selected strains of Propionibacterium freudenreichii in the gastrointestinal tract of human microbiota-associated rats. *Br. J. Nutr.* **97**, 714–724 (2007).
59. Laroui, H. *et al.* Dextran sodium sulfate (DSS) induces colitis in mice by forming nano-lipocomplexes with medium-chain-length fatty acids in the colon. *PloS One* **7**, e32084 (2012).
60. Nonnenmacher, C., Dalpke, A., Mutters, R. & Heeg, K. Quantitative detection of periodontopathogens by real-time PCR. *J. Microbiol. Methods* **59**, 117–125 (2004).

Supplementary Information is available for this paper.

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Part II: Characterization of MAIT cells in the ileal environment

The intestinal tract is composed of different ecological niches shaped by dynamic parameters that determine bacterial colonisation and metabolism. Examples of variations from the proximal to the distal end of the gut, include decreasing concentrations of nutrients, bile acids, antimicrobials and oxygen, and increasing mucus density (Donaldson, Lee, and Mazmanian 2016). Using the exogenous hypoxia marker pimonidazole, we analysed ileal and colon sections from healthy mice for pimonidazole adduct formation. In contrast to the apical surface of colonocytes, which is hypoxic, ileal epithelial cells did not sustain pimonidazole adduct formation, indicating a relatively high oxygen concentration in that location (Fig. 1). These results are consistent with the proposed oxygen gradient along the intestinal tract. The contribution of riboflavin to bacterial respiration and oxidative stress resistance, suggested increased needs for riboflavin in the ileum. In view of this, the production of riboflavin metabolites may be higher in the ileum as compared to the colon, potentially affecting MAIT cell function in each location.

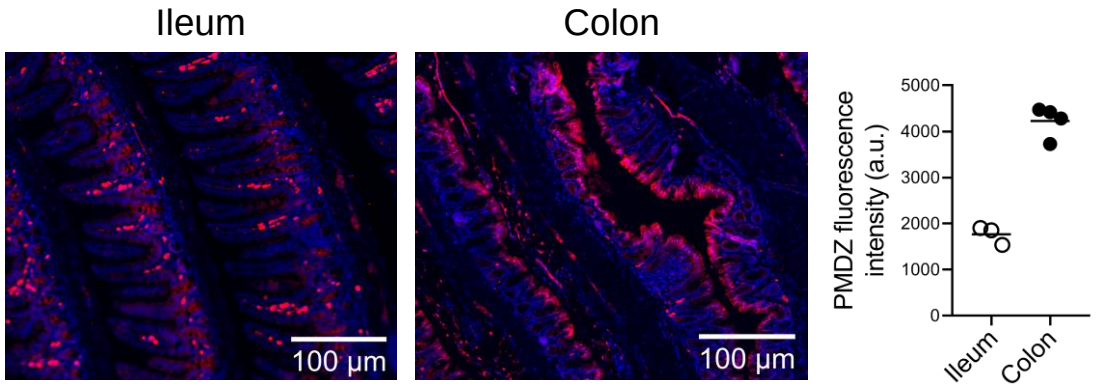


Figure 1: Immunohistochemical visualization of pimonidazole adducts. Right: Representative images showing pimonidazole (PMDZ) adduct (red) and DNA (blue) staining of the ileum and colon of healthy mice. Left: Quantification of PMDZ adducts staining at the epithelium surface of the ileum and colon, each dot represents a mouse.

To detect MAIT antigens along the gut of healthy mice, we used the *in vitro* assay using 5-OP-RU:MR1-specific reporter T cells and MR1-expressing antigen-presenting cells loaded with luminal contents from the ileum, cecum, colon, or feces. Reporter T cells were not activated with contents from the ileum, but up-regulated the activation markers CD25 and CD69 in the presence of cecum, colon contents and feces extracts (Fig. 2). These findings suggested that bacteria colonizing the ileum do not produce sufficient levels of metabolites that are required for the activation of reporter cells *in vitro*.

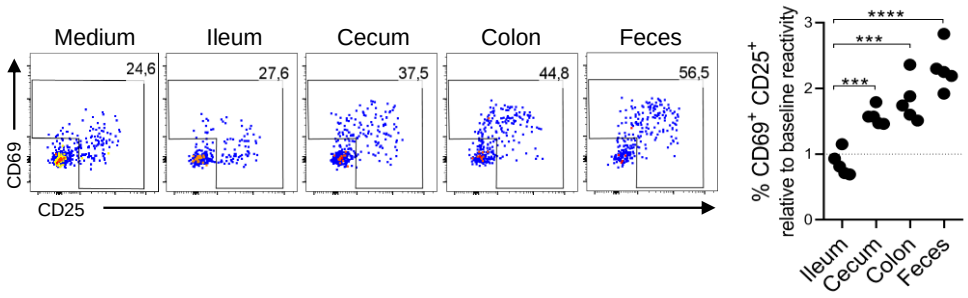


Figure 2: Gradient of MAIT antigens in the gut of healthy mice. Left: Representative flow cytometry of MR1:5-OP-RU-specific reporter T cells after overnight incubation with WT3-MR1 cells pulsed with 100 mg of feces or luminal contents from the indicated segments of healthy mice. Right: Summary of the relative expression of CD25 and CD69 on reporter T cells. ***p<0.001 and ****p<0.0001 by Student's *t* test.

We then characterized MAIT cells in the ileum and compared them to colon MAIT cells. In the ileum, MAIT cells were present in the lamina propria at lower frequencies than in the colon. By contrast, iNKT cells, a subset with similar transcriptional program but distinct specificity (Salou et al. 2019), were not enriched in the colon (Fig. 3a). Just as in the colon, ileal MAIT cells do not circulate between 5-weeks parabiotic mice, indicating residency properties of MAIT cells within the gut (Fig. 3b). In contrast to the thymus, spleen and lung where RORγt and Tbet expression by MAIT cells appears to be mutually exclusive (Rahimpour et al. 2015), in the ileum and the colon, MAIT cells coexpressed RORγt and Tbet (Fig. 3c). However, the proportion of RORγt$^+$ Tbet$^-$ was higher in the ileum, and localization in the colon was associated with reduced RORγt and increased Tbet expression (Fig. 3c,d), suggesting different effector programs within the gut. We then examined the activation state of MAIT cells along the gut. MAIT cell expression of the activation marker CD25 was lower in the ileum compared to the colon, but higher than the iNKT expression of CD25 (Fig. 3e). These data suggested that MAIT cells are less activated in the

ileum where MAIT antigens were not detected. We further assessed TCR signalling in MAIT cells, using the *Nr4a1*-GFP reporter mouse. In the ileum, MAIT cells expressed the lowest levels of GFP compared to MAIT cells from other tissues such as spleen (Fig. 3f), and to conventional T cells and iNKT cells from the ileum (Fig. 3g).

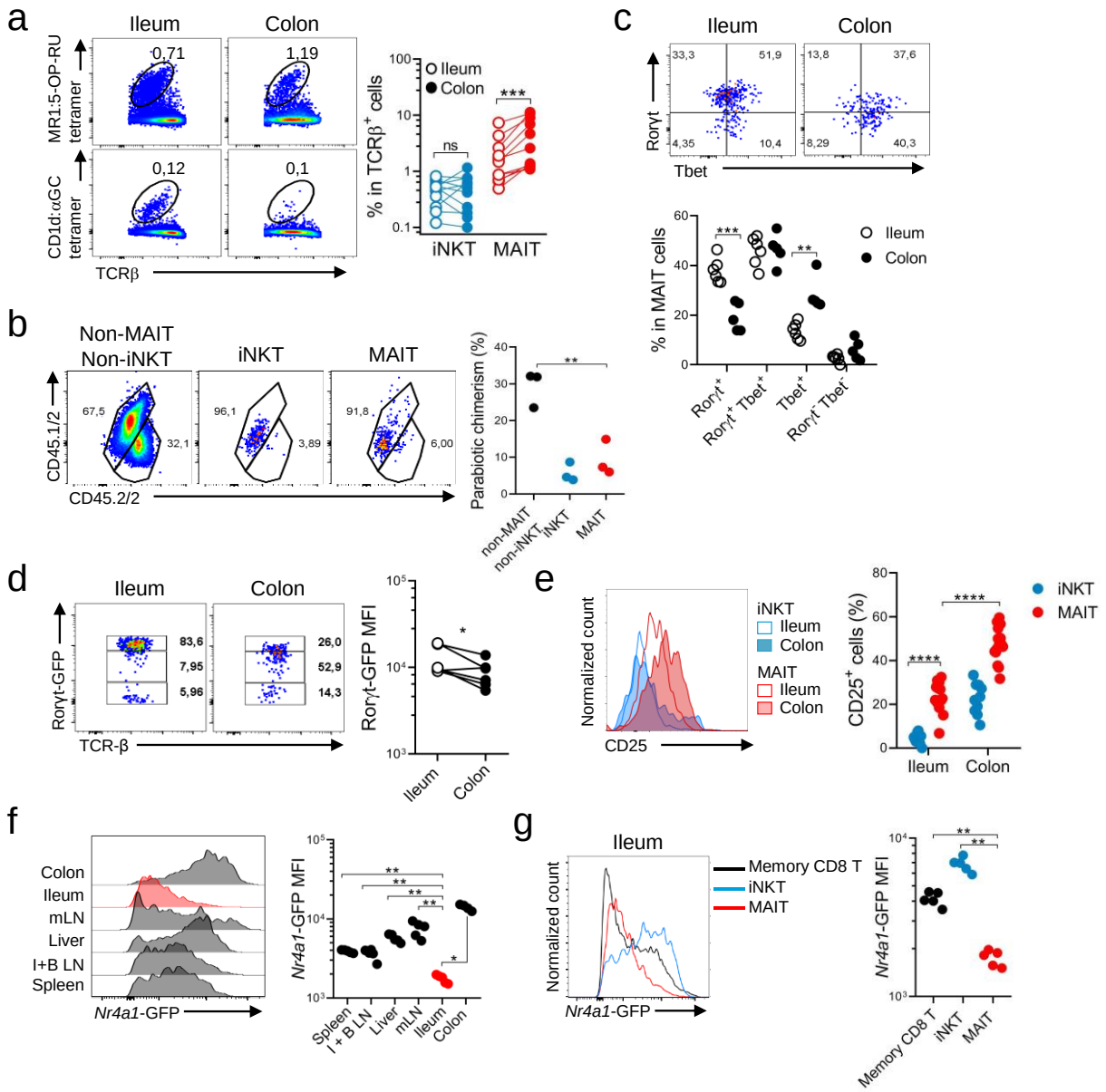


Figure 3: Distinct MAIT phenotypic profiles in the ileum. a. Left: Representative flow cytometry of B6-MAIT^{Cast} lamina propria T cells in the ileum and the colon. Right: Summary of CD1d:αGC tetramer+ (iNKT) and MR1:5-OP-RU tetramer+ (MAIT) cell frequencies in the ileum and colon lamina propria. **b.** CD45.1/2 and CD45.2/2 congenic B6-MAIT^{Cast} mice joined in parabiotic pairs were examined 5 weeks later. Left: Representative flow cytometry of the indicated T cell subsets in the ileum. Right: Summary of chimerism of the indicated T cells subsets. **c.** Top: Representative flow cytometry of MAIT cell expression of RORγt and Tbet in the ileum and colon of healthy mice. Bottom: Summary of the percentages of MAIT cells expressing the indicated transcription factors in the ileum and the colon. **d.** Left: Representative flow cytometry of MAIT cells in the ileum and the colon. Right: Summary of RORγt-GFP mean fluorescence intensity (MFI) in the indicated tissues. **e.** Left: Representative flow cytometry of iNKT and MAIT cells in the ileum and the colon. Right: Summary of CD25+ cell frequencies in the indicated cell subsets and tissues. **f.** Left: Representative flow cytometry of MAIT cells in the indicated tissues. I+B LN: Inguinal and brachial lymph nodes. mLN: mesenteric lymph nodes. Right: Summary of *Nr4a1*-GFP MFI in MAIT cells from the indicated tissues. **g.** Left: Representative flow cytometry of memory (CD44+) CD8+ T cells, iNKT and MAIT cells in the ileum. Right: Summary of *Nr4a1*-GFP MFI in the indicated cell subsets from the ileum. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 by Wilcoxon (**a**), Student's *t* (**b**, **c**, **d** and **e**) and by Mann-Whitney *U* (**f** and **g**) test.

These weak TCR signals in MAIT cells exclusively in the ileum suggested a mechanism controlling ileal MAIT cell activation at steady state. MAIT TCR stimulation in the ileum may be muted either by low production of riboflavin pathway-derived antigens by the microbiota and/or by low expression of MR1 in the lamina propria. We sequenced the 16S rRNA gene to characterize bacterial communities along the gut of healthy B6 mice. As compared to the cecum and colon samples, the ileal lumen of healthy mice had *~10 times* lower numbers of CFU equivalents per gram (Fig. 4a). At the phylum level, *Bacteroidetes* and *Firmicutes* dominated the different regions along the mice intestinal tract (Fig. 4b). *Bacteroidetes*, *Firmicutes* and other phyla such as *Proteobacteria* were similarly abundant in all three anatomical regions (Fig. 4b). We note that intestinal bacteria from the *Bacteroidetes* and *Proteobacteria* phyla can produce large amounts of riboflavin pathway-derived MAIT antigens (Tastan et al. 2018), suggesting that MAIT-stimulating bacteria can be found in the ileum. We analysed expression of the riboflavin

ribD gene in the feces of mice, using a published metatranscriptomic dataset (Ilott et al. 2016b). Except for *Prevotellaceae* and *Deferribacteraceae*, all the families contributing to *ribD* expression in the feces including strong *ribD* contributors such as *Muribaculaceae*, *Lachnospiraceae, Lactobacillaceae, Desulfovibrionaceae, Bacteroidaceae* and *Clostridiaceae* (Fig. 4c), were as abundant in the ileum as in the feces (Fig. 4d). The high MAIT TCR-stimulators belonging to *Enterobacteriaceae* were enriched in the ileum (Fig. 4d). Although the total number of bacteria is lower in the ileum (Fig. 4a), Tastan *et al*. observed no significant correlation between the bacterial counts and the level of MAIT antigens they secreted *in vitro* (Tastan et al. 2018). Thus, the ileum may be populated by strong MAIT-TCR stimulators. However, further experiments are needed to directly assess bacterial transcriptional activity in the ileum.

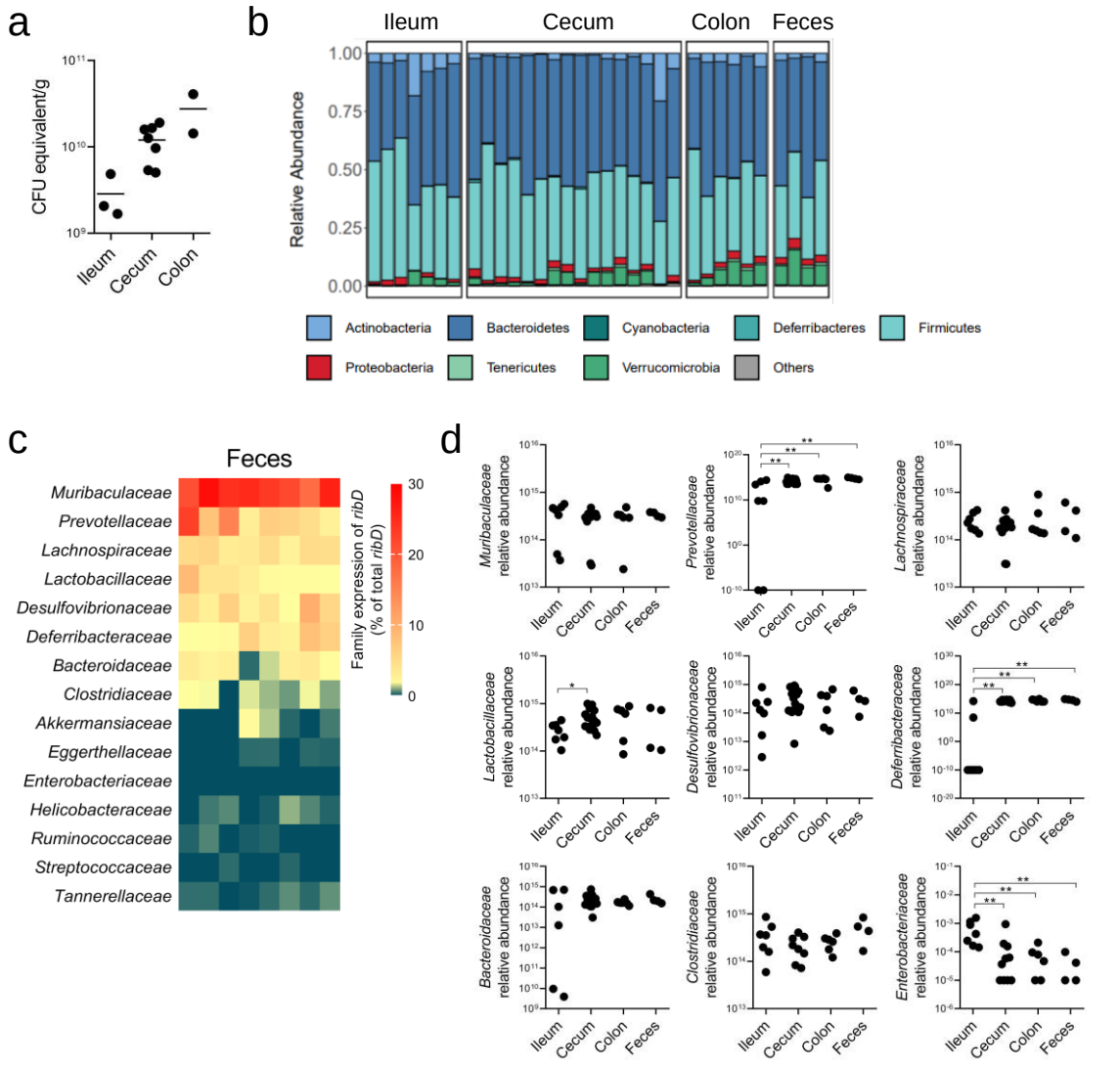


Figure 4: The gut microbiota composition along the gut of healthy mice. a. Summary of colony-forming unit (CFU) equivalents per gram in the indicated tissues, determined by qPCR quantification of the 16S rRNA gene. **b.** Relative abundance of the indicated bacterial phyla in the indicated regions determined by 16S rRNA gene sequencing. **c.** Heatmap showing *ribD* expression in the indicated families in fecal samples. Data are expressed as percentage of total *ribD* expression. Columns indicate individual mice. **d.** Relative abundance of *Muribaculaceae*, *Prevotellaceae*, *Lachnospiraceae*, *Lactobacillaceae, Desulfovibrionaceae*, *Deferribacteraceae*, *Bacteroidaceae*, *Clostridiaceae* and *Enterobacteriaceae* in the indicated regions as determined by 16S rRNA gene sequencing. **p<0.01 by Mann-Whitney *U* test.

We next assessed whether MAIT TCR signals in the ileum are limited by the expression of MR1. To test this hypothesis, we compared the ability of total cells from the thymus (positive control), small intestine and colon lamina propria to capture and present synthetic 5-OP-RU in vitro. In contrast to thymic and colon lamina propria cells harvested from *Mr1*$^{+/+}$ mice, small intestine lamina propria cells were not efficient at activating reporter cells *in vitro* (Fig. 5a). These results suggested low MR1 expression in the small intestine, which correlates with the low MAIT TCR stimulations in the ileum (Fig. 3f,g). We further investigated whether MR1 expression could impact on MAIT cell activation in the ileum. We used the transgenic LSL-MR1 x Villin-CreER mouse that we generated to increase MR1 expression in gut epithelial cells. LSL-MR1 x Villin-CreER mice were crossed to the *Nr4a1*-GFP reporter mouse to assess TCR signalling in MAIT cells after MR1 induction with tamoxifen. Inducible MR1 expression elicited TCR signalling in MAIT cells from the ileum, and increased TCR signalling in colon MAIT cells (Fig. 5b). Although no MAIT antigens were detected in the ileum contents using the *in vitro* assay (Fig. 2), these data suggested that some MAIT ligands were produced in the ileum, possibly at concentrations below the detection limits of our in vitro assay. Robust TCR signalling in MAIT cells from the ileum resulted in reduced RORγt and increased Tbet expression (Fig. 5c). These results indicated that Tbet expression in intestinal MAIT cells is a consequence of TCR triggering by riboflavin-derived metabolites. These results are consistent with previous data showing increased Tbet expression in lung MAIT cells after infection with bacteria that encode the riboflavin pathway (H. Wang et al. 2019). In the colon, MAIT cells were constantly activated through TCR, which resulted in the basal expression of Tbet. Further increase in MR1 expression did not change MAIT cell effector programs in the colon (Fig. 5c).

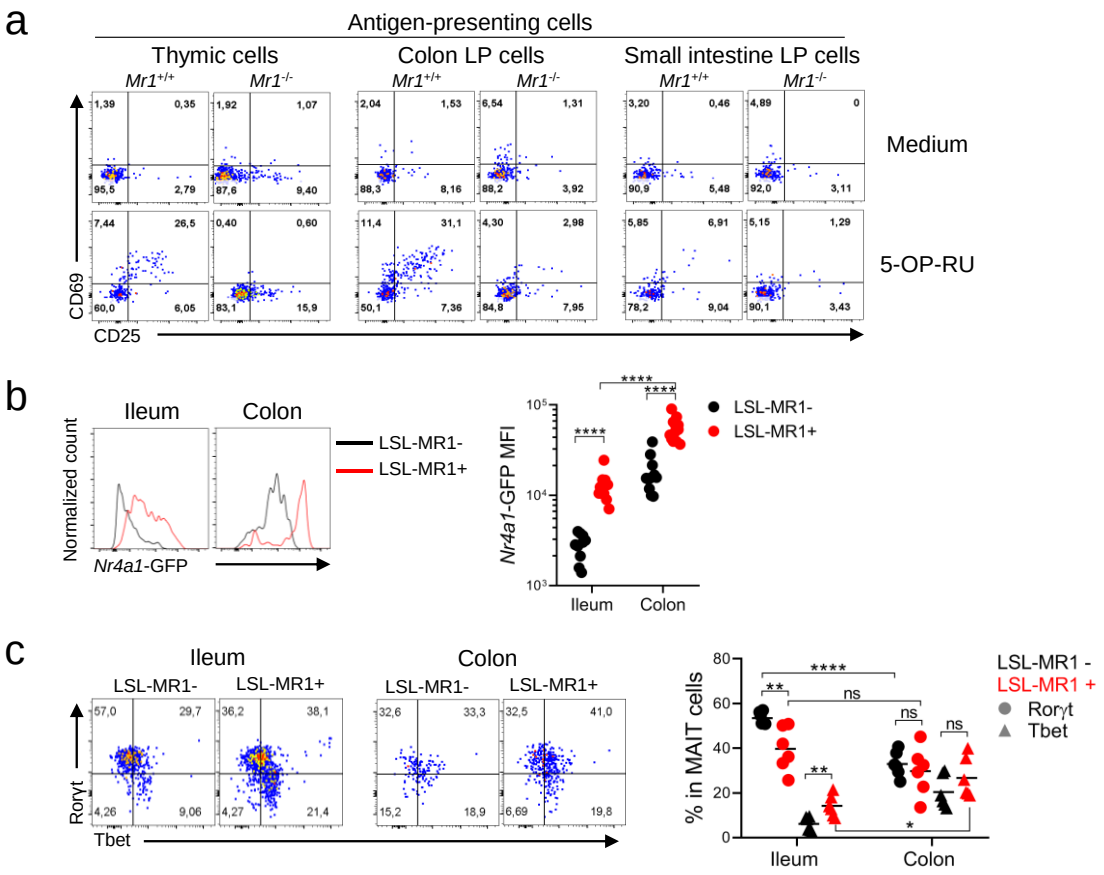


Figure 5: MR1 is a limiting factor controlling MAIT cell activation. a. Activation (CD25 and CD69 expression measured by flow cytometry) of C+ Vβ8+ Ca-/- MR1-/- reporter T cells after overnight incubation with total *Mr1*$^{+/+}$ or *Mr1*$^{-/-}$ cells from the indicated tissues in the presence or absence of 5-OP-RU. **b.** Left: Representative flow cytometry of MAIT cells from the indicated tissues. Both LSL-MR1+ and LSL-MR1- mice received tamoxifen injections. Right: *Nr4a1*-GFP expression in MAIT cells from the indicated tissues. **c**. Left: Representative flow cytometry of MAIT cell expression of RORγt and Tbet in the ileum and colon of LSL-MR1+ and LSL-MR1- mice treated both with tamoxifen. Right: Summary of the percentages of MAIT cells expressing RORγt and Tbet in the ileum and the colon of the indicated mice. ns p>0.5, **p<0.01 and ****p<0.0001 by Mann-Whitney *U* (**b**) or by Student's *t* (**c**) tests.

Finally, we asked whether additional factors drive phenotypic differences in ileal MAIT cells. The adaptor MyD88 is required for TLR and IL1R family signalling and contributes to the maintenance of intestinal homeostasis (Frantz et al. 2012). We examined whether MAIT cell functional program was altered in the gut of *MyD88*$^{-/-}$ mice. In the ileum, MyD88$^{-/-}$ mice had a marked reduction in the proportion of RORγt^{+} MAIT cells, and increased Tbet^{+} MAIT cells compared with WT mice (Fig. 6a). In contrast, in the colon, there was no difference in MAIT cell expression of RORγt and Tbet in WT versus *MyD88*$^{-/-}$ mice (Fig. 6b). These experiments need to be repeated, and may suggest a role of MyD88 for the maintenance of RORγt expression by MAIT cells in the ileum. NOD-like receptor (NLR) family member, Nlrp6, is essential for the production of mature IL-18 (Elinav et al. 2011), and plays a critical role in maintaining gut homeostasis (G. Y. Chen et al. 2011). By contrast with MyD88, NLRP6 did not affect MAIT cell functional program in the ileum and the colon (Fig. 6c-d).

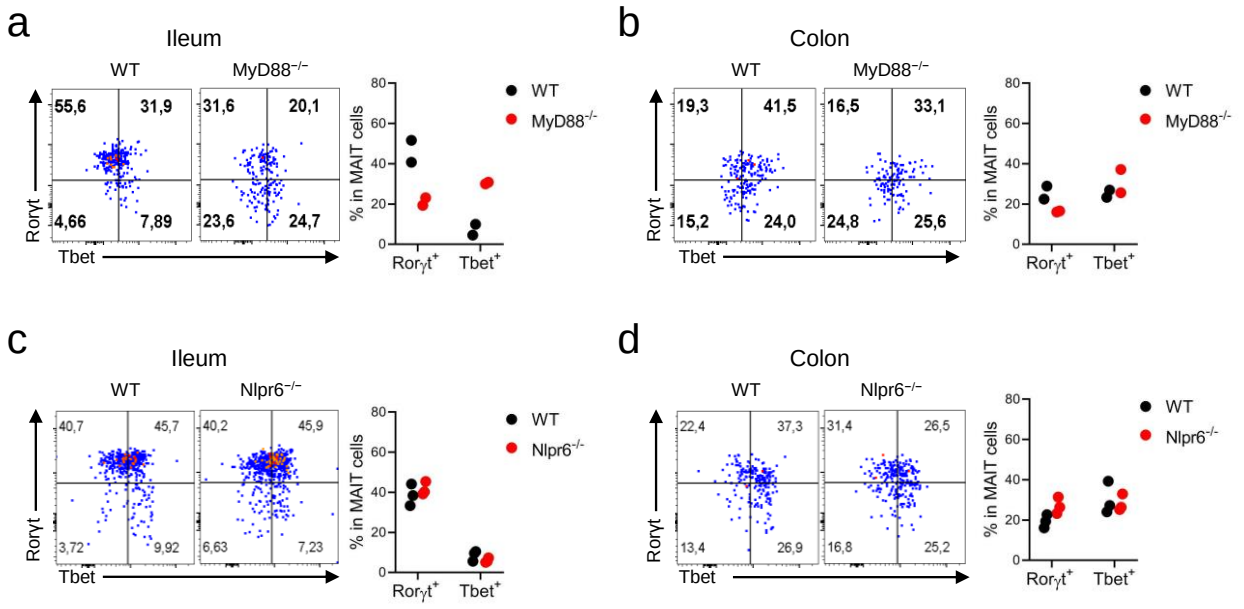


Figure 6: MyD88 signalling controls MAIT cell functional profile in the ileum. a-d. Left: Representative flow cytometry of MAIT cell expression of RORγt and Tbet in the ileum (a,c) and colon (b,d) of indicated mice. Right: Summary of the percentages of MAIT cells expressing of RORγt and Tbet in the ileum (a,c) and the colon (b,d) of indicated mice. Prior to inclusion in experimental groups, WT (B6) and MyD88$^{-/-}$ or Nlpr6$^{-/-}$ mice were co-housed, or cage contents were mixed for at least two weeks in order to normalize microbiota.

Collectively, the ileum is characterized by high levels of oxygen at steady state and is colonized by a range of bacteria including bacteria producing high concentrations of MAIT antigens in vitro. However, MAIT cells are not activated through TCR engagement and express distinct phenotypic profiles in the ileum. MAIT TCR stimulation may be muted by low levels of MR1 expression in the ileum. Alternatively, dietary MAIT antagonists such as folic acid-derived metabolites may be more abundant in the ileum than in the colon and may compete with microbial antigens. Further experiments will be necessary to define how MAIT cells are inhibited in the ileum.

Discussion and perspectives

The microbiota has a strong influence on the host immune system and correspondingly, the immune system shapes the composition and localization of the microbiota. Yet, how the immune system monitors alterations in the composition and function of the microbiota remains poorly understood. This question is of particular importance for MAIT cells, which are predominantly found in tissues colonized by the microbiota and recognize microbial metabolites derived from a microbial riboflavin precursor. MAIT cells have been implicated in pathologies associated with intestinal dysbiosis such as inflammatory bowel diseases, but their exact role in the pathology remains unknown. This work aimed to understand the interaction between MAIT cells and the microbiota and to decipher the role of MAIT cells in the gut.

Our results show that MAIT cells directly monitor a bacterial metabolism associated with colitis and provide host protection. We establish that riboflavin over-production by intestinal bacteria is a hallmark of intestinal inflammation, and results in the production of antigens for MAIT cells. The gene *ribD*, which controls MAIT antigen production, was over-expressed by *Bacteroidaceae*, *Clostridiaceae* and *Enterobacteriacea* during colitis. Riboflavin precursor adducts crossed the intestinal barrier and induced TCR signalling in MAIT cells, which produced the tissue-repair mediator amphiregulin and reduced colitis severity. We propose that riboflavin over-production by intestinal bacteria is a consequence of increased oxygen in the gut environment during colitis. Our study explains for the first time why the immune system monitors the activity of the riboflavin biosynthetic pathway. More generally the study describes a new molecular interaction between the microbiota and mammalian immune system. In the second part of this work, we characterized MAIT cells in the ileal environment wherein high levels of oxygen have been observed at steady state. A range of bacteria that encode the riboflavin pathway populated the ileum, however ileal MAIT cells were not activated through TCR engagement and expressed distinct phenotypic profiles.

Here, I will discuss these results and integrate future studies that will be needed to understand MAIT-microbiota interactions in the gut.

1. Potential of oxygen to induce riboflavin over-production

The *in vitro* MAIT-TCR activation assay we used enabled us to quantify MAIT antigens in the gut. Although mucosal scraps contained fewer bacteria compared to the lumen, we found significantly increased amounts of riboflavin pathway-derived MAIT antigens in the mucosa. Consistent with our observation, the riboflavin biosynthesis pathway was enriched in the macaque mucosal community as compared to the lumen (Yasuda et al. 2015). Riboflavin derivatives such as FMN and FAD contribute to bacterial respiration and oxidative stress resistance, thus bacterial production of riboflavin at the oxic-anoxic interface near the intestinal epithelium may reflect a mechanism by which the gut microbiome responds to oxygen and oxidative stress, or a selection of the bacteria able to survive in these environmental conditions. This notion was supported by the data showing over-expression of the enzymes responsible for riboflavin production upon air exposure *in vitro* and upon hypoxia disruption *in vivo*. Thus, we propose that riboflavin over-production by intestinal bacteria is a consequence of increased oxygen in the gut environment. However, we acknowledge that more experiments are necessary to get a better insight. As described earlier, butyrate is the preferred energy substrate for colonocytes and the primary determinant of physiologic hypoxia in the colon by promoting intestinal epithelial O2 consumption. Antibiotic treatment and colitis are associated with a depletion of butyrate-producing bacteria (Byndloss et al. 2017; Sokol et al. 2009), which results in the loss of epithelial hypoxia and in increased amounts of oxygen emanating from the mucosal surface (Byndloss et al. 2017; Litvak, Byndloss, and Bäumler 2018). Even though butyrate supplementation was able to restore hypoxia (Byndloss et al. 2017), it did not abrogate the increase in MAIT antigens following antibiotic treatment (data not shown). To get a better insight, staining for hypoxia and metagenomic and metatranscriptomic profiling of the gut microbiome after butyrate supplementation would be necessary in future studies.

We and others (Neumann-Schaal et al. 2018) have provided evidence for the potential of oxygen to induce riboflavin biosynthesis gene expression in the strict anaerobes *C. difficile*. The induction of riboflavin seems to be a conserved response in *Clostridia* under microaerobiosis, as *C. butyricum* and *C. perfingens* overexpressed also riboflavin genes upon air exposure. Although expression of *ribD* in *Streptococcus pneumoniae* isolates correlated with MAIT cell response (Hartmann et al. 2018), suggesting differences in the production of MAIT antigens, we did not detect higher frequencies of MAIT antigens in the supernatants of *C. difficile* and *C. perfingens* after *ribD* over-expression (data not shown).

One possible explanation is that these bacteria do not produce or release sufficient amounts of metabolites that are required for the activation of reporter cells *in vitro*. Mass spectrometry analysis of recombinant MR1 protein refolded in the presence of bacterial supernatant could be an alternative way to detect MAIT antigens in the supernatants. The enzymes responsible for riboflavin metabolism (RibD, RibH, RibB, and RibE) are encoded on a single operon in *Clostridia*. Thus, it is also plausible that the absence of increased riboflavin derived metabolites in bacterial supernatants could result from over-expression of *ribB or RibE,* which encodes the enzyme that consumes 5-A-RU. Accordingly, over-expression of *ribB* by a strain of *Salmonella typhimurium* (strain ST313 lineage 2) results in lower production of MAIT antigens, and has been proposed as an escape mechanism (Preciado-Llanes et al. 2020).

2. Modifications in bacterial community structure and MAIT cell activation in human diseases

An important finding in colitic mice was the bloom of *Bacteroidaceae (Bacteroidetes)* and *Enterobacteriaceae* (*Proteobacteria*) which we and others (Tastan et al. 2018) have found to produce high levels of MAIT antigens *in vitro*, as compared to bacterial species from *Firmicutes* phylum. It is also worth noting that in contrast to *Firmicutes*, almost all *Bacteroidetes* and *Proteobacteria* encode the riboflavin pathway (Magnúsdóttir et al. 2015). Interestingly, the *Bacteroidetes to Firmicutes* ratio is perturbed in patients with Crohn's disease who exhibit an increase in the *Bacteroidetes to Firmicutes* ratio compared with healthy controls, as well as an increase in *Enterobacteriaceae* (Wright et al. 2015). Colorectal adenoma, the precursor for the majority of colorectal cancer (CRC), and CRC cancer are other examples of such a shift in gut microbiota (Lu et al. 2016; T. Wang et al. 2012). Although COVID-19 is primarily a respiratory illness, gut microbiota composition of patients with COVID-19 during hospitalization and after recovery is significantly altered. Particularly, members of the *Bacteroidetes* were enriched in patients with COVID-19 (Yeoh et al. 2021). Thus, we speculate that the increase in ratio of high-stimulator to low-stimulators of MAIT cells could be associated with increased concentrations of MAIT antigens and could be one of the reasons for the activation of MAIT cells in IBDs (Serriari et al. 2014; Haga et al. 2016; Tominaga et al. 2017), CRC (Berzins et al. 2020), and patients with COVID-19 (Flament et al. 2021), although the levels of IL-12 and IL-18 are elevated and likely activate MAIT cells (van Wilgenburg et al. 2016).

On the other hand, numerous studies have investigated gut microbial communities in obese individuals, but the results have not always been consistent. In a majority of studies, obesity is associated with a decrease in *Bacteroidetes* while *Firmicutes* increase in both humans and animal models (reviewed in Abenavoli et al. 2019). Only one in three studies reported greater abundance of *Enterobacteriaceae* in obese participants (Peters et al. 2018; Turnbaugh et al. 2006; Zhang et al. 2009). The increase in the ratio of *Firmicutes* to *Bacteroidetes* could explain the low abundance of genes of riboflavin biosynthesis enzymes in obese mice (Toubal et al. 2020). Post-gastric-bypass individuals had an increase of *Proteobacteria* particularly *Enterobacteriaceae* (Zhang et al. 2009) and increase circulating MAIT cell frequency, which was associated with amelioration in patient's metabolic and inflammatory status (Magalhaes et al. 2015).

An important question is still open as to whether increased MAIT antigen production upon colitis results from upregulation of *rib* genes or from the expansion of strong producers of MAIT antigens. The two possibilities are not mutually exclusive. To address this question, combining metagenomic and metatranscriptomic profiling of the gut microbiome would be valuable in future studies to assess modifications to both bacterial community structure and transcriptional activity.

3. Intestinal permeability to MAIT antigens

A striking finding from our study was the rapid and efficient transfer of 5-OP-RU MAIT antigen across the colonocyte lipid bilayer membrane and the whole intestinal barrier. A previous study from the lab showed that 5-OP-RU delivered by oral gavage is detected shortly after in the thymus (Legoux, Bellet, et al. 2019). These findings are consistent with known properties of other microbiota-derived metabolites, such as microbial SCFAs which can reach distant host tissues (Koh et al. 2016). It is important to note that 5-OP-RU is hydrophilic and may require a transporter given that passive diffusion through the lipid bilayer membrane is low for hydrophilic molecules. *Nr4a1*-GFP expression by MAIT cells in the mLN and liver, suggests a scenario where riboflavin precursor adducts cross the intestinal barrier to reach lymph circulation and portal blood before reaching the general blood circulation. This pathway may lead to a progressive dilution of MAIT antigen concentration and could explain the low TCR signalling in MAIT cells from the spleen at steady state. Nonetheless, upon antibiotic treatment and colitis, where the microbiota produced high concentrations of MAIT antigens, MAIT cells received strong TCR signals in the colon and distant tissues such as spleen.

4. MR1-dependent and -independent activation of MAIT cells upon colitis

This is the first demonstration that the activation of MAIT cells upon colitis involves cognate interaction with their restricting molecule, MR1. In line with published data showing MAIT cell expression of genes linked with the tissue-repair program upon TCR stimulation (Hinks et al. 2019; Lamichhane et al. 2019; Leng et al. 2019), we demonstrated that MAIT TCR activation induced the production of Areg, an important mediator of tissue repair at mucosal sites (Zaiss et al. 2015). However, given that Areg is involved in epithelial malignancies (Chayangsu et al. 2017; Normanno et al. 1995), chronic MAIT TCR stimulation could be deleterious. This raises the possibility that MAIT cells could be helpful or harmful, depending on the microenvironment they are in, or depending on the duration of TCR stimulation.

From the data obtained, we would argue that both MR1-dependent and -independent activation may be involved to trigger robust MAIT cell responses in colitis. Indeed, MAIT cells from colitic mice shift towards RORγt, and show enhanced production of IFNγ, IL-17, IL-22 and Areg in the colon. However, except for Areg, increased expression of RORγt, and cytokine secretion were not observed by colon MAIT cells upon transgenic MR1 expression in intestinal epithelial cells (data not shown), suggesting that stimuli other than the TCR are responsible for IL-17, IFNγ and IL-22 production by MAIT cells during colitis. Previous study has reported that IL-23 induced human Tbet-expressing group 1 innate lymphoid cells (ILCs) differentiation into RORγt-expressing group 3 ILCs, and enhanced IL-22 production (Bernink et al. 2015). In addition, an important role for IL-23 in MAIT cell maintenance of RORγt expression was observed in the lung in a mouse model of infection (H. Wang et al. 2019). These findings and the demonstration that IL-23 is up-regulated in inflamed mucosa (Schmidt et al. 2005), suggests that the local inflammatory milieu may contribute to MAIT cell phenotype and function in the gut. To further define the role of IL-23, mice deficient in IL-23 *(*IL23p19KO) (Ghilardi et al. 2004) have been imported in our animal facility and will be studied.

5. Role of MAIT cells in the inflamed gut

Histopathological analysis showed that MAIT cells attenuate DSS-induced inflammation. Owing to the known tissue-protective properties of effector molecules they produce, MAIT cells likely interact with intestinal epithelial cells and preserve the intestinal barrier. This suggestion is corroborated by the loss of small intestine integrity in mice lacking MAIT cells (Rouxel et al. 2017) and by the *in vitro* (Leng et al. 2019) and *in vivo* (Constantinides et al. 2019) results demonstrating a role of MAIT cells in wound healing.

Whether MAIT cells interact with other immune cells in the inflamed gut, which in return maintain homeostasis, has not been determined. Previous study has shown that γδT17 cells, through IL-17, are important in the recruitment of immunosuppressive Gr-1^{+}CD11b^{+} myeloid cells to the inflamed gut lesions (Sun et al. 2017). Thus, it is possible that MAIT cells promote inflammation resolution through a new immune pathway involving myeloid cells recruitment to the site of colitis inflammation.

On the other hand, we speculate that MAIT cells can reciprocally regulate the microbiota through different mechanisms. For example, MAIT-derived IFNγ, IL-17 and IL-22 can influence the compartmentalization of the intestinal microbiota by inducing expression of mucins and by promoting the secretion of AMPs. Indeed, mice lacking the large gel-forming mucin Muc2 or the antibacterial lectin RegIIIγ, lack spatial segregation of commensal bacteria (Johansson et al. 2008; Vaishnava et al. 2011). It is also possible that MAIT cells inhibit the expansion of colitogenic bacteria, such as *E. coli* (Y.-L. Huang et al. 2015), by preserving the integrity of the intestinal epithelial barrier and thus by promoting oxygen consumption by colonocytes and hypoxia. Analysis of commensal microbiota composition in colitic mice deficient or not in MAIT cells would be valuable in future studies to address these possibilities. Only few studies are available that have characterized gut microbiota composition in mice deficient in MAIT cells. Smith et al. showed a greater proportion of particularly *Bacteroidaceae and Lactobacilliaceae* in *Mr1*$^{-/-}$ mice as compared to WT mice (A. D. Smith et al. 2019). However, in that study, microbiota differences were abrogated upon co-housing of WT and *Mr1*$^{-/-}$ mice, suggesting that the differences observed were weak and due to original breeding location. It is also worth noting that we did not find any differences in MAIT ligand concentration in the gut between WT and *Mr1*$^{-/-}$ mice (data not shown).

6. MAIT cells in the ileal environment

A striking observation from our study was the low TCR signals in MAIT cells from the ileum. Because ileal and colon samples were processed similarly at the same time, the observed difference is probably not an experimental artefact. One possible explanation for the low TCR signals in MAIT cells from the ileum as compared to the colon, was the low expression of MR1. However, further microscopy and in vitro assay experiments are needed to investigate MR1 expression along the gut and to identify the antigen presenting cells. Although inducible MR1 expression in intestinal epithelial cells elicited TCR signalling in MAIT cells from the ileum, MAIT TCR signals in the ileum were lower than in the colon. These data and MAIT cell activation in bioassays suggest low production of MAIT antigens in the ileum. We envision that genes controlling production of the MAIT ligand precursor in some bacteria in the ileum are attenuated by riboflavin originating from food. It is worth noting that some bacteria express riboflavin transporter proteins (Gutiérrez-Preciado et al. 2015) and could bypass the need for *de novo* synthesis of riboflavin which is regulated by FMN riboswitch (Pedrolli et al. 2015; Vitreschak et al. 2002). Analysis of bacterial transcriptional activity along the gut of mice fed a normal or riboflavin-deficient diet would be valuable in future studies to address this possibility. Riboflavin transporters are observed in *Firmicutes* but not in *Bacteroidetes* (Gutiérrez-Preciado et al. 2015) which could explain the high and constant production of riboflavin by species belonging to *Bacteroidetes*. It is also plausible that food-derived antagonists such as folate-derived metabolites, compete with the microbial antigens for binding to MR1 in the small intestine.

7. Contact of intestinal epithelial cells (IECs) with MAIT cells in the intestinal mucosa

While MAIT cells from the lamina propria exist below the basement membrane, in this study we showed that transgenic MR1 expression in intestinal epithelial cells elicited strong TCR signalling in MAIT cells from the lamina propria. This could be explained by IECs contact with MAIT cells in the underlying lamina propria via basolateral projections through the semi-porous basement membrane. In this regard, evidence from a number of studies show that IECs can transfer information from luminal microorganisms and antigens by directly processing and presenting them in highly polarized fashion, to lymphocytes in the underlying lamina propria (Dahan et al. 2007; Hershberg and Mayer 2000).

8. Tbet and RORγt coexpression in the lamina propria

Surprisingly, we found that many MAIT cells co-expressed Tbet and RORγt in the lamina propria, which has not been reported yet in mice at steady-state (Rahimpour et al. 2015; Salou et al. 2019), but found in human MAIT cells from the thymus and periphery (Leeansyah et al. 2015). This suggests a form of plasticity within MAIT17 lineage that occurs post-thymically and in a tissue-specific manner. The importance of Tbet in MAIT17 cells is currently unknown. However, the co-expression of Tbet suggests that MAIT cell may acquire type 1 transcriptional and functional traits which may give the cell an advantage for fighting infections. Regulatory cues governing Tbet expression in MAIT17 are to be defined. The current study shows that expression of Tbet was independent of MyD88 but depends on TCR signalling. Further experiments using floxed MR1 mouse that we recently generated are needed to better investigate such an aspect. Previous studies have provided evidence that STAT5B signalling favors Tbet-expressing γδT17 cells in the lamina propria (Kadekar et al. 2019), raising the possibility that the gut-specific cytokine milieu that activate STAT5 may also regulate Tbet expression in MAIT cells.

Conclusion and therapeutic prospects

In summary, we show that intestinal inflammation drives riboflavin production and the accumulation of metabolite antigens captured by MR1 and presented to MAIT cells in the lamina propria, which provide tissue-repair mediators in return. Overproduction of riboflavin by the microbiota may result from increased oxygen concentration in the colon. The study helps understand how the host immune system monitors and responds to altered microbial metabolism during intestinal inflammation. Our findings establish a new molecular interaction between the microbiota and mammalian hosts. The identification of the riboflavin pathway as a functional marker of colitis may explains why MAIT cells recognize riboflavin metabolites.

The demonstration that MAIT cells protect against colitis may facilitate the development of novel therapeutic approaches in inflammatory bowel diseases. Conditional MR1 transgenic mice (LSL-MR1) crossed to the Villin-Cre mice will be very useful in future experiments to assess the contribution of MAIT TCR activation to protection from colitis. The preparation of MAIT antigens in DMSO was found to improve its stability (Mak et al. 2017) and allows their utilization in preclinical and clinical settings. Therefore, we will assess the therapeutic potential of activating MAIT cells with a stimulating ligand and the effect of blocking MAIT cells function with a non-activating ligand. It is worth noting that in contrast to T cells which are often difficult to exploit therapeutically because the diversity of the TCR repertoire, MAIT cells are an attractive target because they express a semi-invariant TCR that binds to well characterized antigens.

Our findings pave the way to further elucidate the interaction between the gut microbiota and MAIT cells in other pathologies associated with dysbiosis such as colorectal cancer. Previous studies have shown that MAIT cells are present in tumors, and that they are capable of cytokine release in response to in vitro stimulation (Berzins et al. 2020). There is an ongoing debate about whether (1) MAIT cell activation in tumors occurs through direct recognition of microbial antigens or via TCR-independent activation, (2) MAIT cell activation promotes malignancy or inhibit cancer progression. MAIT cells from CRC patients can release IFNγ (Ling et al. 2016) which has been shown to control cancer progression by inducing senescence in cancers (Braumüller et al. 2013).

On the other hand, MAIT cells can also express IL-17 (Ling et al. 2016) which can promote cancer progression (Grivennikov et al. 2012). Our study showed that MAIT cells can produce Areg when stimulated in a TCR-dependent manner. While Areg is important in tissue repair, it can also promote tumor growth (Chayangsu et al. 2017). Therefore, studies are required to address the question of the role of MAIT cells in CRC and the possible correlations between their activation and the course of the disease. Depending on whether MAIT cells are beneficial or deleterious in CRC, they can be activated or inhibited in therapeutic settings.

Les lymphocytes MAIT et la riboflavine dans les interactions hôte-microbiote

Les interactions constantes et mutuelles entre hôte et microbiote jouent un rôle clé dans le maintien de la santé et du bien-être de l'hôte. Dans l'organisme, il existe différents microbiotes, dont le microbiote intestinal surtout dans la partie distale de l'intestin (le côlon) est le plus important d'entre eux. Le microbiote se met en place dès la naissance et sa composition est le résultat d'un processus complexe de sélection par l'hôte. Les cellules épithéliales jouent un rôle clé dans ce processus. Par exemple, la consommation du dioxygène par le métabolisme des cellules épithéliales coliques permet de maintenir l'anaérobie dans la lumière du côlon et donc empêche la croissance de certains micro-organismes. Ainsi, le microbiote intestinal des adultes est dominé par des espèces bactériennes anaérobies strictes appartenant à l'embranchement des Bacteroidetes et des Firmicutes. Cependant, des bactéries anaérobies facultatives comme les Enterobactéries appartenant à l'embrachement des Protéobacteria, sont trouvées mais en nombre relativement faible dans le microbiote adulte.

On sait désormais que le microbiote intestinal joue un rôle important dans la maturation et l'homéostasie du système immunitaire, notamment en relarguant des métabolites microbiens. En conséquence, la dysbiose, c'est-à-dire l'altération qualitative et fonctionnelle de la communauté microbienne intestinale, est associée à de différentes pathologies notamment aux maladies inflammatoires chroniques de l'intestin (MICI). Cette dysbiose coïncide avec des modifications des molécules bactériennes produites, mais la façon dont le système immunitaire module ces modifications n'est pas bien connue. Cette question est particulièrement importante pour les cellules T invariantes associées aux muqueuses (MAIT), qui se trouvent principalement dans les tissus colonisés par le microbiote et reconnaissent des métabolites microbiens dérivés de la voie de synthèse de la riboflavine.

La riboflavine, ou la vitamine B_2, est une vitamine synthétisée par la flore intestinale de manière naturelle et peut être également absorbée par le biais de l'alimentation quotidienne. Sa plus importante fonction biologique est de participer à des reactions rédox par l'intermediaire de FAD et FMN.

Par exemple, le FAD est un cofacteur nécessaire à l'activité du glutathion reductase qui sert à régénérer le glutathion, un puissant anti-oxydant, ce qui fait que la riboflavine participe aux défenses antioxydantes. Presque tous les Bacteroidetes et Proteobacteria codent pour la voie de synthèse de la riboflavine et des bactéries intestinales de l'embranchement des Bacteroidetes et Protéobacteria peuvent produire le ligand des MAIT, suggérant une interaction entre les cellules MAIT et le microbiote.

Les cellules MAIT constituent une population de lymphocytes T non conventionnels et ont la particularité d'exprimer un récepteur des cellules T (TCR) semi-invariant. Ce TCR reconnaît spécifiquement des antigènes (5-OP-RU et 5-OE-RU) derivant d'un intermédiaire du métabolisme de la riboflavine et présentées aux lymphocytes MAIT par la molécule non polymorphe MR1 (MHC-related protein 1) apparentée au CMH de classe I. Ces cellules sont activées soit d'une façon MR1-indépendante, *via* certaines cytokines inflammatoires, notamment l'IL-12 et l'IL-18, soit par une reconnaissance d'antigènes microbiens par l'intermédiaire de MR1. Les cellules MAIT ont un rôle dans les maladies infectieuses et métaboliques, la réparation tissulaire et sont alterées chez les patients MICI. Cependant le rôle des cellules MAIT dans les MICI reste inconnu.

La dysbiose au cours de MICI se caractérise par une augmentation des Entérobactéries et une diminution des Firmicutes (en particulier *Faecalibacterium prausnitzii*). Beaucoup d'arguments scientifiques comme l'expansion des bactéries anaerobies facultatives au cours de MICI, suggèrent une perte de l'hypoxie dans le côlon des patients MICI. Les MICI sont souvent associées à une augmentation du stress oxydant et une diminution des capacités anti-oxydantes. La participation de la riboflavine aux défenses antioxydantes suggère une augmentation des besoins en riboflavine pendant l'inflammation intestinale. En accord avec cette hypothèse, les bactéries prototrophes pour la riboflavine sont enrichies dans la maladie de Crohn. L'objectif de cette thèse était de comprendre l'interaction entre les MAIT et le microbiote et d'identifier le rôle des MAIT dans l'intestin.

Dans une première partie, nous avons exploré l'hypothèse que les cellules MAIT détectent des alterations du métabolisme bactérien associées aux dysbioses dans le colon. Nous avons montré que la dysanaérobiose, lors d'un traitement antibiotique ou d'une colite induite par le dextran sulfate de sodium (DSS), augmente la production du ligand des MAIT dans l'intestin. Cette notion était étayée par les données montrant une concentration plus elevée du ligand des MAIT au niveau de l'interface oxique-anoxique près de l'épithélium intestinal, ainsi qu'une surexpression des enzymes responsables de la production de riboflavine après une exposition à l'air in vitro. Pour identifier les bactéries impliquées, nous avons séquencé les gènes d'ARNr 16S dans l'intestin de souris traitées aux antibiotiques et au DSS, et analysé, en utilisant des données publiées, l'expression des gènes de la voie de la riboflavine en cas de colite induite par l'infection par *Helicobacter hepaticus*. La dysanaérobiose était associée à l'expansion des *Enterobacteriaceae* lors du traitement à la vancomycine, et des *Bacteroidaceae* et *Enterobacteriaceae* en cas de colite. La production de riboflavine était bénéfique pour l'expansion d'*Escherichia coli* dans l'intestin enflammé. Les *Bacteroidaceae* et les *Enterobacteriacea* peuvent produire de grandes quantités de ligands des MAIT *in vitro*. Le gène *ribD*, qui contrôle la production des ligands des MAIT, était surexprimé par les *Bacteroidaceae*, les *Clostridiaceae* et les *Enterobacteriaceae* en cas de colite induite par *H. hepaticus*. Les ligands des MAIT traversaient la barrière intestinale et augmentaient la stimulation TCR des MAIT lors de traitements à la vancomycine et au DSS. La création d'une nouvelle souris transgénique a permis de montrer que la stimulation TCR des MAIT du côlon entraîne la production du facteur de croissance épidermique amphiréguline (Areg). Lors de l'inflammation intestinale chronique, les MAIT du côlon produisaient spontanément les médiateurs protecteurs Areg et l'interféron-gamma (IFNy), l'interleukine (IL) -17 et l'IL-22. En conséquence, les souris $B6^{Cast}$ $Mr1^{-/-}$, dépourvues de MAIT, étaient plus sensibles à la colite que les souris $Mr1^{+}$, ce qui indique que les MAIT protègent contre la colite.

En résumé de cette première partie, les MAIT détectent et répondent directement aux changements du métabolisme bactérien associés à l'inflammation du colon et assurent en retour une protection de l'hôte. La surproduction de riboflavine par le microbiote peut résulter d'une augmentation de la concentration d'oxygène dans le côlon ; toutefois des expériences ultérieures sont nécessaires sur ce point**.** Nos résultats établissent une nouvelle interaction moléculaire entre le microbiote intestinal et son hôte.

La démonstration que les cellules MAIT protègent contre la colite peut faciliter le développement de nouvelles approches thérapeutiques dans les MICI. Il est à noter que contrairement aux cellules T qui sont souvent difficiles à exploiter thérapeutiquement en raison de la diversité du répertoire TCR, les cellules MAIT sont une cible attractive car elles expriment un TCR semi-invariant qui se lie à des antigènes bien caractérisés. Les souris transgéniques LSL-MR1 croisées avec les souris Villin-Cre seront très utiles dans de futures expériences pour évaluer la contribution de l'activation des cellules MAIT par leur TCR à la protection contre la colite. D'autre part, nous évaluerons le potentiel thérapeutique de l'activation des cellules MAIT avec un ligand d'activation et l'effet du blocage de la fonction des cellules MAIT avec un antagoniste.
Nos résultats ouvrent la voie à une meilleure élucidation de l'interaction entre le microbiote intestinal et les cellules MAIT dans d'autres pathologies associées à la dysbiose comme le cancer colorectal. Des études antérieures ont montré que les cellules MAIT sont présentes dans les tumeurs et qu'elles sont capables de libérer des cytokines en réponse à une stimulation in vitro. Cependant le rôle des cellules MAIT dans le cancer colorectal reste inconnu et nécessite d'être evalué.

Dans une seconde partie, nous avons caractérisé les MAIT dans l'iléon, dans lequel des niveaux élevés d'oxygène ont été observés à l'état basal. Plusieurs bactéries codant pour la voie de la riboflavine colonisaient l'iléon, mais les MAIT n'étaient pas activés par leur TCR et exprimaient un phénotype distinct dans l'iléon. Une explication possible était la faible expression de MR1 dans l'iléon. Bien que l'expression inductible de MR1 dans les cellules épithéliales de l'intestin ait déclenché une signalisation TCR dans les cellules MAIT de l'iléon, les signaux MAIT TCR dans l'iléon étaient plus faibles que dans le côlon. Nos données suggèrent une faible production d'antigènes MAIT dans l'iléon. Nous envisageons que les gènes contrôlant la production du précurseur du ligand MAIT dans certaines bactéries de l'iléon soient atténués par la riboflavine provenant de l'alimentation. Il est également plausible que les antagonistes d'origine alimentaire tels que les métabolites dérivés du folate, entrent en compétition avec les antigènes microbiens pour se lier à MR1 dans l'intestin grêle. L'analyse de l'activité transcriptionnelle bactérienne le long de l'intestin de souris nourries au régime déficient en riboflavine ou pauvre en folates serait utile pour aborder ces possibilités.

References

Abbas, Charles, and Andriy Sibirny. 2011. “Genetic Control of Biosynthesis and Transport of Riboflavin and Flavin Nucleotides and Construction of Robust Biotechnological Producers.” *Microbiology and Molecular Biology Reviews : MMBR* 75 (June): 321–60. https://doi.org/10.1128/MMBR.00030-10.

Abenavoli, Ludovico, Emidio Scarpellini, Carmela Colica, Luigi Boccuto, Bahare Salehi, Javad Sharifi-Rad, Vincenzo Aiello, et al. 2019. “Gut Microbiota and Obesity: A Role for Probiotics.” *Nutrients* 11 (11). https://doi.org/10.3390/nu11112690.

Agüero, Mercedes Gomez de, Stephanie C. Ganal-Vonarburg, Tobias Fuhrer, Sandra Rupp, Yasuhiro Uchimura, Hai Li, Anna Steinert, et al. 2016. “The Maternal Microbiota Drives Early Postnatal Innate Immune Development.” *Science* 351 (6279): 1296–1302. https://doi.org/10.1126/science.aad2571.

Ahern, Philip P., and Kevin J. Maloy. 2020. “Understanding Immune-Microbiota Interactions in the Intestine.” *Immunology* 159 (1): 4–14. https://doi.org/10.1111/imm.13150.

Albenberg, L, TV Esipova, CP Judge, K Bittinger, J Chen, A Laughlin, S Grunberg, et al. 2014. “Correlation Between Intraluminal Oxygen Gradient and Radial Partitioning of Intestinal Microbiota in Humans and Mice.” *Gastroenterology* 147 (5): 1055-1063.e8. https://doi.org/10.1053/j.gastro.2014.07.020.

Alexeev, Erica E., Jordi M. Lanis, Daniel J. Kao, Eric L. Campbell, Caleb J. Kelly, Kayla D. Battista, Mark E. Gerich, et al. 2018. “Microbiota-Derived Indole Metabolites Promote Human and Murine Intestinal Homeostasis through Regulation of Interleukin-10 Receptor.” *The American Journal of Pathology* 188 (5): 1183–94. https://doi.org/10.1016/j.ajpath.2018.01.011.

Andoh, Akira, Zhuobin Zhang, Osamu Inatomi, Sanae Fujino, Yasuyuki Deguchi, Yoshio Araki, Tomoyuki Tsujikawa, et al. 2005. “Interleukin-22, a Member of the IL-10 Subfamily, Induces Inflammatory Responses in Colonic Subepithelial Myofibroblasts.” *Gastroenterology* 129 (3): 969–84. https://doi.org/10.1053/j.gastro.2005.06.071.

Ashoori, Marziyeh, and Ahmad Saedisomeolia. 2014. “Riboflavin (Vitamin B_2) and Oxidative Stress: A Review.” *The British Journal of Nutrition* 111 (11): 1985–91. https://doi.org/10.1017/S0007114514000178.

Awad, Wael, Geraldine J. M. Ler, Weijun Xu, Andrew N. Keller, Jeffrey Y. W. Mak, Xin Yi Lim, Ligong Liu, et al. 2020. “The Molecular Basis Underpinning the Potency and Specificity of MAIT Cell Antigens.” *Nature Immunology* 21 (4): 400–411. https://doi.org/10.1038/s41590-020-0616-6.

Bansal, Tarun, Robert C. Alaniz, Thomas K. Wood, and Arul Jayaraman. 2010. “The Bacterial Signal Indole Increases Epithelial-Cell Tight-Junction Resistance and Attenuates Indicators of Inflammation.” *Proceedings of the National Academy of Sciences of the United States of America* 107 (1): 228–33. https://doi.org/10.1073/pnas.0906112107.

Baughn, Anthony D., and Michael H. Malamy. 2004. “The Strict Anaerobe Bacteroides Fragilis Grows in and Benefits from Nanomolar Concentrations of Oxygen.” *Nature* 427 (6973): 441–44. https://doi.org/10.1038/nature02285.

Ben Youssef, Ghada, Marie Tourret, Marion Salou, Liana Ghazarian, Véronique Houdouin, Stanislas Mondot, Yvonne Mburu, et al. 2018. “Ontogeny of Human Mucosal-Associated Invariant T Cells and Related T Cell Subsets.” *The Journal of Experimental Medicine* 215 (2): 459–79. https://doi.org/10.1084/jem.20171739.

Bernink, Jochem H., Lisette Krabbendam, Kristine Germar, Esther de Jong, Konrad Gronke, Michael Kofoed-Nielsen, J. Marius Munneke, et al. 2015. “Interleukin-12 and -23 Control Plasticity of CD127+ Group 1 and Group 3 Innate Lymphoid Cells in the Intestinal Lamina Propria.” *Immunity* 43 (1): 146–60. https://doi.org/10.1016/j.immuni.2015.06.019.

Berzins, Stuart P., Morgan E. Wallace, George Kannourakis, and Jason Kelly. 2020. “A Role for MAIT Cells in Colorectal Cancer.” *Frontiers in Immunology* 11 (May). https://doi.org/10.3389/fimmu.2020.00949.

Beudeker, Boris J. B., Gertine W. van Oord, Joop E. Arends, Julian Schulze zur Wiesch, Marieke S. van der Heide, Robert J. de Knegt, Annelies Verbon, Andre Boonstra, and Mark A. A. Claassen. 2018. “Mucosal- associated Invariant T- cell Frequency and Function in Blood and Liver of HCV Mono- and HCV/HIV Co- infected Patients with Advanced Fibrosis.” *Liver International* 38 (3): 458–68. https://doi.org/10.1111/liv.13544.

Billerbeck, Eva, Yu-Hoi Kang, Lucy Walker, Helen Lockstone, Stefanie Grafmueller, Vicki Fleming, Jonathan Flint, et al. 2010. “Analysis of CD161 Expression on Human CD8+ T Cells Defines a Distinct Functional Subset with Tissue-Homing Properties.” *Proceedings of the National Academy of Sciences of the United States of America* 107 (7): 3006–11. https://doi.org/10.1073/pnas.0914839107.

Boden, Elisa K., and Scott B. Snapper. 2008. “Regulatory T Cells in Inflammatory Bowel Disease.” *Current Opinion in Gastroenterology* 24 (6): 733–41. https://doi.org/10.1097/mog.0b013e328311f26e.

Bolte, Fabian J., Ashley C. O’Keefe, Lauren M. Webb, Elisavet Serti, Elenita Rivera, T. Jake Liang, Marc Ghany, and Barbara Rehermann. 2017. “Intra-Hepatic Depletion of Mucosal-Associated Invariant T Cells in Hepatitis C Virus-Induced Liver Inflammation.” *Gastroenterology* 153 (5): 1392-1403.e2. https://doi.org/10.1053/j.gastro.2017.07.043.

Boudinot, Pierre, Stanislas Mondot, Luc Jouneau, Luc Teyton, Marie-Paule Lefranc, and Olivier Lantz. 2016. “Restricting Nonclassical MHC Genes Coevolve with TRAV Genes Used by Innate-like T Cells in Mammals.” *Proceedings of the National Academy of Sciences of the United States of America* 113 (21): E2983-2992. https://doi.org/10.1073/pnas.1600674113.

Brand, Stephan, Florian Beigel, Torsten Olszak, Kathrin Zitzmann, Sören T. Eichhorst, Jan-Michel Otte, Helmut Diepolder, et al. 2006. “IL-22 Is Increased in Active Crohn’s Disease and Promotes Proinflammatory Gene Expression and Intestinal Epithelial Cell Migration.” *American Journal of Physiology. Gastrointestinal and Liver Physiology* 290 (4): G827-838. https://doi.org/10.1152/ajpgi.00513.2005.

Braumüller, Heidi, Thomas Wieder, Ellen Brenner, Sonja Aßmann, Matthias Hahn, Mohammed Alkhaled, Karin Schilbach, et al. 2013. “T-Helper-1-Cell Cytokines Drive Cancer into Senescence.” *Nature* 494 (7437): 361–65. https://doi.org/10.1038/nature11824.

Byndloss, Mariana X., Erin E. Olsan, Fabian Rivera-Chávez, Connor R. Tiffany, Stephanie A. Cevallos, Kristen L. Lokken, Teresa P. Torres, et al. 2017. “Microbiota-Activated PPAR-γ-Signaling Inhibits Dysbiotic Enterobacteriaceae Expansion.” *Science (New York, N.Y.)* 357 (6351): 570–75. https://doi.org/10.1126/science.aam9949.

CAO, STEWART SIYAN, ELLEN M. ZIMMERMANN, BRANDY–MENGCHIEH CHUANG, BENBO SONG, ANOSIKE NWOKOYE, J. ERBY WILKINSON, KATHRYN A. EATON, and RANDAL J. KAUFMAN. 2013. “The Unfolded Protein Response and Chemical Chaperones Reduce Protein Misfolding and Colitis in Mice.” *Gastroenterology* 144 (5): 989-1000.e6. https://doi.org/10.1053/j.gastro.2013.01.023.

Carolan, Eirin, Laura M. Tobin, Bozgana A. Mangan, Michelle Corrigan, Gadinthsware Gaoatswe, Greg Byrne, Justin Geoghegan, et al. 2015. “Altered Distribution and Increased IL-17 Production by Mucosal-Associated Invariant T Cells in Adult and Childhood Obesity.” *Journal of Immunology (Baltimore, Md.: 1950)* 194 (12): 5775–80. https://doi.org/10.4049/jimmunol.1402945.

Cevallos, Stephanie A., Jee-Yon Lee, Connor R. Tiffany, Austin J. Byndloss, Luana Johnston, Mariana X. Byndloss, and Andreas J. Bäumler. 2019. “Increased Epithelial Oxygenation Links Colitis to an Expansion of Tumorigenic Bacteria.” *MBio* 10 (5). https://doi.org/10.1128/mBio.02244-19.

Chassaing, Benoit, Jesse D. Aitken, Madhu Malleshappa, and Matam Vijay-Kumar. 2014. “Dextran Sulfate Sodium (DSS)-Induced Colitis in Mice.” *Current Protocols in Immunology / Edited by John E. Coligan ... [et Al.]* 104 (February): Unit-15.25. https://doi.org/10.1002/0471142735.im1525s104.

Chayangsu, Chawalit, Siriporn Khunsri, Virote Sriuranpong, and Suebpong Tanasanvimon. 2017. “The Correlations between Serum Amphiregulin and Other Clinicopathological Factors in Colorectal Cancer.” *Journal of Gastrointestinal Oncology* 8 (6): 980–84. https://doi.org/10.21037/jgo.2017.08.15.

Chen, Grace Y., Maochang Liu, Fuyuan Wang, John Bertin, and Gabriel Núñez. 2011. “A Functional Role for Nlrp6 in Intestinal Inflammation and Tumorigenesis.” *Journal of Immunology (Baltimore, Md. : 1950)* 186 (12): 7187–94. https://doi.org/10.4049/jimmunol.1100412.

Chen, Jianwei, and Marc Strous. 2013. “Denitrification and Aerobic Respiration, Hybrid Electron Transport Chains and Co-Evolution.” *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, The evolutionary aspects of bioenergetic systems, 1827 (2): 136–44. https://doi.org/10.1016/j.bbabio.2012.10.002.

Chen, Z., H. Wang, C. D’Souza, S. Sun, L. Kostenko, S. B. G. Eckle, B. S. Meehan, et al. 2017. “Mucosal-Associated Invariant T-Cell Activation and Accumulation after in Vivo Infection Depends on Microbial Riboflavin Synthesis and Co-Stimulatory Signals.” *Mucosal Immunology* 10 (1): 58–68. https://doi.org/10.1038/mi.2016.39.

Chin, Alex C., and Charles A. Parkos. 2006. “Neutrophil Transepithelial Migration and Epithelial Barrier Function in IBD.” *Annals of the New York Academy of Sciences* 1072 (1): 276–87. https://doi.org/10.1196/annals.1326.018.

Chu, Derrick M., Jun Ma, Amanda L. Prince, Kathleen M. Antony, Maxim D. Seferovic, and Kjersti M. Aagaard. 2017. “Maturation of the Infant Microbiome Community Structure and Function across Multiple Body Sites and in Relation to Mode of Delivery.” *Nature Medicine* 23 (3): 314–26. https://doi.org/10.1038/nm.4272.

Cl, Zindl, Lai Jf, Lee Yk, Maynard Cl, Harbour Sn, Ouyang W, Chaplin Dd, and Weaver Ct. 2013. “IL-22-Producing Neutrophils Contribute to Antimicrobial Defense and Restitution of Colonic Epithelial Integrity during Colitis.” Proceedings of the National Academy of Sciences of the United States of America. Proc Natl Acad Sci U S A. July 30, 2013. https://doi.org/10.1073/pnas.1300318110.

Combellick, Joan L., Hakdong Shin, Dongjae Shin, Yi Cai, Holly Hagan, Corey Lacher, Din L. Lin, Kathryn McCauley, Susan V. Lynch, and Maria Gloria Dominguez-Bello. 2018. “Differences in the Fecal Microbiota of Neonates Born at Home or in the Hospital.” *Scientific Reports* 8 (1): 15660. https://doi.org/10.1038/s41598-018-33995-7.

Constantinides, Michael G., Verena M. Link, Samira Tamoutounour, Andrea C. Wong, P. Juliana Perez-Chaparro, Seong-Ji Han, Y. Erin Chen, et al. 2019. “MAIT Cells Are Imprinted by the Microbiota in Early Life and Promote Tissue Repair.” *Science* 366 (6464). https://doi.org/10.1126/science.aax6624.

Corbett, Alexandra J., Sidonia B. G. Eckle, Richard W. Birkinshaw, Ligong Liu, Onisha Patel, Jennifer Mahony, Zhenjun Chen, et al. 2014. “T-Cell Activation by Transitory Neo-Antigens Derived from Distinct Microbial Pathways.” *Nature* 509 (7500): 361–65. https://doi.org/10.1038/nature13160.

Cosgrove, Cormac, James E. Ussher, Andri Rauch, Kathleen Gärtner, Ayako Kurioka, Michael H. Hühn, Krista Adelmann, et al. 2013. “Early and Nonreversible Decrease of CD161++/MAIT Cells in HIV Infection.” *Blood* 121 (6): 951–61. https://doi.org/10.1182/blood-2012-06-436436.

Croxford, J. Ludovic, Sachiko Miyake, Yi-Ying Huang, Michio Shimamura, and Takashi Yamamura. 2006. “Invariant V(Alpha)19i T Cells Regulate Autoimmune Inflammation.” *Nature Immunology* 7 (9): 987–94. https://doi.org/10.1038/ni1370.

Cui, Yue, Katarzyna Franciszkiewicz, Yvonne K. Mburu, Stanislas Mondot, Lionel Le Bourhis, Virginie Premel, Emmanuel Martin, et al. 2015. “Mucosal-Associated Invariant T Cell–Rich Congenic Mouse Strain Allows Functional Evaluation.” American Society for Clinical Investigation. November 2, 2015. https://doi.org/10.1172/JCI82424.

Dahan, Stephanie, Franziska Roth-Walter, Paul Arnaboldi, Shradha Agarwal, and Lloyd Mayer. 2007. “Epithelia: Lymphocyte Interactions in the Gut.” *Immunological Reviews* 215 (February). https://doi.org/10.1111/j.1600-065X.2006.00484.x.

De Weirdt, Rosemarie, and Tom Van de Wiele. 2015. “Micromanagement in the Gut: Microenvironmental Factors Govern Colon Mucosal Biofilm Structure and Functionality.” *NPJ Biofilms and Microbiomes* 1: 15026. https://doi.org/10.1038/npjbiofilms.2015.26.

Donaldson, Gregory P., S. Melanie Lee, and Sarkis K. Mazmanian. 2016. “Gut Biogeography of the Bacterial Microbiota.” *Nature Reviews. Microbiology* 14 (1): 20–32. https://doi.org/10.1038/nrmicro3552.

Duboc, Henri, Sylvie Rajca, Dominique Rainteau, David Benarous, Marie-Anne Maubert, Elodie Quervain, Ginette Thomas, et al. 2013. “Connecting Dysbiosis, Bile-Acid Dysmetabolism and Gut Inflammation in Inflammatory Bowel Diseases.” *Gut* 62 (4): 531–39. https://doi.org/10.1136/gutjnl-2012-302578.

Dusseaux, Mathilde, Emmanuel Martin, Nacer Serriari, Isabelle Péguillet, Virginie Premel, Delphine Louis, Maud Milder, et al. 2011. “Human MAIT Cells Are Xenobiotic-Resistant, Tissue-Targeted, CD161hi IL-17-Secreting T Cells.” *Blood* 117 (4): 1250–59. https://doi.org/10.1182/blood-2010-08-303339.

Eastaff-Leung, Nicola, Nicholas Mabarrack, Angela Barbour, Adrian Cummins, and Simon Barry. 2010. “Foxp3+ Regulatory T Cells, Th17 Effector Cells, and Cytokine Environment in Inflammatory Bowel Disease.” *Journal of Clinical Immunology* 30 (1): 80–89. https://doi.org/10.1007/s10875-009-9345-1.

Eckle, Sidonia B. G., Richard W. Birkinshaw, Lyudmila Kostenko, Alexandra J. Corbett, Hamish E. G. McWilliam, Rangsima Reantragoon, Zhenjun Chen, et al. 2014. “A Molecular Basis Underpinning the T Cell Receptor Heterogeneity of Mucosal-Associated Invariant T Cells.” *The Journal of Experimental Medicine* 211 (8): 1585–1600. https://doi.org/10.1084/jem.20140484.

Eckle, Sidonia B. G., Alexandra J. Corbett, Andrew N. Keller, Zhenjun Chen, Dale I. Godfrey, Ligong Liu, Jeffrey Y. W. Mak, David P. Fairlie, Jamie Rossjohn, and James McCluskey. 2015. “Recognition of Vitamin B Precursors and Byproducts by Mucosal Associated Invariant T Cells.” *The Journal of Biological Chemistry* 290 (51): 30204–11. https://doi.org/10.1074/jbc.R115.685990.

Elinav, Eran, Till Strowig, Andrew L. Kau, Jorge Henao-Mejia, Christoph A. Thaiss, Carmen J. Booth, David R. Peaper, et al. 2011. “NLRP6 Inflammasome Regulates Colonic Microbial Ecology and Risk for Colitis.” *Cell* 145 (5): 745–57. https://doi.org/10.1016/j.cell.2011.04.022.

Eyerich, Kilian, Valentina Dimartino, and Andrea Cavani. 2017. “IL-17 and IL-22 in Immunity: Driving Protection and Pathology.” *European Journal of Immunology* 47 (4): 607–14. https://doi.org/10.1002/eji.201646723.

Fabbrini, Elisa, Marina Cella, Steve A. McCartney, Anja Fuchs, Nada A. Abumrad, Terri A. Pietka, Zhouji Chen, et al. 2013. “Association between Specific Adipose Tissue CD4+ T-Cell Populations and Insulin Resistance in Obese Individuals.” *Gastroenterology* 145 (2): 366-374.e1-3. https://doi.org/10.1053/j.gastro.2013.04.010.

Fernandez, Caroline S., Thakshila Amarasena, Anthony D. Kelleher, Jamie Rossjohn, James McCluskey, Dale I. Godfrey, and Stephen J. Kent. 2015. “MAIT Cells Are Depleted Early but Retain Functional Cytokine Expression in HIV Infection.” *Immunology and Cell Biology* 93 (2): 177–88. https://doi.org/10.1038/icb.2014.91.

Flament, Héloïse, Matthieu Rouland, Lucie Beaudoin, Amine Toubal, Léo Bertrand, Samuel Lebourgeois, Camille Rousseau, et al. 2021. “Outcome of SARS-CoV-2 Infection Is Linked to MAIT Cell Activation and Cytotoxicity.” *Nature Immunology*, February, 1–14. https://doi.org/10.1038/s41590-021-00870-z.

Franciszkiewicz, Katarzyna, Marion Salou, Francois Legoux, Qian Zhou, Yue Cui, Stéphanie Bessoles, and Olivier Lantz. 2016. “MHC Class I-Related Molecule, MR1, and Mucosal-Associated Invariant T Cells.” *Immunological Reviews* 272 (1): 120–38. https://doi.org/10.1111/imr.12423.

Frantz, A. L., E. W. Rogier, C. R. Weber, L. Shen, D. A. Cohen, L. A. Fenton, M. E. C. Bruno, and C. S. Kaetzel. 2012. “Targeted Deletion of MyD88 in Intestinal Epithelial Cells Results in Compromised Antibacterial Immunity Associated with Downregulation of Polymeric Immunoglobulin Receptor, Mucin-2, and Antibacterial Peptides.” *Mucosal Immunology* 5 (5): 501–12. https://doi.org/10.1038/mi.2012.23.

Fujino, S, A Andoh, S Bamba, A Ogawa, K Hata, Y Araki, T Bamba, and Y Fujiyama. 2003. “Increased Expression of Interleukin 17 in Inflammatory Bowel Disease.” *Gut* 52 (1): 65–70.

Furusawa, Yukihiro, Yuuki Obata, Shinji Fukuda, Takaho A. Endo, Gaku Nakato, Daisuke Takahashi, Yumiko Nakanishi, et al. 2013. “Commensal Microbe-Derived Butyrate Induces the Differentiation of Colonic Regulatory T Cells.” *Nature* 504 (7480): 446–50. https://doi.org/10.1038/nature12721.

Garcia-Angulo, Victor. 2016. “Overlapping Riboflavin Supply Pathways in Bacteria.” *Critical Reviews in Microbiology* 43 (November): 1–14. https://doi.org/10.1080/1040841X.2016.1192578.

Geboes, Lies, Laure Dumoutier, Hilde Kelchtermans, Evelien Schurgers, Tania Mitera, Jean-Christophe Renauld, and Patrick Matthys. 2009. “Proinflammatory Role of the Th17 Cytokine Interleukin-22 in Collagen-Induced Arthritis in C57BL/6 Mice.” *Arthritis and Rheumatism* 60 (2): 390–95. https://doi.org/10.1002/art.24220.

Georgel, Philippe, Mirjana Radosavljevic, Cécile Macquin, and Seiamak Bahram. 2011. “The Non-Conventional MHC Class I MR1 Molecule Controls Infection by Klebsiella Pneumoniae in Mice.” *Molecular Immunology* 48 (5): 769–75. https://doi.org/10.1016/j.molimm.2010.12.002.

Ghilardi, Nico, Noelyn Kljavin, Qi Chen, Sophie Lucas, Austin L. Gurney, and Frederic J. De Sauvage. 2004. “Compromised Humoral and Delayed-Type Hypersensitivity Responses in IL-23-Deficient Mice.” *Journal of Immunology (Baltimore, Md.: 1950)* 172 (5): 2827–33. https://doi.org/10.4049/jimmunol.172.5.2827.

Gold, Marielle C., Stefania Cerri, Susan Smyk-Pearson, Meghan E. Cansler, Todd M. Vogt, Jacob Delepine, Ervina Winata, et al. 2010. “Human Mucosal Associated Invariant T Cells Detect Bacterially Infected Cells.” *PLoS Biology* 8 (6): e1000407. https://doi.org/10.1371/journal.pbio.1000407.

Grimaldi, David, Lionel Le Bourhis, Bertrand Sauneuf, Agnès Dechartres, Christophe Rousseau, Fatah Ouaaz, Maud Milder, et al. 2014. “Specific MAIT Cell Behaviour among Innate-like T Lymphocytes in Critically Ill Patients with Severe Infections.” *Intensive Care Medicine* 40 (2): 192–201. https://doi.org/10.1007/s00134-013-3163-x.

Grivennikov, Sergei I., Kepeng Wang, Daniel Mucida, C. Andrew Stewart, Bernd Schnabl, Dominik Jauch, Koji Taniguchi, et al. 2012. “Adenoma-Linked Barrier Defects and Microbial Products Drive IL-23/IL-17-Mediated Tumour Growth.” *Nature* 491 (7423): 254–58. https://doi.org/10.1038/nature11465.

Gu, Shenghua, Dandan Chen, Jin-Na Zhang, Xiaoman Lv, Kun Wang, Li-Ping Duan, Yong Nie, and Xiao-Lei Wu. 2013. “Bacterial Community Mapping of the Mouse Gastrointestinal Tract.” *PLoS ONE* 8 (10). https://doi.org/10.1371/journal.pone.0074957.

Guaraldi, Federica, and Guglielmo Salvatori. 2012. “Effect of Breast and Formula Feeding on Gut Microbiota Shaping in Newborns.” *Frontiers in Cellular and Infection Microbiology* 2 (October). https://doi.org/10.3389/fcimb.2012.00094.

Gutiérrez-Preciado, Ana, Alfredo Gabriel Torres, Enrique Merino, Hernán Ruy Bonomi, Fernando Alberto Goldbaum, and Víctor Antonio García-Angulo. 2015. “Extensive Identification of Bacterial Riboflavin Transporters and Their Distribution across Bacterial Species.” *PLoS ONE* 10 (5). https://doi.org/10.1371/journal.pone.0126124.

Haberman, Yael, Timothy L. Tickle, Phillip J. Dexheimer, Mi-Ok Kim, Dora Tang, Rebekah Karns, Robert N. Baldassano, et al. 2014. “Pediatric Crohn Disease Patients Exhibit Specific Ileal Transcriptome and Microbiome Signature.” *The Journal of Clinical Investigation* 124 (8): 3617–33. https://doi.org/10.1172/JCI75436.

Haga, Keiichi, Asako Chiba, Tomoyoshi Shibuya, Taro Osada, Dai Ishikawa, Tomohiro Kodani, Osamu Nomura, Sumio Watanabe, and Sachiko Miyake. 2016. “MAIT Cells Are Activated and Accumulated in the Inflamed Mucosa of Ulcerative Colitis.” *Journal of Gastroenterology and Hepatology* 31 (5): 965–72. https://doi.org/10.1111/jgh.13242.

Hang, Saiyu, Donggi Paik, Lina Yao, Eunha Kim, Jamma Trinath, Jingping Lu, Soyoung Ha, et al. 2019. “Bile Acid Metabolites Control T H 17 and T Reg Cell Differentiation.” *Nature* 576 (7785): 143–48. https://doi.org/10.1038/s41586-019-1785-z.

Harper, Richart W., Changhong Xu, Jason P. Eiserich, Yin Chen, Cheng-Yuan Kao, Philip Thai, Henny Setiadi, and Reen Wu. 2005. “Differential Regulation of Dual NADPH Oxidases/Peroxidases, Duox1 and Duox2, by Th1 and Th2 Cytokines in Respiratory Tract Epithelium.” *FEBS Letters* 579 (21): 4911–17. https://doi.org/10.1016/j.febslet.2005.08.002.

Harriff, Melanie J., Meghan E. Cansler, Katelynne Gardner Toren, Elizabeth T. Canfield, Stephen Kwak, Marielle C. Gold, and David M. Lewinsohn. 2014. “Human Lung Epithelial Cells Contain Mycobacterium Tuberculosis in a Late Endosomal Vacuole and Are Efficiently Recognized by $CD8^+$ T Cells.” *PloS One* 9 (5): e97515. https://doi.org/10.1371/journal.pone.0097515.

Harriff, Melanie J., Elham Karamooz, Ansen Burr, Wilmon F. Grant, Elizabeth T. Canfield, Michelle L. Sorensen, Luis F. Moita, and David M. Lewinsohn. 2016. “Endosomal MR1 Trafficking Plays a Key Role in Presentation of Mycobacterium Tuberculosis Ligands to MAIT Cells.” *PLoS Pathogens* 12 (3). https://doi.org/10.1371/journal.ppat.1005524.

Hartmann, Nadine, Curtis McMurtrey, Michelle L. Sorensen, Megan E. Huber, Regina Kurapova, Fadie T. Coleman, Joseph P. Mizgerd, et al. 2018. “Riboflavin Metabolism Variation among Clinical Isolates of Streptococcus Pneumoniae Results in Differential Activation of Mucosal-Associated Invariant T Cells.” *American Journal of Respiratory Cell and Molecular Biology* 58 (6): 767–76. https://doi.org/10.1165/rcmb.2017-0290OC.

He, Guanglong, Ravi A. Shankar, Michael Chzhan, Alexandre Samouilov, Periannan Kuppusamy, and Jay L. Zweier. 1999. “Noninvasive Measurement of Anatomic Structure and Intraluminal Oxygenation in the Gastrointestinal Tract of Living Mice with Spatial and Spectral EPR Imaging.” *Proceedings of the National Academy of Sciences of the United States of America* 96 (8): 4586–91.

Hengst, Julia, Benedikt Strunz, Katja Deterding, Hans-Gustaf Ljunggren, Edwin Leeansyah, Michael P. Manns, Markus Cornberg, Johan K. Sandberg, Heiner Wedemeyer, and Niklas K. Björkström. 2016. “Nonreversible MAIT Cell-Dysfunction in Chronic Hepatitis C Virus Infection despite Successful Interferon-Free Therapy.” *European Journal of Immunology* 46 (9): 2204–10. https://doi.org/10.1002/eji.201646447.

Hershberg, R. M., and L. F. Mayer. 2000. “Antigen Processing and Presentation by Intestinal Epithelial Cells - Polarity and Complexity.” *Immunology Today* 21 (3): 123–28. https://doi.org/10.1016/s0167-5699(99)01575-3.

Hill, David A., Christian Hoffmann, Michael C. Abt, Yurong Du, Dmytro Kobuley, Thomas J. Kirn, Frederic D. Bushman, and David Artis. 2010. “Metagenomic Analyses Reveal Antibiotic-Induced Temporal and Spatial Changes in Intestinal Microbiota with Associated Alterations in Immune Cell Homeostasis.” *Mucosal Immunology* 3 (2): 148–58. https://doi.org/10.1038/mi.2009.132.

Hinks, Timothy S. C., Emanuele Marchi, Maisha Jabeen, Moshe Olshansky, Ayako Kurioka, Troi J. Pediongco, Bronwyn S. Meehan, et al. 2019. “Activation and In Vivo Evolution of the MAIT Cell Transcriptome in Mice and Humans Reveals Tissue Repair Functionality.” *Cell Reports* 28 (12): 3249-3262.e5. https://doi.org/10.1016/j.celrep.2019.07.039.

Hinks, Timothy S. C., and Xia-Wei Zhang. 2020. “MAIT Cell Activation and Functions.” *Frontiers in Immunology* 11 (May). https://doi.org/10.3389/fimmu.2020.01014.

Hollister, Emily B., Chunxu Gao, and James Versalovic. 2014. “Compositional and Functional Features of the Gastrointestinal Microbiome and Their Effects on Human Health.” *Gastroenterology* 146 (6): 1449–58. https://doi.org/10.1053/j.gastro.2014.01.052.

Honda, Kenya, and Dan R. Littman. 2016. “The Microbiota in Adaptive Immune Homeostasis and Disease.” *Nature* 535 (7610): 75–84. https://doi.org/10.1038/nature18848.

Huang, Shouxiong, Emmanuel Martin, Sojung Kim, Lawrence Yu, Claire Soudais, Daved H. Fremont, Olivier Lantz, and Ted H. Hansen. 2009. “MR1 Antigen Presentation to Mucosal-Associated Invariant T Cells Was Highly Conserved in Evolution.” *Proceedings of the National Academy of Sciences of the United States of America* 106 (20): 8290–95. https://doi.org/10.1073/pnas.0903196106.

Huang, Yen-Lin, Christophe Chassard, Martin Hausmann, Mark von Itzstein, and Thierry Hennet. 2015. “Sialic Acid Catabolism Drives Intestinal Inflammation and Microbial Dysbiosis in Mice.” *Nature Communications* 6 (1): 8141. https://doi.org/10.1038/ncomms9141.

Hueber, Wolfgang, Bruce E. Sands, Steve Lewitzky, Marc Vandemeulebroecke, Walter Reinisch, Peter D. R. Higgins, Jan Wehkamp, et al. 2012. “Secukinumab, a Human Anti-IL-17A Monoclonal Antibody, for Moderate to Severe Crohn’s Disease: Unexpected Results of a Randomised, Double-Blind Placebo-Controlled Trial.” *Gut* 61 (12): 1693–1700. https://doi.org/10.1136/gutjnl-2011-301668.

Hughes, Elizabeth R., Maria G. Winter, Breck A. Duerkop, Luisella Spiga, Tatiane Furtado de Carvalho, Wenhan Zhu, Caroline C. Gillis, et al. 2017. “Microbial Respiration and Formate Oxidation as Metabolic Signatures of Inflammation-Associated Dysbiosis.” *Cell Host & Microbe* 21 (2): 208–19. https://doi.org/10.1016/j.chom.2017.01.005.

Ilott, Nicholas Edward, Julia Bollrath, Camille Danne, Chris Schiering, Matthew Shale, Krista Adelmann, Thomas Krausgruber, Andreas Heger, David Sims, and Fiona Powrie. 2016a. “Defining the Microbial Transcriptional Response to Colitis through Integrated Host and Microbiome Profiling.” *The ISME Journal* 10 (10): 2389–2404. https://doi.org/10.1038/ismej.2016.40.

———. 2016b. “Defining the Microbial Transcriptional Response to Colitis through Integrated Host and Microbiome Profiling.” *The ISME Journal* 10 (10): 2389–2404. https://doi.org/10.1038/ismej.2016.40.

Imlay, James A. 2019. “Where in the World Do Bacteria Experience Oxidative Stress?” *Environmental Microbiology* 21 (2): 521–30. https://doi.org/10.1111/1462-2920.14445.

Ishimaru, Naozumi, Akiko Yamada, Masayuki Kohashi, Rieko Arakaki, Tetsuyuki Takahashi, Keisuke Izumi, and Yoshio Hayashi. 2008. “Development of Inflammatory Bowel Disease in Long-Evans Cinnamon Rats Based on CD4+CD25+Foxp3+ Regulatory T Cell Dysfunction.” *Journal of Immunology (Baltimore, Md.: 1950)* 180 (10): 6997–7008. https://doi.org/10.4049/jimmunol.180.10.6997.

Jiang, Jing, Xinjing Wang, Hongjuan An, Bingfen Yang, Zhihong Cao, Yanhua Liu, Jinwen Su, et al. 2014. “Mucosal-Associated Invariant T-Cell Function Is Modulated by Programmed Death-1 Signaling in Patients with Active Tuberculosis.” *American Journal of Respiratory and Critical Care Medicine* 190 (3): 329–39. https://doi.org/10.1164/rccm.201401-0106OC.

Jiang, Jing, Bingfen Yang, Hongjuan An, Xinjing Wang, Yanhua Liu, Zhihong Cao, Fei Zhai, Ruo Wang, Yan Cao, and Xiaoxing Cheng. 2016. “Mucosal-Associated Invariant T Cells from Patients with Tuberculosis Exhibit Impaired Immune Response.” *The Journal of Infection* 72 (3): 338–52. https://doi.org/10.1016/j.jinf.2015.11.010.

Jo, Juandy, Anthony T. Tan, James E. Ussher, Elena Sandalova, Xin-Zi Tang, Alfonso Tan-Garcia, Natalie To, et al. 2014. “Toll-Like Receptor 8 Agonist and Bacteria Trigger Potent Activation of Innate Immune Cells in Human Liver.” *PLoS Pathogens* 10 (6). https://doi.org/10.1371/journal.ppat.1004210.

Johansson, Malin E. V., Mia Phillipson, Joel Petersson, Anna Velcich, Lena Holm, and Gunnar C. Hansson. 2008. “The Inner of the Two Muc2 Mucin-Dependent Mucus Layers in Colon Is Devoid of Bacteria.” *Proceedings of the National Academy of Sciences of the United States of America* 105 (39): 15064–69. https://doi.org/10.1073/pnas.0803124105.

Juárez, Oscar, Mark J. Nilges, Portia Gillespie, Jennifer Cotton, and Blanca Barquera. 2008. “Riboflavin Is an Active Redox Cofactor in the Na+-Pumping NADH:Quinone Oxidoreductase (Na+-NQR) from Vibrio Cholerae.” *The Journal of Biological Chemistry* 283 (48): 33162–67. https://doi.org/10.1074/jbc.M806913200.

Kadekar, Darshana, Rasmus Agerholm, John Rizk, Heidi Neubauer, Tobias Suske, Barbara Maurer, Monica Torrellas Viñals, et al. 2019. “The Neonatal Microenvironment Programs Conventional and Intestinal Tbet+ ΓδT17 Cells through the Transcription Factor STAT5.” *BioRxiv*, June, 658542. https://doi.org/10.1101/658542.

Kanehisa, Minoru, Susumu Goto, Shuichi Kawashima, and Akihiro Nakaya. 2002. “The KEGG Databases at GenomeNet.” *Nucleic Acids Research* 30 (1): 42–46. https://doi.org/10.1093/nar/30.1.42.

Kelly, C. J., L. E. Glover, E. L. Campbell, D. J. Kominsky, S. F. Ehrentraut, B. E. Bowers, A. J. Bayless, B. J. Saeedi, and S. P. Colgan. 2013. “Fundamental Role for HIF-1α in Constitutive Expression of Human β Defensin-1.” *Mucosal Immunology* 6 (6): 1110–18. https://doi.org/10.1038/mi.2013.6.

Kelly, Caleb J., Leon Zheng, Eric L. Campbell, Bejan Saeedi, Carsten C. Scholz, Amanda J. Bayless, Kelly E. Wilson, et al. 2015. “Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function.” *Cell Host & Microbe* 17 (5): 662–71. https://doi.org/10.1016/j.chom.2015.03.005.

Khan, M. Tanweer, Sylvia H. Duncan, Alfons J. M. Stams, Jan Maarten van Dijl, Harry J. Flint, and Hermie J. M. Harmsen. 2012. “The Gut Anaerobe Faecalibacterium Prausnitzii Uses an Extracellular Electron Shuttle to Grow at Oxic-Anoxic Interphases.” *The ISME Journal* 6 (8): 1578–85. https://doi.org/10.1038/ismej.2012.5.

Kinugasa, Tetsushi, Takanori Sakaguchi, Xuibin Gu, and Hans–Christian Reinecker. 2000. “Claudins Regulate the Intestinal Barrier in Response to Immune Mediators.” *Gastroenterology* 118 (6): 1001–11. https://doi.org/10.1016/S0016-5085(00)70351-9.

Kitajima, S., S. Takuma, and M. Morimoto. 1999. “Changes in Colonic Mucosal Permeability in Mouse Colitis Induced with Dextran Sulfate Sodium.” *Experimental Animals* 48 (3): 137–43. https://doi.org/10.1538/expanim.48.137.

Kjer-Nielsen, Lars, Alexandra J. Corbett, Zhenjun Chen, Ligong Liu, Jeffrey Yw Mak, Dale I. Godfrey, Jamie Rossjohn, David P. Fairlie, James McCluskey, and Sidonia Bg Eckle. 2018. “An Overview on the Identification of MAIT Cell Antigens.” *Immunology and Cell Biology* 96 (6): 573–87. https://doi.org/10.1111/imcb.12057.

Kjer-Nielsen, Lars, Onisha Patel, Alexandra J. Corbett, Jérôme Le Nours, Bronwyn Meehan, Ligong Liu, Mugdha Bhati, et al. 2012. “MR1 Presents Microbial Vitamin B Metabolites to MAIT Cells.” *Nature* 491 (7426): 717–23. https://doi.org/10.1038/nature11605.

Klose, Christoph S. N., Elina A. Kiss, Vera Schwierzeck, Karolina Ebert, Thomas Hoyler, Yannick d’Hargues, Nathalie Göppert, et al. 2013a. “A T-Bet Gradient Controls the Fate and Function of CCR6 − RORγt + Innate Lymphoid Cells.” *Nature* 494 (7436): 261–65. https://doi.org/10.1038/nature11813.

Klose, Christoph S. N., Elina A. Kiss, Vera Schwierzeck, Karolina Ebert, Thomas Hoyler, Yannick d’Hargues, Nathalie Göppert, et al. 2013b. “A T-Bet Gradient Controls the Fate and Function of CCR6-RORγt+ Innate Lymphoid Cells.” *Nature* 494 (7436): 261–65. https://doi.org/10.1038/nature11813.

Koay, Hui-Fern, Nicholas A. Gherardin, Anselm Enders, Liyen Loh, Laura K. Mackay, Catarina F. Almeida, Brendan E. Russ, et al. 2016. “A Three-Stage Intrathymic Development Pathway for the Mucosal-Associated Invariant T Cell Lineage.” *Nature Immunology* 17 (11): 1300–1311. https://doi.org/10.1038/ni.3565.

Kobayashi, Taku, S Okamoto, Tadakazu Hisamatsu, Nobuhiko Kamada, H Chinen, R Saito, M Kitazume, et al. 2008. “IL23 Differentially Regulates the Th1/Th17 Balance in Ulcerative Colitis and Crohn’s Disease.” *Gut* 57 (December): 1682–89. https://doi.org/10.1136/gut.2007.135053.

Koh, Ara, Filipe De Vadder, Petia Kovatcheva-Datchary, and Fredrik Bäckhed. 2016. “From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites.” *Cell* 165 (6): 1332–45. https://doi.org/10.1016/j.cell.2016.05.041.

Kotlarz, Daniel, Rita Beier, Dhaarini Murugan, Jana Diestelhorst, Ole Jensen, Kaan Boztug, Dietmar Pfeifer, et al. 2012. “Loss of Interleukin-10 Signaling and Infantile Inflammatory Bowel Disease: Implications for Diagnosis and Therapy.” *Gastroenterology* 143 (2): 347–55. https://doi.org/10.1053/j.gastro.2012.04.045.

Kurioka, A., J. E. Ussher, C. Cosgrove, C. Clough, J. R. Fergusson, K. Smith, Y.-H. Kang, et al. 2015. “MAIT Cells Are Licensed through Granzyme Exchange to Kill Bacterially Sensitized Targets.” *Mucosal Immunology* 8 (2): 429–40. https://doi.org/10.1038/mi.2014.81.

Kurioka, Ayako, Lucy J. Walker, Paul Klenerman, and Christian B. Willberg. 2016. “MAIT Cells: New Guardians of the Liver.” *Clinical & Translational Immunology* 5 (8): e98. https://doi.org/10.1038/cti.2016.51.

Lamichhane, Rajesh, Marion Schneider, Sara M. de la Harpe, Thomas W. R. Harrop, Rachel F. Hannaway, Peter K. Dearden, Joanna R. Kirman, Joel D. A. Tyndall, Andrea J. Vernall, and James E. Ussher. 2019. “TCR- or Cytokine-Activated CD8+ Mucosal-Associated Invariant T Cells Are Rapid Polyfunctional Effectors That Can Coordinate Immune Responses.” *Cell Reports* 28 (12): 3061-3076.e5. https://doi.org/10.1016/j.celrep.2019.08.054.

Langille, Morgan G. I., Jesse Zaneveld, J. Gregory Caporaso, Daniel McDonald, Dan Knights, Joshua A. Reyes, Jose C. Clemente, et al. 2013. “Predictive Functional Profiling of Microbial Communities Using 16S RRNA Marker Gene Sequences.” *Nature Biotechnology* 31 (9): 814–21. https://doi.org/10.1038/nbt.2676.

Lavelle, A., G. Lennon, O. O’Sullivan, N. Docherty, A. Balfe, A. Maguire, H. E. Mulcahy, et al. 2015. “Spatial Variation of the Colonic Microbiota in Patients with Ulcerative Colitis and Control Volunteers.” *Gut* 64 (10): 1553–61. https://doi.org/10.1136/gutjnl-2014-307873.

Lavelle, Aonghus, and Harry Sokol. 2020. “Gut Microbiota-Derived Metabolites as Key Actors in Inflammatory Bowel Disease.” *Nature Reviews Gastroenterology & Hepatology* 17 (4): 223–37. https://doi.org/10.1038/s41575-019-0258-z.

Le Bourhis, Lionel, Mathilde Dusseaux, Armelle Bohineust, Stéphanie Bessoles, Emmanuel Martin, Virginie Premel, Maxime Coré, et al. 2013. “MAIT Cells Detect and Efficiently Lyse Bacterially-Infected Epithelial Cells.” *PLoS Pathogens* 9 (10): e1003681. https://doi.org/10.1371/journal.ppat.1003681.

Le Bourhis, Lionel, Emmanuel Martin, Isabelle Péguillet, Amélie Guihot, Nathalie Froux, Maxime Coré, Eva Lévy, et al. 2010. “Antimicrobial Activity of Mucosal-Associated Invariant T Cells.” *Nature Immunology* 11 (8): 701–8. https://doi.org/10.1038/ni.1890.

Le Nours, Jérôme, Nicholas A. Gherardin, Sri H. Ramarathinam, Wael Awad, Florian Wiede, Benjamin S. Gully, Yogesh Khandokar, et al. 2019. “A Class of Γδ T Cell Receptors Recognize the Underside of the Antigen-Presenting Molecule MR1.” *Science (New York, N.Y.)* 366 (6472): 1522–27. https://doi.org/10.1126/science.aav3900.

Leeansyah, Edwin, Anupama Ganesh, Máire F. Quigley, Anders Sönnerborg, Jan Andersson, Peter W. Hunt, Ma Somsouk, et al. 2013. “Activation, Exhaustion, and Persistent Decline of the Antimicrobial MR1-Restricted MAIT-Cell Population in Chronic HIV-1 Infection.” *Blood* 121 (7): 1124–35. https://doi.org/10.1182/blood-2012-07-445429.

Leeansyah, Edwin, Liyen Loh, Douglas F. Nixon, and Johan K. Sandberg. 2014. “Acquisition of Innate-like Microbial Reactivity in Mucosal Tissues during Human Fetal MAIT-Cell Development.” *Nature Communications* 5 (January). https://doi.org/10.1038/ncomms4143.

Leeansyah, Edwin, Jenny Svärd, Joana Dias, Marcus Buggert, Jessica Nyström, Máire F. Quigley, Markus Moll, Anders Sönnerborg, Piotr Nowak, and Johan K. Sandberg. 2015. “Arming of MAIT Cell Cytolytic Antimicrobial Activity Is Induced by IL-7 and Defective in HIV-1 Infection.” *PLoS Pathogens* 11 (8): e1005072. https://doi.org/10.1371/journal.ppat.1005072.

Legoux, François, Déborah Bellet, Celine Daviaud, Yara El Morr, Aurelie Darbois, Kristina Niort, Emanuele Procopio, et al. 2019. “Microbial Metabolites Control the Thymic Development of Mucosal-Associated Invariant T Cells.” *Science* 366 (6464): 494–99. https://doi.org/10.1126/science.aaw2719.

Legoux, François, Jules Gilet, Emanuele Procopio, Klara Echasserieau, Karine Bernardeau, and Olivier Lantz. 2019. “Molecular Mechanisms of Lineage Decisions in Metabolite-Specific T Cells.” *Nature Immunology* 20 (9): 1244–55. https://doi.org/10.1038/s41590-019-0465-3.

Legoux, François, Marion Salou, and Olivier Lantz. 2020. “MAIT Cell Development and Functions: The Microbial Connection.” *Immunity* 53 (4): 710–23. https://doi.org/10.1016/j.immuni.2020.09.009.

Leng, Tianqi, Hossain Delowar Akther, Carl-Philipp Hackstein, Kate Powell, Thomas King, Matthias Friedrich, Zoe Christoforidou, et al. 2019. “TCR and Inflammatory Signals Tune Human MAIT Cells to Exert Specific Tissue Repair and Effector Functions.” *Cell Reports* 28 (12): 3077-3091.e5. https://doi.org/10.1016/j.celrep.2019.08.050.

Leung, Daniel T., Taufiqur R. Bhuiyan, Naoshin S. Nishat, Mohammad Rubel Hoq, Amena Aktar, M. Arifur Rahman, Taher Uddin, et al. 2014. “Circulating Mucosal Associated Invariant T Cells Are Activated in Vibrio Cholerae O1 Infection and Associated with Lipopolysaccharide Antibody Responses.” *PLoS Neglected Tropical Diseases* 8 (8): e3076. https://doi.org/10.1371/journal.pntd.0003076.

Levy, Maayan, Aleksandra A. Kolodziejczyk, Christoph A. Thaiss, and Eran Elinav. 2017. “Dysbiosis and the Immune System.” *Nature Reviews Immunology* 17 (4): 219–32. https://doi.org/10.1038/nri.2017.7.

Ley, Ruth E., Catherine A. Lozupone, Micah Hamady, Rob Knight, and Jeffrey I. Gordon. 2008. “Worlds within Worlds: Evolution of the Vertebrate Gut Microbiota.” *Nature Reviews. Microbiology* 6 (10): 776–88. https://doi.org/10.1038/nrmicro1978.

Li, Y.-Z, K.-X Sun, and C. Zhao. 2006. “Expression of Foxp3 MRNA in Peripheral Blood Monocytes of Patients with Ulcerative Colitis” 14 (April): 810–13.

Lichtenstein, Gary R. 2013. “Comprehensive Review: Antitumor Necrosis Factor Agents in Inflammatory Bowel Disease and Factors Implicated in Treatment Response.” *Therapeutic Advances in Gastroenterology* 6 (4): 269–93. https://doi.org/10.1177/1756283X13479826.

Ling, Limian, Yuyang Lin, Wenwen Zheng, Sen Hong, Xiuqi Tang, Pingwei Zhao, Ming Li, et al. 2016. “Circulating and Tumor-Infiltrating Mucosal Associated Invariant T (MAIT) Cells in Colorectal Cancer Patients.” *Scientific Reports* 6 (February). https://doi.org/10.1038/srep20358.

Litvak, Yael, Mariana X. Byndloss, and Andreas J. Bäumler. 2018. “Colonocyte Metabolism Shapes the Gut Microbiota.” *Science* 362 (6418). https://doi.org/10.1126/science.aat9076.

Loh, Liyen, Zhongfang Wang, Sneha Sant, Marios Koutsakos, Sinthujan Jegaskanda, Alexandra J. Corbett, Ligong Liu, et al. 2016. “Human Mucosal-Associated Invariant T Cells Contribute to Antiviral Influenza Immunity via IL-18–Dependent Activation.” *Proceedings of the National Academy of Sciences* 113 (36): 10133–38. https://doi.org/10.1073/pnas.1610750113.

Louis, Nancy A., Kathryn E. Hamilton, Geraldine Canny, Laurie L. Shekels, Samuel B. Ho, and Sean P. Colgan. 2006. “Selective Induction of Mucin-3 by Hypoxia in Intestinal Epithelia.” *Journal of Cellular Biochemistry* 99 (6): 1616–27. https://doi.org/10.1002/jcb.20947.

Lu, Yingying, Jing Chen, Junyuan Zheng, Guoyong Hu, Jingjing Wang, Chunlan Huang, Lihong Lou, Xingpeng Wang, and Yue Zeng. 2016. “Mucosal Adherent Bacterial Dysbiosis in Patients with Colorectal Adenomas.” *Scientific Reports* 6 (1): 26337. https://doi.org/10.1038/srep26337.

Ma, Hak-Ling, Spencer Liang, Jing Li, Lee Napierata, Tom Brown, Stephen Benoit, Mayra Senices, et al. 2008. “IL-22 Is Required for Th17 Cell-Mediated Pathology in a Mouse Model of Psoriasis-like Skin Inflammation.” *The Journal of Clinical Investigation* 118 (2): 597–607. https://doi.org/10.1172/JCI33263.

Magalhaes, Isabelle, Karine Pingris, Christine Poitou, Stéphanie Bessoles, Nicolas Venteclef, Badr Kiaf, Lucie Beaudoin, et al. 2015. “Mucosal-Associated Invariant T Cell Alterations in Obese and Type 2 Diabetic Patients.” *Journal of Clinical Investigation* 125 (4): 1752–62. https://doi.org/10.1172/JCI78941.

Magnúsdóttir, Stefanía, Dmitry Ravcheev, Valérie de Crécy-Lagard, and Ines Thiele. 2015. “Systematic Genome Assessment of B-Vitamin Biosynthesis Suggests Co-Operation among Gut Microbes.” *Frontiers in Genetics* 6 (April). https://doi.org/10.3389/fgene.2015.00148.

Mak, Jeffrey Y. W., Weijun Xu, Robert C. Reid, Alexandra J. Corbett, Bronwyn S. Meehan, Huimeng Wang, Zhenjun Chen, et al. 2017. “Stabilizing Short-Lived Schiff Base Derivatives of 5-Aminouracils That Activate Mucosal-Associated Invariant T Cells.” *Nature Communications* 8 (1): 1–13. https://doi.org/10.1038/ncomms14599.

Marchesi, Julian R., Elaine Holmes, Fatima Khan, Sunil Kochhar, Pauline Scanlan, Fergus Shanahan, Ian D. Wilson, and Yulan Wang. 2007. “Rapid and Noninvasive Metabonomic Characterization of Inflammatory Bowel Disease.” *Journal of Proteome Research* 6 (2): 546–51. https://doi.org/10.1021/pr060470d.

Martin, Emmanuel, Emmanuel Treiner, Livine Duban, Lucia Guerri, Hélène Laude, Cécile Toly, Virginie Premel, et al. 2009. “Stepwise Development of MAIT Cells in Mouse and Human.” *PLOS Biology* 7 (3): e1000054. https://doi.org/10.1371/journal.pbio.1000054.

Maxwell, Joseph R., Yu Zhang, William A. Brown, Carole L. Smith, Fergus R. Byrne, Mike Fiorino, Erin Stevens, et al. 2015. “Differential Roles for Interleukin-23 and Interleukin-17 in Intestinal Immunoregulation.” *Immunity* 43 (4): 739–50. https://doi.org/10.1016/j.immuni.2015.08.019.

McWilliam, Hamish E. G., Sidonia B. G. Eckle, Alex Theodossis, Ligong Liu, Zhenjun Chen, Jacinta M. Wubben, David P. Fairlie, et al. 2016. “The Intracellular Pathway for the Presentation of Vitamin B–Related Antigens by the Antigen-Presenting Molecule MR1.” *Nature Immunology* 17 (5): 531–37. https://doi.org/10.1038/ni.3416.

McWilliam, Hamish E. G., and Jose A. Villadangos. 2017. “How MR1 Presents a Pathogen Metabolic Signature to Mucosal-Associated Invariant T (MAIT) Cells.” *Trends in Immunology* 38 (9): 679–89. https://doi.org/10.1016/j.it.2017.06.005.

Meierovics, Anda I., and Siobhán C. Cowley. 2016. “MAIT Cells Promote Inflammatory Monocyte Differentiation into Dendritic Cells during Pulmonary Intracellular Infection.” *The Journal of Experimental Medicine* 213 (12): 2793–2809. https://doi.org/10.1084/jem.20160637.

Meierovics, Anda, Wei-Jen Chua Yankelevich, and Siobhán C. Cowley. 2013. “MAIT Cells Are Critical for Optimal Mucosal Immune Responses during in Vivo Pulmonary Bacterial Infection.” *Proceedings of the National Academy of Sciences of the United States of America* 110 (33): E3119-3128. https://doi.org/10.1073/pnas.1302799110.

Michielan, Andrea, and Renata D’Incà. 2015. “Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut.” Review Article. Mediators of Inflammation. Hindawi. October 25, 2015. https://doi.org/10.1155/2015/628157.

Miyazaki, Yusei, Sachiko Miyake, Asako Chiba, Olivier Lantz, and Takashi Yamamura. 2011. “Mucosal-Associated Invariant T Cells Regulate Th1 Response in Multiple Sclerosis.” *International Immunology* 23 (9): 529–35. https://doi.org/10.1093/intimm/dxr047.

Mondot, Stanislas, Pierre Boudinot, and Olivier Lantz. 2016. “MAIT, MR1, Microbes and Riboflavin: A Paradigm for the Co-Evolution of Invariant TCRs and Restricting MHCI-like Molecules?” *Immunogenetics* 68 (8): 537–48. https://doi.org/10.1007/s00251-016-0927-9.

Morgan, Xochitl C., Timothy L. Tickle, Harry Sokol, Dirk Gevers, Kathryn L. Devaney, Doyle V. Ward, Joshua A. Reyes, et al. 2012. “Dysfunction of the Intestinal Microbiome in Inflammatory Bowel Disease and Treatment.” *Genome Biology* 13 (9): R79. https://doi.org/10.1186/gb-2012-13-9-r79.

Muñoz, Melba, Markus M. Heimesaat, Kerstin Danker, Daniela Struck, Uwe Lohmann, Rita Plickert, Stefan Bereswill, et al. 2009. “Interleukin (IL)-23 Mediates Toxoplasma Gondii-Induced Immunopathology in the Gut via Matrixmetalloproteinase-2 and IL-22 but Independent of IL-17.” *The Journal of Experimental Medicine* 206 (13): 3047–59. https://doi.org/10.1084/jem.20090900.

Neumann-Schaal, Meina, Nicole G. Metzendorf, Daniel Troitzsch, Aaron Mischa Nuss, Julia Danielle Hofmann, Michael Beckstette, Petra Dersch, Andreas Otto, and Susanne Sievers. 2018. “Tracking Gene Expression and Oxidative Damage of O2-Stressed Clostridioides Difficile by a Multi-Omics Approach.” *Anaerobe* 53 (October): 94–107. https://doi.org/10.1016/j.anaerobe.2018.05.018.

Neurath, Markus F. 2014. “Cytokines in Inflammatory Bowel Disease.” *Nature Reviews. Immunology* 14 (5): 329–42. https://doi.org/10.1038/nri3661.

Nguyen, Thi Loan Anh, Sara Vieira-Silva, Adrian Liston, and Jeroen Raes. 2015. “How Informative Is the Mouse for Human Gut Microbiota Research?” *Disease Models & Mechanisms* 8 (1): 1–16. https://doi.org/10.1242/dmm.017400.

Normanno, Nicola, Mouna P. Selvam, Caterina Bianco, Vincenzo Damiano, Ernesto de Angelis, Michele Grassi, Gabriella Magliulo, Giampaolo Tortora, David S. Salomon, and Fortunato Ciardiello. 1995. “Amphiregulin Anti-Sense Oligodeoxynucleotides Inhibit Growth and Transformation of a Human Colon Carcinoma Cell Line.” *International Journal of Cancer* 62 (6): 762–66. https://doi.org/10.1002/ijc.2910620619.

O’Connor, William, Masahito Kamanaka, Carmen J Booth, Terrence Town, Susumu Nakae, Yoichiro Iwakura, Jay K Kolls, and Richard A Flavell. 2009. “A Protective Function for Interleukin 17A in T Cell–Mediated Intestinal Inflammation.” *Nature Immunology* 10 (6): 603–9. https://doi.org/10.1038/ni.1736.

Ogawa, Atsuhiro, Akira Andoh, Yoshio Araki, Tadao Bamba, and Yoshihide Fujiyama. 2004. “Neutralization of Interleukin-17 Aggravates Dextran Sulfate Sodium-Induced Colitis in Mice.” *Clinical Immunology (Orlando, Fla.)* 110 (1): 55–62. https://doi.org/10.1016/j.clim.2003.09.013.

Papandreou, Ioanna, Rob A. Cairns, Lucrezia Fontana, Ai Lin Lim, and Nicholas C. Denko. 2006. “HIF-1 Mediates Adaptation to Hypoxia by Actively Downregulating Mitochondrial Oxygen Consumption.” *Cell Metabolism* 3 (3): 187–97. https://doi.org/10.1016/j.cmet.2006.01.012.

Paquin-Proulx, Dominic, Vivian Lida Avelino-Silva, Bianca A. N. Santos, Nathália Silveira Barsotti, Fabiana Siroma, Jessica Fernandes Ramos, Adriana Coracini Tonacio, et al. 2018. “MAIT Cells Are Activated in Acute Dengue Virus Infection and after in Vitro Zika Virus Infection.” *PLOS Neglected Tropical Diseases* 12 (1): e0006154. https://doi.org/10.1371/journal.pntd.0006154.

Paquin-Proulx, Dominic, Benjamin C. Greenspun, Emanuela A. S. Costa, Aluisio C. Segurado, Esper G. Kallas, Douglas F. Nixon, and Fabio E. Leal. 2017. “MAIT Cells Are Reduced in Frequency and Functionally Impaired in Human T Lymphotropic Virus Type 1 Infection: Potential Clinical Implications.” *PLoS ONE* 12 (4). https://doi.org/10.1371/journal.pone.0175345.

Parrot, Tiphaine, Jean-Baptiste Gorin, Andrea Ponzetta, Kimia T. Maleki, Tobias Kammann, Johanna Emgård, André Perez-Potti, et al. 2020. “MAIT Cell Activation and Dynamics Associated with COVID-19 Disease Severity.” *Science Immunology* 5 (51). https://doi.org/10.1126/sciimmunol.abe1670.

Pedrolli, Danielle, Simone Langer, Birgit Hobl, Julia Schwarz, Masayuki Hashimoto, and Matthias Mack. 2015. “The RibB FMN Riboswitch from Escherichia Coli Operates at the Transcriptional and Translational Level and Regulates Riboflavin Biosynthesis.” *The FEBS Journal* 282 (16): 3230–42. https://doi.org/10.1111/febs.13226.

Pereira, Fátima C., and David Berry. 2017. “Microbial Nutrient Niches in the Gut.” *Environmental Microbiology* 19 (4): 1366–78. https://doi.org/10.1111/1462-2920.13659.

Peters, Brandilyn A., Jean A. Shapiro, Timothy R. Church, George Miller, Chau Trinh-Shevrin, Elizabeth Yuen, Charles Friedlander, Richard B. Hayes, and Jiyoung Ahn. 2018. “A Taxonomic Signature of Obesity in a Large Study of American Adults.” *Scientific Reports* 8 (1): 9749. https://doi.org/10.1038/s41598-018-28126-1.

Poole, Leslie B. 2005. “Bacterial Defenses against Oxidants: Mechanistic Features of Cysteine-Based Peroxidases and Their Flavoprotein Reductases.” *Archives of Biochemistry and Biophysics*, Highlight issue on Enzyme Mechanisms, 433 (1): 240–54. https://doi.org/10.1016/j.abb.2004.09.006.

Preciado-Llanes, Lorena, Anna Aulicino, Rocío Canals, Patrick J. Moynihan, Xiaojun Zhu, Ndaru Jambo, Tonney S. Nyirenda, et al. 2020. “Evasion of MAIT Cell Recognition by the African Salmonella Typhimurium ST313 Pathovar That Causes Invasive Disease.” *Proceedings of the National Academy of Sciences* 117 (34): 20717–28. https://doi.org/10.1073/pnas.2007472117.

Rahimpour, Azad, Hui Fern Koay, Anselm Enders, Rhiannon Clanchy, Sidonia B. G. Eckle, Bronwyn Meehan, Zhenjun Chen, et al. 2015. “Identification of Phenotypically and Functionally Heterogeneous Mouse Mucosal-Associated Invariant T Cells Using MR1 Tetramers.” *The Journal of Experimental Medicine* 212 (7): 1095–1108. https://doi.org/10.1084/jem.20142110.

Rigottier-Gois, Lionel. 2013. “Dysbiosis in Inflammatory Bowel Diseases: The Oxygen Hypothesis.” *The ISME Journal* 7 (7): 1256–61. https://doi.org/10.1038/ismej.2013.80.

Rocha, E. R., and C. J. Smith. 1999. “Role of the Alkyl Hydroperoxide Reductase (AhpCF) Gene in Oxidative Stress Defense of the Obligate Anaerobe Bacteroides Fragilis.” *Journal of Bacteriology* 181 (18): 5701–10.

Roediger, W. E. W. 1980. “THE COLONIC EPITHELIUM IN ULCERATIVE COLITIS: AN ENERGY-DEFICIENCY DISEASE?” *The Lancet* 316 (8197): 712–15. https://doi.org/10.1016/S0140-6736(80)91934-0.

Rooks, Michelle G., and Wendy S. Garrett. 2016. “Gut Microbiota, Metabolites and Host Immunity.” *Nature Reviews. Immunology* 16 (6): 341–52. https://doi.org/10.1038/nri.2016.42.

Rouxel, Ophélie, Isabelle Nel, Jennifer DaSilva, Lucie Beaudoin, Céline Tard, Badr Kiaf, Lucie Cagninacci, et al. 2017. “Dual Role of Mucosal-Associated Invariant T Cells in Type 1 Diabetes.” *Nature Immunology* 18 (12): 1321–31. https://doi.org/10.1038/ni.3854.

Sabatino, A. Di, R. Morera, R. Ciccocioppo, P. Cazzola, S. Gotti, F. P. Tinozzi, S. Tinozzi, and G. R. Corazza. 2005. “Oral Butyrate for Mildly to Moderately Active Crohn’s Disease.” *Alimentary Pharmacology & Therapeutics* 22 (9): 789–94. https://doi.org/10.1111/j.1365-2036.2005.02639.x.

Saeidi, Alireza, Vicky L. Tien Tien, Rami Al-Batran, Haider A. Al-Darraji, Hong Y. Tan, Yean K. Yong, Sasheela Ponnampalavanar, et al. 2015. “Attrition of TCR Vα7.2+ CD161++ MAIT Cells in HIV-Tuberculosis Co-Infection Is Associated with Elevated Levels of PD-1 Expression.” *PloS One* 10 (4): e0124659. https://doi.org/10.1371/journal.pone.0124659.

Safran, Michal, William Y. Kim, Fionnuala O'Connell, Lee Flippin, Volkmar Günzler, James W. Horner, Ronald A. Depinho, and William G. Kaelin. 2006. "Mouse Model for Noninvasive Imaging of HIF Prolyl Hydroxylase Activity: Assessment of an Oral Agent That Stimulates Erythropoietin Production." *Proceedings of the National Academy of Sciences of the United States of America* 103 (1): 105–10. https://doi.org/10.1073/pnas.0509459103.

Salerno-Goncalves, Rosângela, David Luo, Stephanie Fresnay, Laurence Magder, Thomas C. Darton, Claire Jones, Claire S. Waddington, et al. 2017. "Challenge of Humans with Wild-Type Salmonella Enterica Serovar Typhi Elicits Changes in the Activation and Homing Characteristics of Mucosal-Associated Invariant T Cells." *Frontiers in Immunology* 8: 398. https://doi.org/10.3389/fimmu.2017.00398.

Salou, Marion, Katarzyna Franciszkiewicz, and Olivier Lantz. 2017. "MAIT Cells in Infectious Diseases." *Current Opinion in Immunology* 48 (October): 7–14. https://doi.org/10.1016/j.coi.2017.07.009.

Salou, Marion, and Olivier Lantz. 2019. "A TCR-Dependent Tissue Repair Potential of MAIT Cells." *Trends in Immunology* 40 (11): 975–77. https://doi.org/10.1016/j.it.2019.09.001.

Salou, Marion, François Legoux, Jules Gilet, Aurélie Darbois, Anastasia du Halgouet, Ruby Alonso, Wilfrid Richer, et al. 2019. "A Common Transcriptomic Program Acquired in the Thymus Defines Tissue Residency of MAIT and NKT Subsets." *The Journal of Experimental Medicine* 216 (1): 133–51. https://doi.org/10.1084/jem.20181483.

Salou, Marion, Bryan Nicol, Alexandra Garcia, Daniel Baron, Laure Michel, Annie Elong-Ngono, Philippe Hulin, et al. 2016. "Neuropathologic, Phenotypic and Functional Analyses of Mucosal Associated Invariant T Cells in Multiple Sclerosis." *Clinical Immunology (Orlando, Fla.)* 166–167: 1–11. https://doi.org/10.1016/j.clim.2016.03.014.

Sandborn, William J., Christopher Gasink, Long-Long Gao, Marion A. Blank, Jewel Johanns, Cynthia Guzzo, Bruce E. Sands, et al. 2012. "Ustekinumab Induction and Maintenance Therapy in Refractory Crohn's Disease." *New England Journal of Medicine* 367 (16): 1519–28. https://doi.org/10.1056/NEJMoa1203572.

Sands, Bruce E., Jingjing Chen, Brian G. Feagan, Mark Penney, William A. Rees, Silvio Danese, Peter D. R. Higgins, et al. 2017. "Efficacy and Safety of MEDI2070, an Antibody Against Interleukin 23, in Patients With Moderate to Severe Crohn's Disease: A Phase 2a Study." *Gastroenterology* 153 (1): 77-86.e6. https://doi.org/10.1053/j.gastro.2017.03.049.

Sanidad, Katherine Z, and Melody Y Zeng. 2020. "Neonatal Gut Microbiome and Immunity." *Current Opinion in Microbiology*, Microbe – Microbe Interactions • Microbiota, 56 (August): 30–37. https://doi.org/10.1016/j.mib.2020.05.011.

Savage, Adam K., Michael G. Constantinides, Jin Han, Damien Picard, Emmanuel Martin, Bofeng Li, Olivier Lantz, and Albert Bendelac. 2008. "The Transcription Factor PLZF Directs the Effector Program of the NKT Cell Lineage." *Immunity* 29 (3): 391–403. https://doi.org/10.1016/j.immuni.2008.07.011.

Scheppach, W., H. Sommer, T. Kirchner, G. M. Paganelli, P. Bartram, S. Christl, F. Richter, G. Dusel, and H. Kasper. 1992. "Effect of Butyrate Enemas on the Colonic Mucosa in Distal Ulcerative Colitis." *Gastroenterology* 103 (1): 51–56. https://doi.org/10.1016/0016-5085(92)91094-k.

Schmidt, Carsten, Thomas Giese, Bianca Ludwig, Ina Mueller-Molaian, Thomas Marth, Stefan Zeuzem, Stefan C. Meuer, and Andreas Stallmach. 2005. "Expression of Interleukin-12-Related Cytokine Transcripts in Inflammatory Bowel Disease: Elevated Interleukin-23p19 and Interleukin-27p28 in Crohn's Disease but Not in Ulcerative Colitis." *Inflammatory Bowel Diseases* 11 (1): 16–23. https://doi.org/10.1097/00054725-200501000-00003.

Seach, Natalie, Lucia Guerri, Lionel Le Bourhis, Yvonne Mburu, Yue Cui, Stéphanie Bessoles, Claire Soudais, and Olivier Lantz. 2013. “Double-Positive Thymocytes Select Mucosal-Associated Invariant T Cells.” *Journal of Immunology (Baltimore, Md.: 1950)* 191 (12): 6002–9. https://doi.org/10.4049/jimmunol.1301212.

Segata, Nicola, Jacques Izard, Levi Waldron, Dirk Gevers, Larisa Miropolsky, Wendy S. Garrett, and Curtis Huttenhower. 2011. “Metagenomic Biomarker Discovery and Explanation.” *Genome Biology* 12 (6): R60. https://doi.org/10.1186/gb-2011-12-6-r60.

Serriari, N-E, M Eoche, L Lamotte, J Lion, M Fumery, P Marcelo, D Chatelain, et al. 2014. “Innate Mucosal-Associated Invariant T (MAIT) Cells Are Activated in Inflammatory Bowel Diseases.” *Clinical and Experimental Immunology* 176 (2): 266–74. https://doi.org/10.1111/cei.12277.

Shen, Chong, Carol J. Landers, Carrie Derkowski, Charles O. Elson, and Stephan R. Targan. 2008. “Enhanced CBir1-Specific Innate and Adaptive Immune Responses in Crohn’s Disease.” *Inflammatory Bowel Diseases* 14 (12): 1641–51. https://doi.org/10.1002/ibd.20645.

Singh, Nagendra, Ashish Gurav, Sathish Sivaprakasam, Evan Brady, Ravi Padia, Huidong Shi, Muthusamy Thangaraju, et al. 2014. “Activation of Gpr109a, Receptor for Niacin and the Commensal Metabolite Butyrate, Suppresses Colonic Inflammation and Carcinogenesis.” *Immunity* 40 (1): 128–39. https://doi.org/10.1016/j.immuni.2013.12.007.

Singh, Nagendra, Muthusamy Thangaraju, Puttur D. Prasad, Pamela M. Martin, Nevin A. Lambert, Thomas Boettger, Stefan Offermanns, and Vadivel Ganapathy. 2010. “Blockade of Dendritic Cell Development by Bacterial Fermentation Products Butyrate and Propionate through a Transporter (Slc5a8)-Dependent Inhibition of Histone Deacetylases.” *The Journal of Biological Chemistry* 285 (36): 27601–8. https://doi.org/10.1074/jbc.M110.102947.

Smith, Ashley D., Elissa D. Foss, Irma Zhang, Jessica L. Hastie, Nicole P. Giordano, Lusine Gasparyan, Lam Phuc VinhNguyen, et al. 2019. “Microbiota of MR1 Deficient Mice Confer Resistance against Clostridium Difficile Infection.” *PLoS ONE* 14 (9). https://doi.org/10.1371/journal.pone.0223025.

Smith, Daniel J., Geoffrey R. Hill, Scott C. Bell, and David W. Reid. 2014. “Reduced Mucosal Associated Invariant T-Cells Are Associated with Increased Disease Severity and Pseudomonas Aeruginosa Infection in Cystic Fibrosis.” *PloS One* 9 (10): e109891. https://doi.org/10.1371/journal.pone.0109891.

Smith, Patrick M., Michael R. Howitt, Nicolai Panikov, Monia Michaud, Carey Ann Gallini, Mohammad Bohlooly-Y, Jonathan N. Glickman, and Wendy S. Garrett. 2013. “The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic Treg Cell Homeostasis.” *Science* 341 (6145): 569–73. https://doi.org/10.1126/science.1241165.

Sobkowiak, Michał J., Haleh Davanian, Robert Heymann, Anna Gibbs, Johanna Emgård, Joana Dias, Soo Aleman, et al. 2019. “Tissue-Resident MAIT Cell Populations in Human Oral Mucosa Exhibit an Activated Profile and Produce IL-17.” *European Journal of Immunology* 49 (1): 133–43. https://doi.org/10.1002/eji.201847759.

Sokol, H., P. Seksik, J. P. Furet, O. Firmesse, I. Nion-Larmurier, L. Beaugerie, J. Cosnes, G. Corthier, P. Marteau, and J. Doré. 2009. “Low Counts of Faecalibacterium Prausnitzii in Colitis Microbiota.” *Inflammatory Bowel Diseases* 15 (8): 1183–89. https://doi.org/10.1002/ibd.20903.

Song, Xinyang, Dai Dai, Xiao He, Shu Zhu, Yikun Yao, Hanchao Gao, Jingjing Wang, et al. 2015. “Growth Factor FGF2 Cooperates with Interleukin-17 to Repair Intestinal Epithelial Damage.” *Immunity* 43 (3): 488–501. https://doi.org/10.1016/j.immuni.2015.06.024.

Song, Xinyang, Ximei Sun, Sungwhan F. Oh, Meng Wu, Yanbo Zhang, Wen Zheng, Naama Geva-Zatorsky, et al. 2020. “Microbial Bile Acid Metabolites Modulate Gut RORγ + Regulatory T Cell Homeostasis.” *Nature* 577 (7790): 410–15. https://doi.org/10.1038/s41586-019-1865-0.

Sonnenberg, Gregory F., Lynette A. Fouser, and David Artis. 2011. “Border Patrol: Regulation of Immunity, Inflammation and Tissue Homeostasis at Barrier Surfaces by IL-22.” *Nature Immunology* 12 (5): 383–90. https://doi.org/10.1038/ni.2025.

Soudais, Claire, Fatoumata Samassa, Manal Sarkis, Lionel Le Bourhis, Stéphanie Bessoles, Didier Blanot, Mireille Hervé, Frédéric Schmidt, Dominique Mengin-Lecreulx, and Olivier Lantz. 2015. “In Vitro and In Vivo Analysis of the Gram-Negative Bacteria-Derived Riboflavin Precursor Derivatives Activating Mouse MAIT Cells.” *Journal of Immunology (Baltimore, Md.: 1950)* 194 (10): 4641–49. https://doi.org/10.4049/jimmunol.1403224.

Strugala, V., P. W. Dettmar, and J. P. Pearson. 2008. “Thickness and Continuity of the Adherent Colonic Mucus Barrier in Active and Quiescent Ulcerative Colitis and Crohn’s Disease.” *International Journal of Clinical Practice* 62 (5): 762–69. https://doi.org/10.1111/j.1742-1241.2007.01665.x.

Sugimoto, Ken, Atsuhiro Ogawa, Emiko Mizoguchi, Yasuyo Shimomura, Akira Andoh, Atul K. Bhan, Richard S. Blumberg, Ramnik J. Xavier, and Atsushi Mizoguchi. 2008. “IL-22 Ameliorates Intestinal Inflammation in a Mouse Model of Ulcerative Colitis.” *The Journal of Clinical Investigation* 118 (2): 534–44. https://doi.org/10.1172/JCI33194.

Sun, Xuan, Yihua Cai, Chris Fleming, Zan Tong, Zhenglong Wang, Chuanlin Ding, Minye Qu, Huang-ge Zhang, Jian Suo, and Jun Yan. 2017. “Innate ΓδT17 Cells Play a Protective Role in DSS-Induced Colitis via Recruitment of Gr-1+CD11b+ Myeloid Suppressor Cells.” *OncoImmunology* 6 (5): e1313369. https://doi.org/10.1080/2162402X.2017.1313369.

Tang, Xin-Zi, Juandy Jo, Anthony T. Tan, Elena Sandalova, Adeline Chia, Kai Chah Tan, Kang Hoe Lee, Adam J. Gehring, Gennaro De Libero, and Antonio Bertoletti. 2013. “IL-7 Licenses Activation of Human Liver Intrasinusoidal Mucosal-Associated Invariant T Cells.” *Journal of Immunology (Baltimore, Md.: 1950)* 190 (7): 3142–52. https://doi.org/10.4049/jimmunol.1203218.

Tap, Julien, Stanislas Mondot, Florence Levenez, Eric Pelletier, Christophe Caron, Jean-Pierre Furet, Edgardo Ugarte, et al. 2009. “Towards the Human Intestinal Microbiota Phylogenetic Core.” *Environmental Microbiology* 11 (10): 2574–84. https://doi.org/10.1111/j.1462-2920.2009.01982.x.

Tastan, Cihan, Ece Karhan, Wei Zhou, Elizabeth Fleming, Anita Y. Voigt, Xudong Yao, Lei Wang, et al. 2018. “Tuning of Human MAIT Cell Activation by Commensal Bacteria Species and MR1 Dependent T Cell Presentation.” *Mucosal Immunology* 11 (6): 1591–1605. https://doi.org/10.1038/s41385-018-0072-x.

Tilloy, F., E. Treiner, S. H. Park, C. Garcia, F. Lemonnier, H. de la Salle, A. Bendelac, M. Bonneville, and O. Lantz. 1999. “An Invariant T Cell Receptor Alpha Chain Defines a Novel TAP-Independent Major Histocompatibility Complex Class Ib-Restricted Alpha/Beta T Cell Subpopulation in Mammals.” *The Journal of Experimental Medicine* 189 (12): 1907–21. https://doi.org/10.1084/jem.189.12.1907.

Tominaga, Kentaro, Satoshi Yamagiwa, Toru Setsu, Naruhiro Kimura, Hiroki Honda, Hiroteru Kamimura, Yutaka Honda, et al. 2017. “Possible Involvement of Mucosal-Associated Invariant T Cells in the Progression of Inflammatory Bowel Diseases.” *Biomedical Research (Tokyo, Japan)* 38 (2): 111–21. https://doi.org/10.2220/biomedres.38.111.

Toubal, Amine, Badr Kiaf, Lucie Beaudoin, Lucie Cagninacci, Moez Rhimi, Blandine Fruchet, Jennifer da Silva, et al. 2020. “Mucosal-Associated Invariant T Cells Promote Inflammation and Intestinal Dysbiosis Leading to Metabolic Dysfunction during Obesity.” *Nature Communications* 11 (1): 3755. https://doi.org/10.1038/s41467-020-17307-0.

Turnbaugh, Peter J., Ruth E. Ley, Michael A. Mahowald, Vincent Magrini, Elaine R. Mardis, and Jeffrey I. Gordon. 2006. “An Obesity-Associated Gut Microbiome with Increased Capacity for Energy Harvest.” *Nature* 444 (7122): 1027–31. https://doi.org/10.1038/nature05414.

Turtle, Cameron J., Jeff Delrow, Rochelle C. Joslyn, Hillary M. Swanson, Ryan Basom, Laura Tabellini, Colleen Delaney, Shelly Heimfeld, John A. Hansen, and Stanley R. Riddell. 2011. “Innate Signals Overcome Acquired TCR Signaling Pathway Regulation and Govern the Fate of Human CD161(Hi) CD8α^{+} Semi-Invariant T Cells.” *Blood* 118 (10): 2752–62. https://doi.org/10.1182/blood-2011-02-334698.

Uchimura, Y., T. Fuhrer, H. Li, M.A. Lawson, M. Zimmermann, B. Yilmaz, J. Zindel, et al. 2018. “Antibodies Set Boundaries Limiting Microbial Metabolite Penetration and the Resultant Mammalian Host Response.” *Immunity* 49 (3): 545-559.e5. https://doi.org/10.1016/j.immuni.2018.08.004.

Ussher, James E, Matthew Bilton, Emma Attwod, Jonathan Shadwell, Rachel Richardson, Catherine de Lara, Elisabeth Mettke, et al. 2014. “CD161++CD8+ T Cells, Including the MAIT Cell Subset, Are Specifically Activated by IL-12+IL-18 in a TCR-Independent Manner.” *European Journal of Immunology* 44 (1): 195–203. https://doi.org/10.1002/eji.201343509.

Ussher, James E., Bonnie van Wilgenburg, Rachel F. Hannaway, Kerstin Ruustal, Prabhjeet Phalora, Ayako Kurioka, Ted H. Hansen, Christian B. Willberg, Rodney E. Phillips, and Paul Klenerman. 2016. “TLR Signaling in Human Antigen-Presenting Cells Regulates MR1-Dependent Activation of MAIT Cells.” *European Journal of Immunology* 46 (7): 1600–1614. https://doi.org/10.1002/eji.201545969.

Vaishnava, Shipra, Miwako Yamamoto, Kari M. Severson, Kelly A. Ruhn, Xiaofei Yu, Omry Koren, Ruth Ley, Edward K. Wakeland, and Lora V. Hooper. 2011. “The Antibacterial Lectin RegIIIgamma Promotes the Spatial Segregation of Microbiota and Host in the Intestine.” *Science (New York, N.Y.)* 334 (6053): 255–58. https://doi.org/10.1126/science.1209791.

Vitreschak, Alexey G., Dmitry A. Rodionov, Andrey A. Mironov, and Mikhail S. Gelfand. 2002. “Regulation of Riboflavin Biosynthesis and Transport Genes in Bacteria by Transcriptional and Translational Attenuation.” *Nucleic Acids Research* 30 (14): 3141–51. https://doi.org/10.1093/nar/gkf433.

Wang, Gang, Shuo Huang, Yuming Wang, Shuang Cai, Haitao Yu, Hongbing Liu, Xiangfang Zeng, Guolong Zhang, and Shiyan Qiao. 2019. “Bridging Intestinal Immunity and Gut Microbiota by Metabolites.” *Cellular and Molecular Life Sciences: CMLS* 76 (20): 3917–37. https://doi.org/10.1007/s00018-019-03190-6.

Wang, Huimeng, Criselle D’Souza, Xin Yi Lim, Lyudmila Kostenko, Troi J. Pediongco, Sidonia B. G. Eckle, Bronwyn S. Meehan, et al. 2018. “MAIT Cells Protect against Pulmonary Legionella Longbeachae Infection.” *Nature Communications* 9 (1): 1–15. https://doi.org/10.1038/s41467-018-05202-8.

Wang, Huimeng, Lars Kjer-Nielsen, Mai Shi, Criselle D’Souza, Troi J. Pediongco, Hanwei Cao, Lyudmila Kostenko, et al. 2019. “IL-23 Costimulates Antigen-Specific MAIT Cell Activation and Enables Vaccination against Bacterial Infection.” *Science Immunology* 4 (41). https://doi.org/10.1126/sciimmunol.aaw0402.

Wang, Tingting, Guoxiang Cai, Yunping Qiu, Na Fei, Menghui Zhang, Xiaoyan Pang, Wei Jia, Sanjun Cai, and Liping Zhao. 2012. “Structural Segregation of Gut Microbiota between Colorectal Cancer Patients and Healthy Volunteers.” *The ISME Journal* 6 (2): 320–29. https://doi.org/10.1038/ismej.2011.109.

Wilgenburg, Bonnie van, Liyen Loh, Zhenjun Chen, Troi J. Pediongco, Huimeng Wang, Mai Shi, Zhe Zhao, et al. 2018. “MAIT Cells Contribute to Protection against Lethal Influenza Infection in Vivo.” *Nature Communications* 9 (1): 1–9. https://doi.org/10.1038/s41467-018-07207-9.

Wilgenburg, Bonnie van, Iris Scherwitzl, Edward C. Hutchinson, Tianqi Leng, Ayako Kurioka, Corinna Kulicke, Catherine de Lara, et al. 2016. “MAIT Cells Are Activated during Human Viral Infections.” *Nature Communications* 7 (1): 1–11. https://doi.org/10.1038/ncomms11653.

Willing, Anne, Oliver A. Leach, Friederike Ufer, Kathrine E. Attfield, Karin Steinbach, Nina Kursawe, Melanie Piedavent, and Manuel A. Friese. 2014. “$CD8^+$ MAIT Cells Infiltrate into the CNS and Alterations in Their Blood Frequencies Correlate with IL-18 Serum Levels in Multiple Sclerosis.” *European Journal of Immunology* 44 (10): 3119–28. https://doi.org/10.1002/eji.201344160.

Wright, Emily K., Michael A. Kamm, Shu Mei Teo, Michael Inouye, Josef Wagner, and Carl D. Kirkwood. 2015. “Recent Advances in Characterizing the Gastrointestinal Microbiome in Crohn’s Disease: A Systematic Review.” *Inflammatory Bowel Diseases* 21 (6): 1219–28. https://doi.org/10.1097/MIB.0000000000000382.

Wu, Ze Ang, and Hai Xia Wang. 2019. “A Systematic Review of the Interaction Between Gut Microbiota and Host Health from a Symbiotic Perspective.” *SN Comprehensive Clinical Medicine* 1 (3): 224–35. https://doi.org/10.1007/s42399-018-0033-4.

Xiao, Xingxing, Kun Li, Xueting Ma, Baohong Liu, Xueyang He, Shunli Yang, Wenqing Wang, Baoyu Jiang, and Jianping Cai. 2019. “Mucosal-Associated Invariant T Cells Expressing the TRAV1-TRAJ33 Chain Are Present in Pigs.” *Frontiers in Immunology* 10 (September). https://doi.org/10.3389/fimmu.2019.02070.

Yang, Qianting, Qian Xu, Qi Chen, Jin Li, Mingxia Zhang, Yi Cai, Haiying Liu, et al. 2015. “Discriminating Active Tuberculosis from Latent Tuberculosis Infection by Flow Cytometric Measurement of CD161-Expressing T Cells.” *Scientific Reports* 5 (December): 17918. https://doi.org/10.1038/srep17918.

Yasuda, Koji, Keunyoung Oh, Boyu Ren, Timothy L. Tickle, Eric A. Franzosa, Lynn M. Wachtman, Andrew D. Miller, et al. 2015. “Biogeography of the Intestinal Mucosal and Lumenal Microbiome in the Rhesus Macaque.” *Cell Host & Microbe* 17 (3): 385–91. https://doi.org/10.1016/j.chom.2015.01.015.

Yatsunenko, Tanya, Federico E. Rey, Mark J. Manary, Indi Trehan, Maria Gloria Dominguez-Bello, Monica Contreras, Magda Magris, et al. 2012. “Human Gut Microbiome Viewed across Age and Geography.” *Nature* 486 (7402): 222–27. https://doi.org/10.1038/nature11053.

Yeoh, Yun Kit, Tao Zuo, Grace Chung-Yan Lui, Fen Zhang, Qin Liu, Amy YL Li, Arthur CK Chung, et al. 2021. “Gut Microbiota Composition Reflects Disease Severity and Dysfunctional Immune Responses in Patients with COVID-19.” *Gut* 70 (4): 698–706. https://doi.org/10.1136/gutjnl-2020-323020.

Zaiss, Dietmar M.W., William C. Gause, Lisa C. Osborne, and David Artis. 2015. “Emerging Functions of Amphiregulin in Orchestrating Immunity, Inflammation and Tissue Repair.” *Immunity* 42 (2): 216–26. https://doi.org/10.1016/j.immuni.2015.01.020.

Zelante, Teresa, Rossana G. Iannitti, Cristina Cunha, Antonella De Luca, Gloria Giovannini, Giuseppe Pieraccini, Riccardo Zecchi, et al. 2013. “Tryptophan Catabolites from Microbiota Engage Aryl Hydrocarbon Receptor and Balance Mucosal Reactivity via Interleukin-22.” *Immunity* 39 (2): 372–85. https://doi.org/10.1016/j.immuni.2013.08.003.

Zhang, Husen, John K. DiBaise, Andrea Zuccolo, Dave Kudrna, Michele Braidotti, Yeisoo Yu, Prathap Parameswaran, et al. 2009. “Human Gut Microbiota in Obesity and after Gastric Bypass.” *Proceedings of the National Academy of Sciences of the United States of America* 106 (7): 2365–70. https://doi.org/10.1073/pnas.0812600106.

Zheng, Leon, Caleb J. Kelly, and Sean P. Colgan. 2015. “Physiologic Hypoxia and Oxygen Homeostasis in the Healthy Intestine. A Review in the Theme: Cellular Responses to Hypoxia.” *American Journal of Physiology - Cell Physiology* 309 (6): C350–60. https://doi.org/10.1152/ajpcell.00191.2015.

Zheng, Yan, Dimitry M. Danilenko, Patricia Valdez, Ian Kasman, Jeffrey Eastham-Anderson, Jianfeng Wu, and Wenjun Ouyang. 2007. “Interleukin-22, a T(H)17 Cytokine, Mediates IL-23-Induced Dermal Inflammation and Acanthosis.” *Nature* 445 (7128): 648–51. https://doi.org/10.1038/nature05505.

Zoetendal, Erwin G, Jeroen Raes, Bartholomeus van den Bogert, Manimozhiyan Arumugam, Carien CGM Booijink, Freddy J Troost, Peer Bork, Michiel Wels, Willem M de Vos, and Michiel Kleerebezem. 2012. “The Human Small Intestinal Microbiota Is Driven by Rapid Uptake and Conversion of Simple Carbohydrates.” *The ISME Journal* 6 (7): 1415–26. https://doi.org/10.1038/ismej.2011.212.

Zúñiga, Luis A., Wen-Jun Shen, Barbara Joyce-Shaikh, Ekaterina A. Pyatnova, Andrew G. Richards, Colin Thom, Sofia M. Andrade, Daniel J. Cua, Fredric B. Kraemer, and Eugene C. Butcher. 2010. “IL-17 Regulates Adipogenesis, Glucose Homeostasis, and Obesity.” *Journal of Immunology (Baltimore, Md.: 1950)* 185 (11): 6947–59. https://doi.org/10.4049/jimmunol.1001269.

RÉSUMÉ

Les cellules MAIT sont des lymphocytes T innés reconnaissant des métabolites microbiens dérivés de la voie de synthèse de la riboflavine, présentés par MR1. Des bactéries intestinales de l'embranchement des *Bacteroidetes* et *Proteobacteria* peuvent produire le ligand des MAIT, suggérant une interaction entre les MAIT et le microbiote. Chez l'homme, les MAIT ont été impliqués dans des pathologies associées à une dysbiose intestinale tel que les maladies inflammatoires de l'intestin, mais le rôle des MAIT dans ces maladies reste inconnu. La dysanaérobiose et le stress oxidatif caractérisent les maladies inflammatoires de l'intestin. La riboflavine contribue à la respiration aérobie et à la résistance des bactéries au stress oxydatif, suggérant une augmentation des besoins en riboflavine pendant l'inflammation intestinale. En accord avec cette hypothèse, les bactéries prototrophes pour la riboflavine sont enrichies dans la maladie de Crohn.

Dans une première partie, nous avons exploré l'hypothèse que les MAIT détectent des alterations du métabolisme bactérien associées aux dysbioses dans le colon. Nous avons montré que la dysanaérobiose, lors d'un traitement antibiotique ou d'une colite induite par le dextran sulfate de sodium (DSS), augmente la production du ligand des MAIT dans l'intestin. Nous avons séquencé les gènes d'ARNr 16S dans l'intestin de souris traitées aux antibiotiques et au DSS, et analysé l'expression des gènes de la voie de la riboflavine en cas de colite induite par l'infection par *Helicobacter hepaticus*. La dysanaérobiose était associée à l'expansion des *Enterobacteriaceae* lors du traitement à la vancomycine, et des *Bacteroidaceae* et *Enterobacteriaceae* en cas de colite. La production de riboflavine était bénéfique pour l'expansion d'*Escherichia coli* dans l'intestin enflammé. Les *Bacteroidaceae* et les *Enterobacteriacea* peuvent produire de grandes quantités de ligands des MAIT *in vitro*. Le gène *ribD*, qui contrôle la production des ligands des MAIT, était surexprimé par les *Bacteroidaceae*, les *Clostridiaceae* et les *Enterobacteriaceae* en cas de colite induite par *H. hepaticus*. Le ligand des MAIT traversaient la barrière intestinale et augmentaient la stimulation TCR des MAIT qui produisent en conséquence amphiréguline et protégeaint contre la colite. Dans une seconde partie, nous avons caractérisé les MAIT dans l'iléon, dans lequel des niveaux élevés d'oxygène ont été observés à l'état basal. Plusieurs bactéries codant pour la voie de la riboflavine colonisaient l'iléon, mais les MAIT n'étaient pas activés par leur TCR et exprimaient un phénotype distinct dans l'iléon. Nous discutons les études futures qui permettront de comprendre les interactions MAIT-microbiote dans l'iléon.

En résumé, les MAIT détectent et répondent directement aux changements du métabolisme bactérien associés à l'inflammation du colon et assurent en retour une protection de l'hôte. Cette nouvelle interaction hôte-microbiote peut expliquer l'activation des MAIT dans d'autres pathologies

MOTS CLÉS

MAIT, lamina propria, microbiote, riboflavine, colite, souris.