REVIEW ARTICLE

The dialogue between unconventional T cells and the microbiota

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The mammalian immune system is equipped with unconventional T cells that respond to microbial molecules such as glycolipids and small-molecule metabolites, which are invisible to conventional CD4 and CD8 T cells. Unconventional T cells include invariant natural killer T (iNKT) cells and mucosa-associated invariant T (MAIT) cells, which are involved in a wide range of infectious and non-infectious diseases, such as cancer and autoimmunity. In addition, their high conservation across mammals, their restriction by non-polymorphic antigen-presenting molecules, and their immediate and robust responses make these 'innate' T cells appealing targets for the development of one-size-fits-all immunotherapies. In this review, we discuss how iNKT and MAIT cells directly and indirectly detect the presence of and respond to pathogenic and commensal microbes. We also explore the current understanding of the bidirectional relationship between the microbiota and innate T cells, and how this crosstalk shapes the immune response in disease.

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INTRODUCTION – UNCONVENTIONAL T CELLS AND THEIR FUNCTIONS

Several populations of unconventional T cells have been identified in mammals that possess both innate and adaptive characteristics, including $\gamma\delta$ T cells (not discussed here), invariant natural killer T (iNKT) cells and mucosa-associated invariant T (MAIT) cells.¹ Unlike conventional T cells, which typically respond to peptide antigens presented in the context of major histocompatibility complex (MHC) molecules, iNKT and MAIT cells are specialized in the recognition of glycolipid antigens and microbial vitamin derivatives, respectively, two antigenic reservoirs that are invisible to conventional T cells.¹

There are several phenotypic and functional features that are strikingly similar between these two populations. iNKT and MAIT cells express restricted T cell receptor (TCR) repertoires, characterized by a canonical and invariant TCR α chain. Mouse and human iNKT cells express a TRAV11-TRAJ18- and TRAV10-TRAJ18-containing TCR α chain, respectively. Both mouse and human MAIT cells express a TRAV1.2-TRAJ33-containing TCR α chain. In addition, these cells are restricted by non-polymorphic MHC class I-related proteins, namely CD1d for iNKT cells² and MR1 for MAIT cells.³ Both cell types share similar transcriptomic profiles^{4,5} and are generally considered tissue-resident. Finally, iNKT and MAIT cells exhibit rapid helper and cytotoxic effector functions that are reminiscent of innate responses and are often considered to be innate T lymphocytes.

iNKT cells vastly outnumber MAIT cells in mice, whereas MAIT cells are more prevalent in humans. The most notable difference between iNKT and MAIT cells lies in the biochemical nature of the antigens they recognize. iNKT cells respond to glycolipid antigens such as α -galactosylceramide (aGC) presented by CD1d,^{6,7} while

MAIT cells respond to riboflavin (vitamin B2) metabolic derivatives, which includes 5-(2-oxopropylideneamino)-6-D-ribitylamouracil (5-OP-RU), presented in the context of MR1.⁸

iNKT and MAIT cells develop in the thymus and require expression of the transcription factor promyelocytic leukaemia zinc finger (PLZF).^{9–12} They differentiate into discrete effector subsets that correspond to CD4 helper T (T_H) cell subsets^{13,14} and are characterized by the expression of signature transcription factors. iNKT1 cells are T-bet⁺ PLZF^{low} and preferentially produce IFN- γ , while iNKT17 are ROR- γ t⁺ PLZF^{int} and produce IL-17. Similarly, MAIT1 and MAIT17 cells are characterized by their respective expression of T-bet and ROR- γ t. iNKT2 cells are PLZF^{high} T-bet^{neg} and ROR- γ t^{neg} and produce IL-4. No T_H2 functional equivalent of MAIT cells has yet been identified. Multiple factors have been shown to control iNKT and MAIT cell effector differentiation, including TCR signal strength and cytokines.^{15–18} It is important to note that in humans, iNKT cell subsets have not yet been identified and MAIT cells do not exhibit the clear MAIT1/17 dichotomy found in mice.^{13,19}

The roles iNKT and MAIT cells play in health and disease have been the focus of intense work and the subject of excellent recent reviews.^{1,20–22} Briefly, these cells actively participate in anti-infectious immunity against bacteria, viruses, and other pathogens, and it is arguable that such specialized T cells have evolved in mammals in response to pathogen exposure from their environment. In addition, these cells exert potent functions in tumor immunity^{20,22–24} as well as autoimmune, allergic and inflammatory diseases such as type 1 diabetes and Crohn's disease.^{25–32} In this review, we will focus on the interactions between microbes and unconventional T cells and how these interactions influence their function in disease.

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THE MICROBIOTA AND THE IMMUNE SYSTEM

Mammals are hosts to a multitude of microbes that colonize their epithelial surfaces to form heterogeneous, complex and dynamic ecosystems.^{33–35} The composition of the microbiota is highly personalized and acts as an individual microbial ‘fingerprint’.³⁴ Increased microbiota diversity (or richness) and resilience (i.e., the ability to recover from perturbations) are generally associated with better health.^{36–38} Shifts in microbial composition and diversity from the steady-state—a phenomenon known as dysbiosis—have been associated with numerous diseases, such as obesity, inflammatory bowel disease, and cancer.^{39–42} In some instances, disease susceptibility can be transferred upon colonization with the dysbiotic microbiota.^{43–45} The microbiota influences host physiological functions, taking part in essential biological processes, such as nutrient absorption and metabolism,⁴⁶ barrier function⁴⁷, and immune education.^{48–51}

The immune system and the microbiota are engaged in an intimate and interdependent relationship. The loss of microbial diversity and the presence of pathobionts have been associated with inflammation and immune activation,⁵¹ which can feed back and fuel further dysbiosis.⁵² Specific pro-inflammatory microbes have been described, including *Clostridium difficile*,^{53,54} certain members of Prevotellaceae,⁵⁵ the murine norovirus⁵⁶ and protozoa of the *Trichomonas* genus.^{57,58} Recent studies suggest that individual components of the innate immune system exert little influence on shaping microbiota composition at steady-state,^{59,60} however ongoing immune or inflammatory responses can impact microbiota communities through the recruitment and activation of immune cells (e.g., neutrophils, macrophages), the production of pro-inflammatory cytokines, antimicrobial peptides, immunoglobulin (Ig) A and reactive oxygen and nitrogen species.⁵²

In addition, the microbiota exerts a tremendous influence on the development, maturation, and function of the immune system, including T cells. A key role that the microbiota exerts on T cells is the local control of regulatory T (T_{REG}) and T_H17 CD4 T cell populations, which balances immune tolerance and pro-inflammatory responses. For example, certain members of the microbiota (e.g., SFB) or pathogens (e.g., *Citrobacter rodentium*) induce the development of intestinal T_H17 cells.^{61–63} Colonization of mice by the protozoa *Trichomonas* similarly exacerbates mucosal and systemic T_H1 and T_H17 responses.^{57,58} On the other hand, certain commensals such as *Clostridium* species have been shown to induce the generation of T_{REG} cells.^{64–66} Finally, monocolonization of germ-free (GF) mice by *Bacteroides fragilis* has been shown to restore the T_H1/T_H2 balance.⁶⁷ *B. fragilis* also reduced *Helicobacter hepaticus*-induced inflammation by suppressing T_H17 cells and inducing interleukin (IL)-10-producing T_{REG} cells.⁶⁸ Although iNKT and MAIT cells exert potent antimicrobial functions, how these unconventional T cells and the microbiota influence each other is only beginning to emerge.

UNCONVENTIONAL T CELLS DETECT MICROBIAL SIGNATURE MOLECULES

iNKT and MAIT cells are a key component of antimicrobial responses as they detect antigens that are invisible to conventional T cells. The microbial realm contains CD1d- and MR1-binding ligands that potentially activate iNKT and MAIT cells through their TCRs. Such ligands can, therefore, be viewed as microbial-associated molecular patterns (MAMPs) and drive specific immune outputs from these innate T cells to shape the resulting immune response to pathogens.

Microbial glycolipids

Several CD1d-restricted antigens have been identified in bacteria and other microbes. For example, α -galacturonosylceramides have been isolated from *Sphingomonas* and *Ehrlichia* species,^{69,70} while α -galactosyldiacylglycerols and α -glucosyldiacylglycerols have been isolated from *Borrelia burgdorferi*⁷¹ and *Streptococcus*

pneumoniae,⁷² respectively. These ligands have been extensively validated through biological, biochemical, and structural approaches. Other lipid antigens capable of activating iNKT cells include glycolipids from *Chlamydia muridarum*⁷³ and *Helicobacter pylori*,⁷⁴ as well as phospholipids from *Mycobacterium bovis* bacillus Calmette-Guerin⁷⁵ and the parasite *Leishmania donovani*.⁷⁶ Importantly, α -galactosylceramides have been recently isolated from the cecal and colonic contents of mice⁷⁷ and from the commensal *Bacteroides fragilis*.^{78,79} These findings suggest that the intestinal microbiota may represent an unexplored reservoir of ligands capable of modulating iNKT cell activation and function.

Vitamin B metabolites

Riboflavin or vitamin B2 is essential for energy generation in all organisms.⁸⁰ Although mammals acquire this metabolite through their diet, bacteria are able to obtain riboflavin through import and/or *de novo* biosynthesis.⁸¹ Bacteria that possess riboflavin biosynthetic pathways potentially activate MAIT cells through their TCR. These include *S. pneumoniae*, *Klebsiella pneumoniae*, *Salmonella enterica* serovar Typhimurium, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis*,^{8,82,83} as well as several bacterial commensal species from the Bacteroidetes and Proteobacteria phyla.⁸⁴ The genetic ablation or inhibition of the riboflavin metabolism in several of these bacteria abolished MAIT cell responses, confirming that this metabolic pathway produces MAIT cell antigens.^{8,85,86} Riboflavin biosynthesis, therefore, constitutes a telltale sign of microbial activity that MAIT cells can respond to, with the riboflavin derivatives 5-OP-RU and 5-(2-oxoethylideneamino)-6-D-ribitylamino-uracil (5-OE-RU) being potent agonists.⁸⁵ It is important to note that not all MR1-binding ligands are capable of activating MAIT cells. For example, although the folic acid (vitamin B9) metabolite 6-formylpterin (6-FP), the aspirin analog 3-formylsalicylic acid (3-FSA), and a derivative of the drug methotrexate all bind within the MR1 antigen-binding pocket, they do not stimulate MAIT cells, suggesting that non-agonist and agonist ligands may compete to regulate MAIT cell activation.^{8,87}

MICROBES REGULATE iNKT AND MAIT CELL ACTIVATION

Aside from the detection of glycolipid and vitamin metabolites, the activation and response of iNKT and MAIT cells can be controlled by many microbe- and host-derived factors.

Microbe-induced pro-inflammatory cytokines are essential co-signals

iNKT and MAIT cells can indirectly detect the presence of microbes through the recognition of MAMPs. Antigen-presenting cells (APCs) activated by natural or synthetic ligands of the toll-like receptors (TLR) 2, TLR3, TLR4, TLR5, TLR8, and TLR9 release pro-inflammatory cytokines that can activate MAIT cells to produce IFN- γ .^{88–90} Similarly, stimulation of TLR4, TLR7 and TLR9, Dectin-1, Rlg-I, and Nod1/2 on APCs leads to IFN- γ production by iNKT cells.^{70,91–96} In these instances, MAIT and iNKT cells are activated by IL-12 and/or IL-18 produced by activated APCs such as dendritic cells and monocytes/macrophages. In addition, iNKT and MAIT cells can respond to IL-1 β , IL-7, IL-15, IL-18, IL-21, IL-23, IL-25, and IL-33 alone or in certain combinations.^{97–104} Whereas some of these cytokines have proliferative effects (e.g. IL-7 and IL-15), others induce the production of helper cytokines (e.g., IL-1 β , IL-23, IL-25, and IL-33). Pro-inflammatory cytokines can activate innate T cells on their own or act as co-signals to potentiate weak TCR-mediated signals^{90,105–108} and, at least for iNKT cells, appear to be the main activation pathway in response to bacteria.¹⁰⁵ Pro-inflammatory signals also underpin iNKT and MAIT cell activation during viral infection and sterile inflammation, such as during cancer and autoimmunity.^{1,20–22}

Microbes regulate CD1d and MR1 expression

Regulating the expression of CD1d and MR1 molecules by microbes and their by-products constitutes another mechanism to control iNKT and MAIT cell activation and potentially prevent unwanted responses towards commensals. MR1 is essentially sequestered within the endoplasmic reticulum at steady-state^{109,110} and its expression at the plasma membrane can, in the presence of riboflavin derivatives such as 5-OP-RU, be induced or reinforced by MAMPs and/or type I and II interferons.^{111–114} In addition, viruses such as herpes virus can downregulate surface MR1 levels and lead to impaired TCR-mediated activation.¹¹⁵ CD1d is expressed quite ubiquitously and its expression can be increased by type I and II interferons, MAMPs such as LPS, all-trans retinoic acid, the short-chain fatty acid butyrate, and potentially vitamin D.^{116–123} Viruses such as herpes virus or human immunodeficiency virus-1 are also able to regulate CD1d expression, perhaps as a mechanism for immune evasion.^{118,124,125}

Bile acids and microbial by-products may influence iNKT and MAIT cell responses

Some microbes may regulate iNKT and MAIT cell activation and function through their production of metabolites as well as modulation of bile acids within the intestine (Fig. 1). Primary bile acids are secreted into the small intestine during digestion and

metabolized by the gut microbiota into secondary bile acids upon reaching the large intestine. Lithocholic acid (LCA) is a secondary bile acid produced by *Clostridium* and *Eubacterium* species that inhibits the function of T_H1 CD4 T cells by binding to the vitamin D receptor.¹²⁶ Since the vitamin D receptor is expressed by both iNKT and MAIT cells and is required for the normal development and function of iNKT cells,^{4,122,127} it is possible that LCA similarly modulates iNKT and MAIT cell function.

Indole is a catabolite of tryptophan produced by Gram-negative and Gram-positive microbes containing tryptophanase, including *E. coli*, *Bacteroides*, *Clostridium*, and *Vibrio* species.¹²⁸ Indole inhibits T_H17 CD4 T cell differentiation through interactions with ROR γ t,¹²⁹ and may similarly inhibit the differentiation and/or function of iNKT17 and MAIT17 cells. Other tryptophan catabolites such as tryptamine, indolelactic acid, and indoleacetic acid are ligands for the aryl hydrocarbon receptor (AhR). AhR is expressed by human iNKT cells and has been shown to regulate their IL-17 and IL-22 production,¹³⁰ and AhR deficiency results in increased IFN- γ production by iNKT cells and exacerbates ConA-induced liver injury in mice.¹³¹

Oxazoles are heterocyclic aromatic organic compounds that are found in certain diets as well as in microcins, which are antimicrobial peptides produced by *E. coli*. Oxazoles can induce tryptophan metabolism and AhR activation in intestinal epithelial

Fig. 1 Modulation of unconventional T cell function by microbe-derived metabolites and other molecules. Lithocholic acid (LCA), a by-product of bile acid metabolism, is a ligand for the vitamin D receptor (VDR), which is expressed by iNKT and MAIT cells. Binding of LCA to VDR may inhibit their production of T_H1 cytokines (e.g., IFN- γ and TNF- α). Indole is a tryptophan catabolite that may regulate IL-17 and IL-22 production by unconventional T cells through interaction with the transcription factor ROR γ t. The short-chain fatty acid (SCFA) butyrate regulates gene expression through inhibition of histone deacetylases (HDAC). Butyrate may inhibit HDAC3 in iNKT cells, which has been shown to inhibit their cytokine secretion. Other tryptophan catabolites may regulate iNKT cell cytokine response through interaction with the aryl hydrocarbon receptor (AhR). Finally, inhibition of AhR within intestinal epithelial cells (IEC) alters interactions with iNKT cells. This results in the loss of IL-10 production by IECs and exacerbated IL-13/IFN- γ responses in iNKT cells, which leads to intestinal inflammation.

cells, and result in exacerbated pro-inflammatory responses of iNKT cells and colitis.¹³²

Butyrate is a short-chain fatty acid derived from the fermentation of indigestible food by certain bacterial taxa belonging to Firmicutes and Bacteroidetes.^{133–135} Butyrate has gained increasing attention in recent years due to its capacity to regulate diseases such as inflammatory bowel disease and colorectal cancer.^{136,137} The primary mechanism of action of butyrate is the epigenetic regulation of gene expression via inhibition of histone deacetylase (HDAC),¹³⁸ among which HDAC3 is required for iNKT cell development¹³⁹ and has been shown to inhibit cytokine secretion by iNKT cells in a model of antibody-induced arthritis.¹⁴⁰ Therefore, butyrate-producing microbes can participate in the control of iNKT cells via the butyrate-HDAC3 axis.

Although relatively little is known compared to conventional T cells,^{141,142} the emerging data suggest that iNKT and MAIT cells respond to a complex network of microbe-derived molecules and their by-products to fine tune ensuing immune responses. Specific interactions between the microbiota and unconventional T cells are discussed below.

REGULATION OF THE MICROBIOTA BY UNCONVENTIONAL T CELLS

iNKT cell/CD1d-mediated shaping of the microbiota

Analysis of the gut microbiota through 16S rRNA sequencing using TRAJ18^{-/-} mice (lacking iNKT cells) and CD1d^{-/-} mice (lacking all CD1d-restricted T cells, including iNKT cells) has revealed that CD1d and/or iNKT cells may influence the composition of the microbiota (Table 1).^{143–148} Two studies have reported similar alterations in CD1d^{-/-}¹⁴³ and TRAJ18^{-/-} mice,¹⁴⁴ namely an increased prevalence of the Bacteroidetes, Deferribacteres, Proteobacteria, and TM7 phyla and several genera including *Lactobacillus*, *Bacteroides*, *Mucispirillum*, and *Prevotella* relative to wild-type (WT) mice. In a third study, bacteria from the phylum Tenericutes and genera *Bifidobacterium*, *Prevotella*, and *Olsenella* were found to be enriched in TRAJ18^{-/-} compared to WT mice, while the Deferribacteres and Actinobacteria phyla were markedly decreased.¹⁴⁷ It is important to note that environmental factors, such as diet, housing conditions, and maternal transmission are known to have a major impact on microbiota composition and should, therefore, be carefully controlled in all microbiota studies.^{33,34,149–153} Although co-housing of mice after weaning has been widely used in efforts to normalize the microbiota in various experimental settings, a recent study demonstrated that this method is ineffective at normalizing mucosa-associated microbes and that only littermate mice from heterozygous breeding should be used to minimize these confounding effects.¹⁵² In support of this, we found that adjacently bred non-littermate CD1d^{-/-} and WT mice had major microbiota alterations, whereas CD1d^{+/-}, CD1d^{+/-} and CD1d^{-/-} littermate mice had similar bacterial composition.¹⁴³ Two of the studies discussed in this section lacked information on the experimental designs used and likely compared non-littermate mice, which puts the relevance of the findings into question.^{144,147} In addition, the TRAJ18^{-/-} used in these studies were recently shown to have a less diverse T cell population and to completely lack MAIT cells as an artifact of the genetic targeting method employed.^{154,155} Several new strains of TRAJ18^{-/-} have been generated to alleviate the unintended consequences of the genetic manipulation, which should help clarify the biological functions of iNKT cells in disease and in shaping the intestinal microbiota.^{156–158}

An initial study found that CD1d^{-/-} mice had altered microbial communities and increased bacterial translocation compared to WT littermates, which was attributed to altered Paneth cell function.¹⁴⁵ However, this study contained no statistical analyses and it is unclear how much the genotype contributed to community dissimilarity. Additionally, a more recent study

reported microbial alterations between CD1d^{-/-} and CD1d^{+/-} littermates, between CD1d^{fl/fl} CD11c-cre (which conditionally lack CD1d only on CD11c-expressing cells) and CD1d^{fl/fl} littermates, and following oral αGC administration in WT mice.¹⁴⁶ However, it is unclear whether the genotype or other factors such as maternal transmission, caging or sex primarily explained the observed shifts in bacterial composition. In sum, it is presently unclear whether the presence and/or activation of iNKT cells exert a strong influence in shaping the commensal microbial communities in mice.

Influence of MAIT cells and MR1 on the microbiota

Only a few studies have addressed whether MAIT cells or MR1 shape the microbiota thus far. Recent studies showed that MR1^{-/-} mice exhibited differences in the relative abundance of several bacterial families, including Bacteroidaceae and Lactobacillaceae, compared to WT mice, which conferred resilience to antibiotic perturbations and was associated with increased production of bile acids and resistance to *Clostridium difficile* infection.^{159,160} However, it is important to note that these authors initially compared MR1^{-/-} mice bred in house with commercially obtained WT mice, while additional analyses were performed on mice that were co-housed for four weeks post-weaning (Table 1). As discussed above, co-housing is not a reliable method to normalize environmental differences.¹⁵² In addition, the relative contribution of genotype and other factors to the observed variation in microbiota composition was not analyzed. An important message that can be drawn from these studies is that littermate-controlled experiments carefully designed to minimize variability and robust statistical methods are needed in order to draw biologically meaningful conclusions on the immune-mediated shaping of the gut microbiota.⁵⁹

MODULATION OF INNATE T CELLS BY THE MICROBIOTA

Microbial colonization impacts innate T cell development and homeostasis

The microbiota is important for the education of the adaptive immune system, and early life exposure ensures proper T and B cell maturation, differentiation, and tolerance.^{49,161,162} This also appears to be true for iNKT and MAIT cells. Although the development and tissue homing capabilities of iNKT cells are largely unaffected by the absence of the microbiota in GF mice, their prevalence is increased in the lungs, small intestine and colon.^{27,163–165} In addition, iNKT cells in GF mice have an altered TCRβ repertoire and are overall less mature and hyporesponsive to αGC stimulation.¹⁶³ Similar defects are also observed in mice harboring a restricted microbiota characterized by a reduced prevalence of *Sphingomonas* species, which are known to produce activating iNKT cell ligands.¹⁶⁴ However, whether these changes are specific to certain iNKT cell subsets and their tissue distribution is not known. Interestingly, mono-colonization of GF mice with *Sphingomonas yanoikuyake*, but not *E. coli*, corrected the defects, suggesting that the commensal-derived antigenic load directly participates in iNKT cell homeostasis and/or function.¹⁶³ Furthermore, the accumulation of iNKT cells in the lungs and intestines of GF mice can be reversed by microbiota colonization early in life but not after weaning.²⁷

The development of MAIT cells is profoundly affected by the absence of the microbiota in GF mice^{3,10,13,82,166} (Fig. 2). Although colonization of GF mice with a complex human microbiota is able to restore MAIT cell numbers to that of specific pathogen-free (SPF) mice, they are unable to reach the higher levels normally found in humans.¹⁰ Similarly, mono-association of GF mice with *Enterobacter cloacae*, *Lactobacillus casei*, *Bacteroides thetaiotaomicron*, *Bifidobacterium animalis*, but not *E. faecalis*, also restores MAIT cell numbers to SPF levels.⁸² In addition, early-life exposure to riboflavin-synthesizing bacteria, such as Enterobacteriaceae,

Table 1. Summary of 16S rRNA sequencing studies assessing the role of INKT and MAIT cells in shaping the intestinal microbiota composition.

Study	Comparison	Littermate	Source of bacterial DNA	Sequencing method	Analysis method	Findings	Reference
1	WT (n = 5) vs. CD1d ^{-/-} (n = 5)	Yes	Small intestine	ABI 3730-Capillary Sequencer: Region of amplification unknown	OTU alignment: Ribosomal Database Project II Phylogenetic distance: UniFrac	CD1d ^{-/-} mice have a different microbiota than WT mice characterized by increased Bacteroidetes and Firmicutes (Clostridia) and decreased Deferribacteres and Firmicutes (Bacilli)	Nieuwenhuis et al., ¹⁴⁵ J. Clin. Invest.
2	WT (n = 7) vs. CD1d ^{-/-} (n = 8)	No	Proximal colon	Illumina MiSeq2: V4 region	OTU alignment: Greengenes database Phylogenetic distance: Weighted UniFrac	CD1d ^{-/-} mice have a different microbiota than WT mice characterized by increased Proteobacteria, Deferribacteria and TM7	Selvanantham et al., ¹⁴³ J. Immunol.
3	CD1d ^{+/-} (n = 6) vs. CD1d ^{-/-} (n = 4)	Yes	Ileal wall, ileal lumen and cecum	Illumina MiSeq: V1-V3 region	OTU alignment: SILVA database Phylogenetic distance: Yue-Clayton	CD1d ^{-/-} mice have a different microbiota than CD1d ^{+/-} mice characterized by decreased Bacteroidetes in the ileal lumen but not in the ileal wall and cecum	Saez de Guinoa et al., ¹⁴⁶ EMBO J.
	CD1d ^{fl/fl} CD11c ^{Cre-} (n = 4-5) vs. CD1d ^{fl/fl} CD11c ^{Cre+} (n = 4-5)		Ileal lumen			CD1d ^{fl/fl} CD11c ^{Cre+} mice have a different microbiota than CD1d ^{fl/fl} CD11c ^{Cre-} mice characterized by decreased Bacteroidetes	
4	WT (n = 10) vs. TRAJ18 ^{-/-} (n = 9)	No	Stool (location unspecified)	Illumina MiSeq: V4 region	OTU alignment: Greengenes database Phylogenetic distance: Unweighted and weighted UniFrac	TRAJ18 ^{-/-} mice have a different microbiota than WT mice characterized by increased Tenericutes and decreased Deferribacteres and Actinobacteria; Microbial α -diversity was comparable between WT and TRAJ18 ^{-/-} mice	Shen et al., ¹⁴⁷ Front. Immunol.
5	WT (n = 60) vs. TRAJ18 ^{-/-} (n = 26) vs. CD1d ^{-/-} (n = 13)	No	Fecal pellet	Illumina MiSeq: V4 region	OTU alignment: Greengenes database Phylogenetic distance: Unweighted and weighted UniFrac	TRAJ18 ^{-/-} mice have a different microbiota than WT mice characterized by increased Proteobacteria, Cyanobacteria, Deferribacteres and TM7, as well as decreased Verrucomicrobia; Microbial α -diversity was higher in both TRAJ18 ^{-/-} and CD1d ^{-/-} mice than in WT mice	Maricic et al., ¹⁴⁴ J. Immunol.
6	WT (n = 6) vs. TRAJ18 ^{-/-} (n = 5)	No	Colon	Illumina MiSeq: V3-V4 region	OTU alignment: Greengenes database Phylogenetic distance: Unspecified	TRAJ18 ^{-/-} mice have a different microbiota than WT mice characterized by increased Bacteroidetes and reduced Lactobacillaceae and Lactobacillus murinus.	de Aguiar et al., ¹⁴⁸ Inflamm. Bowel. Dis.
7	WT ^a (n = 6) vs. MR1 ^{-/-} (n = 6)	No	Stool (location unspecified)	Illumina MiSeq2: V4 region	OTU alignment: SILVA database Phylogenetic distance: Yue-Clayton	MR1 ^{-/-} mice have a different microbiota than WT ^a mice characterized by the presence of Bacteroidaceae, Desulfovibrionaceae and unclassified Burkholderiales, as well as increased Porphyromonadaceae, Lactobacillaceae and Lachnospiraceae; After antibiotic treatment, MR1 ^{-/-} mice retained a similar microbial profile while WT mice showed loss of diversity and domination by Enterococcaceae and Bacteroidaceae	Smith et al., ¹⁵⁹ PLoS ONE
	WT ^b (n = 5) vs. MR1 ^{-/-} (n = 6)					Co-housing with MR1 ^{-/-} mice led to a shift in the microbiota of WT ^b mice, which partially reverted back to the original state after separation; Microbiota of MR1 ^{-/-} did not show substantial change throughout co-housing	

WT wild-type.

^aPurchased from The Jackson Laboratory.

^bCo-housed with MR1^{-/-} for 4 weeks then separated for 3 weeks; age of co-housing unknown.

Fig. 2 Early-life microbial colonization stimulates MAIT cell development and maturation. MAIT cells are virtually absent in germ-free (GF) mice. Early-life colonization of GF mice with diverse microbiota from mice or humans restores MAIT cell development. Similarly, oral, intraperitoneal, or topical administration of riboflavin-producing bacteria or the MAIT ligand 5-OP-RU restores MAIT cell development and homeostasis. Interestingly, exogenous 5-OP-RU can traffic to the thymus to stimulate MAIT cell ontogeny.

can restore MAIT cell development in GF mice, whereas colonization of adult GF mice has no effect.¹⁰⁰ Together, these findings suggest that riboflavin-synthesizing commensals can stimulate MAIT cell development and/or homeostasis (Fig. 2). Likewise, topical application of 5-OP-RU to the skin of GF mice early in life, but not in adulthood, is sufficient to restore MAIT cell numbers.¹⁰⁰ Another recent study in mice has suggested that microbial metabolites have the potential to translocate to the thymus, upon which they can be captured by MR1-expressing cells and presented to developing thymocytes to induce MAIT cell maturation.¹⁶⁶ In humans, MAIT found in the umbilical cord blood are immature, whereas some mature MAIT cells can be found in peripheral blood after birth and accumulate with age.^{167,168} This suggests that MAIT cell maturation likely involves microbial antigens and appears to be fine-tuned by the TCR β chain.^{167,168} Collectively, these findings suggest that early-life colonization by the microbiota has a profound and long-lasting impact on the development and homeostasis of both iNKT and MAIT cells.

The microbiota influences innate T cell function in disease
The gut microbiota modulates iNKT cell function in disease. The accumulation of iNKT cells in the lungs and intestines of GF mice was correlated with exacerbated ovalbumin-driven asthma and oxazolone-induced colitis, which could be improved by administration of neutralizing anti-CD1d antibodies.²⁷ The authors of this study went on to show that GF mice mono-colonized with *B. fragilis* from birth were protected from chemically induced colitis in adulthood as a result of normalized abundance of colonic iNKT cells.⁷⁹ 1. The protective effect of *B. fragilis* was dependent on the expression of the glycosphingolipid GSL-Bf717, as GF mice colonized with mutant *B. fragilis* lacking GSL-Bf717 were not protected.^{143,169,170} Although iNKT cell functional response (e.g., cytokine production) was not directly assessed, these studies demonstrated that early-life microbial colonization is necessary to tune down the pro-inflammatory functions of iNKT cells at mucosal sites.

Additional hints on how the gut microbiota functionally impacts iNKT cells and their ability to regulate disease can be gleaned from a few other studies. For example, glycolipid-mediated activation of iNKT cells during the course of dextran sulfate sodium (DSS)-induced colitis has shown inconsistent results between studies.^{143,169,170} The discrepancies may be attributed to different experimental designs, including the dose, frequency, and route of glycolipid injection, as well as environmental factors such as housing conditions, diet, and microbiota differences between animal facilities. In a previous study, we demonstrated that WT mice born from dams transplanted with two discrete microbial communities have different clinical outcomes in response to the synthetic iNKT cell ligand OCH9, which suggests that iNKT cell responses and/or their ability to modulate immune and inflammatory responses are regulated by the intestinal microbiota.¹⁴³ Short-term broad spectrum antibiotic treatment in adult mice increased colonic iNKT cell numbers, and subsequent reconstitution with a dysbiotic microbiota (isolated from DSS-treated mice) skewed the cells towards a pro-inflammatory phenotype.¹⁷¹ This microbiota-driven skewing of iNKT cell phenotype was also observed in ulcerative colitis patients.²⁸ Antibiotic treatment also had similar effects on liver iNKT cells. In mice, accumulation of IFN- γ -producing iNKT cells in the liver was driven by the upregulation of CXCL16 in liver sinusoidal endothelial cells as a result of the reduction in microbiota-mediated primary to secondary bile acid conversion in the gut, and in this case was associated with protective function against intrahepatic tumor growth.¹⁷² Taken together, these studies suggest that the gut microbiota has the capacity to regulate iNKT cell homeostasis in certain tissues, as well as their functional response during inflammation and tumor development (Fig. 3).

MAIT cells have also been shown to play a role in multiple infectious and sterile diseases, however to what extent and how the microbiota regulates their function remains unclear. MAIT cells provide initial immunity against *Mycobacterium tuberculosis* infection due to their robust granzyme B production.¹⁷³ Interestingly, their capacity to produce granzyme B correlates with the abundance of specific bacterial species, namely *B. ovatus* and *P. merdae* of the class Bacteroidia. Furthermore, antibiotic-induced dysbiosis was found to increase early infection levels of *M. tuberculosis* in mice, which correlated with reduced MAIT cell numbers in the lungs.¹⁷⁴ The inoculation of a complex microbiota into antibiotic-treated mice restored MAIT cell numbers and conferred protection, suggesting that the microbiota modulates MAIT cell proliferation and activity in response to infection. In addition, the loss of intestinal epithelial integrity and bacterial translocation in patients with alcohol-related liver disease led to reduced circulating MAIT cell numbers and impaired IL-17 and granzyme B production.¹⁷⁵ Similar MAIT cell defects were found in children with type 1 diabetes, which is associated with a loss of intestinal barrier integrity.²⁵ Furthermore, MR1^{-/-} non-obese diabetic (NOD) mice have greater intestinal permeability, decreased expression of tight-junction proteins, abnormal mucus distribution, and accelerated disease progression compared to MR1^{+/-} NOD mice.²⁵ Therefore, there is mounting evidence that the microbiota can modulate MAIT cell function during infectious and inflammatory diseases.

CONCLUDING REMARKS

While our understanding of the biology of iNKT and MAIT cells has seen tremendous progress in recent years, it has for the most part focused on how host-derived factors control the development and function of these innate T cells, with the influence of environmental factors, such as the microbiota being mainly overlooked. Whereas it remains unclear whether innate T cells influence the composition of the microbiota in mammals, the influence of the microbiota on iNKT and MAIT cells has now come into clearer focus. These findings raise several fundamental and practical

Fig. 3 The microbiota modulates iNKT cell function in disease. iNKT cells in GF mice are pro-inflammatory and fuel allergen-induced airway inflammation and colitis. Early-life colonization with a complex microbiota, or the sphingolipid-producing commensal *Bacteroides fragilis* restores normal iNKT cell functions and limits inflammation. Bile acids produced by the liver are modified by the microbiota in the gut and recirculate back to the liver where they recruit iNKT cells via the chemokine CXCL16. Accumulated iNKT cells provide antitumor immunity in the liver.

questions: Can the heterogeneity observed in innate T cell compartments between individuals and populations be explained, at least in part, by environmental differences such as microbiota composition? Are microbiota-innate T cell interactions relevant to human disease and therapies targeting these cells? What are the microbial determinants that modulate innate T cell homeostasis and functional properties? And most importantly, can we manipulate the gut microbiota or harness their by-products in order to modulate iNKT and MAIT cell function in disease? Innate T cells constitute an integral component of the immunologist's toolkit to develop novel targeted immunotherapies,²⁴ and prospects of their selective manipulation learned through their dialogue with the gut microbiota are certainly worthy of careful examination.

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AUTHOR CONTRIBUTIONS

Q.L., M.K., D.J.P., and T.M. wrote the paper and T.M., M.K., and Q.L. prepared the figures.

ADDITIONAL INFORMATION

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