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REVIEW

Interactions between the microbiota and innate and innate-like lymphocytes

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Summary sentence: Review of how the microbiota promotes homeostatic immunity through the regulation of innate and innate-like lymphocytes.

Abstract

The microbiota, which consists of commensal bacteria, fungi, and viruses, limits the colonization of pathogens at barrier tissues and promotes immune homeostasis. The latter is accomplished through the induction and regulation of both innate and adaptive immune responses. Innate lymphocytes, which include the type-1 innate lymphoid cell (ILC1), NK cell, type-2 innate lymphoid cell (ILC2), type-3 innate lymphoid cell (ILC3), and lymphoid tissue inducer (LTi) cell populations, and innate-like lymphocytes, such as NKT cells, mucosal-associated invariant T (MAIT) cells, and $\gamma\delta$ T cells, are uniquely capable of responding to the microbiota due to their tissue localization and rapid primary responses. In turn, through their effector functions, these lymphocyte populations modulate the composition of the microbiota and maintain the segregation of commensals. This review will focus on how innate and innate-like lymphocytes mediate the crosstalk with the microbiome.

KEYWORDS
commensal, ILC, MAIT, microbiome, NKT, $\gamma\delta$

1 | INTRODUCTION

The diverse array of bacteria, fungi, and viruses present at barrier sites is collectively termed the microbiome.[1] Most commensals reside in the gastrointestinal tract, with estimates of approximately 10^{14} bacteria in the human colon,[2] whereas the skin harbors about 10^{12} bacteria and another 10^{12} bacteria are present at other mucosal sites.[3] Each barrier site is populated with a unique microbial community that displays remarkable temporal stability.[4–6] In addition to inhibiting colonization by pathogens through competition and the induction of immune responses, commensals promote immune homeostasis via the release of microbial products and their constant interaction with host cells.[1] Although colonization primarily occurs at birth, the dialog between the microbiota and the host begins in utero, through the transfer of microbial products derived from the mother's microbiota and limited colonization of the amniotic fluid.[7,8] Thus, while neonatal immunity is initially type-2 helper T cell (Th2) skewed, early exposure to the microbiota shifts the immune response toward type-1 helper T cell (Th1), reducing the possibility of allergy and autoimmunity through adulthood.[9] Understanding how the microbiome modulates the immune response is paramount to harnessing its therapeutic potential.

Immunity can be broadly classified into innate and adaptive components. The innate immune system relies on germline-encoded pattern-recognition receptors for the identification of pathogen-associated molecular patterns and is present in both animals and plants, suggesting that it evolved prior to the divergence of these two kingdoms.[10] Conversely, the adaptive immune system is only present in vertebrates and these cells recombine their antigen receptors to generate a broader range of antigen specificities.[10] Innate lymphoid cells (ILCs) exhibit lymphoid morphology, yet lack RAG-dependent antigen receptors characteristically expressed by B and T cells. As their innate designation would suggest, these lymphocytes mount a rapid primary response and localize primarily to tissues. Based on their production of Th1-, Th2-, and Th17/22-associated cytokines, ILCs have been categorized into three distinct subsets, termed group 1, group 2, and group 3 ILCs, respectively.[11,12] While innate-like populations such as NKT cells, mucosal-associated invariant T (MAIT) cells, and $\gamma\delta$ T cells, use RAG-mediated recombination to generate their TCRs, they preferentially utilize specific TCR genes, resulting in the expression of semi-invariant receptors that limit the range of antigens these cells recognize.[13]

Abbreviations: AhR, aryl hydrocarbon receptor; AMP, antimicrobial peptide; Areg, amphiregulin; CSF2, colony-stimulating factor 2; DC, dendritic cell; I3C, indol-3-carbinol; IEL, intraepithelial lymphocyte; ILC, innate lymphoid cell; ILC1, type-1 innate lymphoid cell; ILC2, type-2 innate lymphoid cell; ILC3, type-3 innate lymphoid cell; iNKT cell, invariant NKT cell; iNKT1 cell, type-1 invariant NKT cell; iNKT17 cell, type-17 invariant NKT cell; iNKT2 cell, type-2 invariant NKT cell; LTi cell, lymphoid tissue inducer cell; MAIT cell, mucosal-associated invariant T cell; MCMV, mouse cytomegalovirus; MZ, marginal zone; NCR, natural cytotoxicity receptor; SAA, serum amyloid A; SCFA, short-chain fatty acid; Th1, type-1 helper T cell; Th17, type-17 helper T cell; Th2, type-2 helper T cell; TSLP, thymic stromal lymphopoietin; γ_c, gamma chain

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Furthermore, like ILCs, innate-like cells acquire their effector characteristics during their development and predominately localize to tissues, where they respond immediately upon antigen recognition.[13] Innate-like lymphocytes do not exhibit immunologic memory either, as there is no marked difference in their activity following subsequent antigen exposure.[13]

Owing to their tissue localization, innate and innate-like lymphocytes are in close proximity to the microbiota. These populations also seed barrier sites prior to adaptive populations, with NK cells and group 2 and 3 ILCs observed in the intestines of human fetuses prior to T cells.[14] Since many innate and innate-like populations are enriched in mucosal tissues during neonatal colonization,[7] these cells are crucial for establishing the localization and composition of the microbial communities. Conversely, the microbiome promotes the development and/or function of many innate and innate-like lymphocytes. This review will summarize both aspects of the dialog between host and commensal.

2 | GROUP 1 ILCS

Group 1 ILCs are defined by their expression of the T-box transcription factor T-bet and their ability to produce the Th1-associated cytokines IFN-γ and TNF, which enables them to promote immunity to intracellular bacteria, viruses, and parasites.[11,12] This group is composed of recirculating NK cells, which are present in blood and broadly distributed within secondary lymphoid and peripheral organs, and tissue-resident type-1 innate lymphoid cells (ILC1s) that predominantly reside within mucosal sites.[15] NK cells recognize target cells through activating and inhibitory surface receptors, including NKp46, NKG2D, and in some mouse strains NK1.1. Upon activation, NK cells release cytotoxic granules that contain the pore-forming protein perforin and proteases called granzymes, which result in apoptosis or osmotic cell lysis of the target. While ILC1s also express activating surface receptors, they are devoid of inhibitory receptors that recognize MHC-I. Furthermore, though ILC1s produce IFN-γ, they are functionally distinct from NK cells in that they lack cytotoxicity.[15]

Though the development of splenic NK cells does not require microbiota and the expression of Ly49 receptors, NKp46, NKG2D, CD122, and α2 integrin are unaltered in the absence of commensals, NK cells exhibit impaired cytotoxicity and IFN-γ production in germ-free or antibiotic-treated mice.[16] This corroborated an early study which found that colonization of germ-free mice with intestinal bacteria increased NK cell cytotoxicity.[17] The diminished NK cell function in mice that lack commensals is due to a reduction in type-I interferons produced by dendritic cells (DCs) and macrophages (Figure 1), which are necessary for priming NK cells via the trans-presentation of IL-15.[16,18] Therefore, the impaired antiviral immune responses observed in germ-free and antibiotic-treated mice are caused by NK cell extrinsic effects of the microbiota.

While innate immune cells are typically thought to lack memory characteristics, it has become evident that NK cells can exhibit "memory-like" qualities following antigen-dependent recognition of viral ligands or haptens, including longevity and enhanced effector

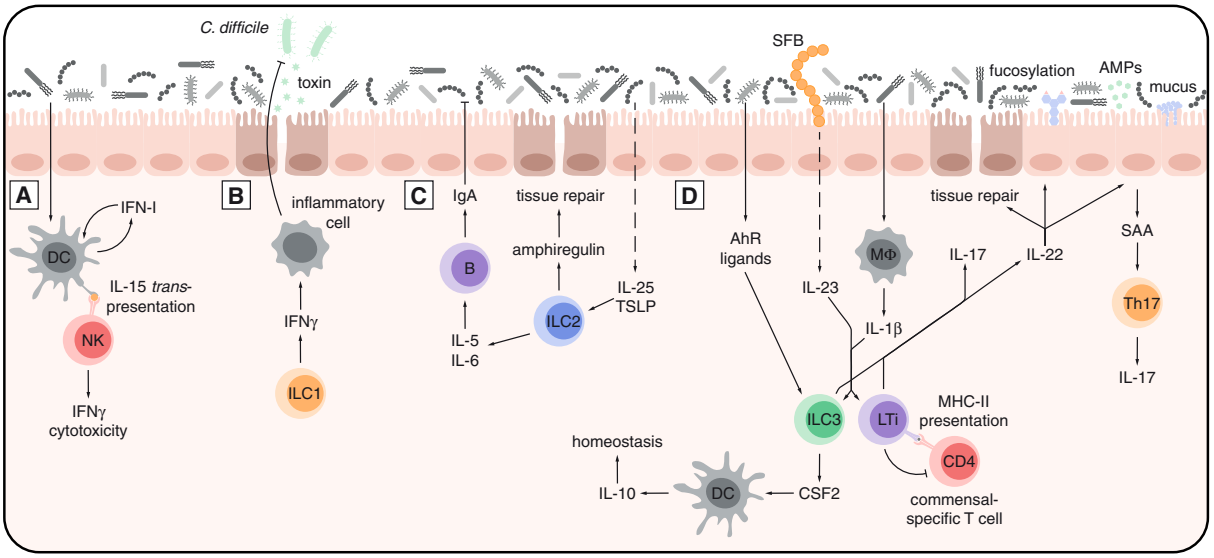

FIGURE 1 **Interactions between innate lymphocytes and the microbiota.** (A) The microbiota induces the release of type-I interferons from DCs and macrophages, which promotes the trans-presentation of IL-15 to NK cells, activating their IFN-γ production and cytotoxicity. (B) Intestinal dysbiosis allows the opportunistic pathogen *C. difficile* to disrupt epithelial integrity via the release of toxins. ILC1s activate infiltrating inflammatory cells through their production of IFN-γ, promoting clearance of the pathogen and return to homeostasis. (C) Commensals induce IL-25 and TSLP, which activates ILC2s to release Areg and IL-5/6. Areg promotes tissue repair, whereas IL-5/6 induce IgA production from B cells, which modulates microbial colonization. (D) Commensal-derived AhR ligands induce IL-22 production from group 3 ILCs, which promotes mucus production, release of AMPs and fucosylation of luminal proteins and lipids that provide energy for the microbiota. Both ILC3s and LTi cells can be activated by IL-1β and IL-23, resulting in the production of IL-17 and IL-22. The intestinal bacteria segmented filamentous bacteria (SFB) causes the release of IL-23, which activates group 3 ILCs and the resulting IL-22 induces epithelial cell production of SAA proteins that license Th17 cells to produce IL-17. LTi cells process and present commensal antigens, which results in the apoptosis of commensal-specific CD4$^+$ T cells. Macrophage-derived IL-1β causes group 3 ILCs to produce CSF2, which promotes homeostasis through the release of IL-10 from DCs

functions.[19] Recent work has shown that commensals activate NOD receptors on nonhematopoitic cells, which promote IL-15 production by myeloid cells.[20] Excess IL-15 results in the terminal maturation of NK cells, thus diminishing the pool of immature KLRG1$^-$ NK cells that generate the memory-like pool.[20] In the absence of T cells, depletion of bacterial commensals improves NK cell antiviral responses. Antibiotic treatment of *Rag1*$^{-/-}$ mice prior to infection with mouse cytomegalovirus (MCMV) leads to a greater persistence of NK cells that express Ly49H, which recognizes the MCMV glycoprotein m157, resulting in decreased viral copies and improved survival.[20] Thus, commensals can indirectly regulate the generation of long-lived NK cells.

Owing to the recent distinction between the NK and ILC1 populations, neither the impact of the microbiota on ILC1 development and function, nor the role of ILC1s in regulating commensals, have been established. While all ILC1s express T-bet, single-cell RNA-sequencing of ILC subsets revealed that the microbiota promotes expression of *Tbx21*, the gene that encodes this transcription factor, in a fraction of ILC1s.[21]

Oral antibiotics frequently leads to dysbiosis of the intestinal microbiota, which can allow the opportunistic enteric pathogen *Clostridium difficile* to establish an infection.[22] Mice deficient in RAG2 and γ_c, which lack both adaptive and innate lymphocytes, are more susceptible to *C. difficile* infection than *Rag1*$^{-/-}$ mice, suggesting that ILCs provide immunity in the absence of T and B cells.[23] Transfer of ILC1s into Rag2$^{-/-}$.$\gamma_c$$^{-/-}$ mice decreased disease progression and improved survival to levels comparable to *Rag1*$^{-/-}$ mice, indicating that ILC1s were the primary contributor to immunity against *C. difficile* (Figure 1).[23] Therefore, ILC1s contribute to returning the intestinal microbiome to homeostasis by suppressing opportunistic pathogens.

3 | GROUP 2 ILCS

Multiple studies have described innate lymphocytes that produce type-2 cytokines, initially referring to these cells as "natural helper cells,"[24] "nuocytes,"[25] or "innate helper type 2 cells,"[26] though they were subsequently shown to be the same lineage and were therefore designated type-2 innate lymphoid cells (ILC2s).[11] These cells are defined by their expression of the transcription factor GATA3 and production of the Th2-associated cytokines IL-4, IL-5, IL-6, IL-9, and IL-13, which promote immunity to helminths through the recruitment of eosinophils, activation of macrophages and granulocytes, mucus production by goblet cells, and smooth muscle contraction.[11,12] ILC2s express receptors for IL-25 (IL-17RB), IL-33 (T1/ST2), and thymic stromal lymphopoietin (TSLP), enabling them to respond to cytokines produced by hematopoietic and epithelial cells during inflammation.[15]

In germ-free mice, the frequency and number of lung ILC2s is comparable to specific-pathogen-free (SPF) animals, and the expression of surface markers characteristically found on ILC2s, including c-Kit, IL-7Rα, and T1/ST2, is unaltered, suggesting that ILC2 development occurs independently of the microbiota.[27] However, the proportion of ILC2s within the small intestine is higher in the absence of commensals,[28] likely due to the decrease in microbe-dependent populations. While the microbiota may not be necessary for ILC2 development, human ILC2s express TLRs, including TLR1, TLR4, and TLR6, enabling them to recognize microbial ligand directly.[29] Additionally, the microbiota can indirectly regulate ILC2 function through cytokines. Commensals induce IL-25 and TSLP production,[30,31] both of which activate ILC2s (Figure 1). While type-I and type-II interferons induced by viral infections can inhibit cytokine production and proliferation of ILC2s,[32] it remains to be seen whether the virome is also capable of modulating ILC2 activity.

Though ILC2s have not been shown to directly target commensals, they indirectly regulate the composition and spatial segregation of the microbiota through antibody responses and mucus production, respectively. ILC2s express IL-5 and IL-6, which promote the production of IgA,[33,34] and coculture of ILC2s with B cells increased the secretion of IgA in vitro,[24] suggesting that ILC2s promote IgA responses (Figure 1). Additionally, IL-5 supports the proliferation of B1 cells, which can modulate commensals.[24,35] ILC2s also minimize translocation of commensals through their production of amphiregulin (Areg), a member of the epidermal growth factor family that promotes epithelial repair (Figure 1).[12]

4 | GROUP 3 ILCS

Group 3 ILCs are defined by their expression of the transcription factor RORγt and production of the Th17/22-associated cytokines IL-17 and/or IL-22, which enable them to promote immunity to extracellular bacteria and fungi, as well as tissue repair.[11,12] The group consists of lymphoid tissue inducer (LTi) cells and ILC3s; the latter of which is separated into two subsets based on expression of the natural cytotoxicity receptor (NCR) NKp46.[11,12,15] LTi cells are necessary for generation of secondary lymphoid organs during embryonic development, including the formation of lymph nodes and Peyer's patches, which are crucial sites for the development adaptive immune responses to commensal and pathogenic microbes.[36,37] Following birth, LTi cells generate cryptopatches, which are transformed into isolated lymphoid follicles in a microbiota-dependent manner,[38] promoting intestinal IgA production. In mice, embryonic LTi cells have been shown to originate from the fetal liver and can be distinguished from ILC3s by their expression of the chemokine receptor CCR6.[11,12] LTi-like cells also arise from bone marrow precursors in adults, though their ability to generate lymphoid tissue has not been established. While all group 3 ILCs respond to the cytokines IL-1β and IL-23, only LTi cells and NCR$^-$ ILC3s produce IL-17 in response to IL-23.[11] Additionally, both NCR$^+$ and NCR$^-$ ILC3s are capable of producing IFN-γ.[39]

There are conflicting reports on whether the microbiota is necessary for the development of group 3 ILCs. One study observed a similar frequency of NKp46$^+$ NK1.1$^{-/int}$ IL-7Rα^+ cells within the CD3$^-$ CD19$^-$ population in germ-free animals,[40] suggesting that NCR$^+$ ILC3s were unaffected, but did not assess the number of group 3 ILCs. Since germ-free animals are known to harbor fewer innate immune cells,[41] an unaltered frequency may not imply that the number of ILC3s was not decreased in the absence of the microbiota. Another study found that germ-free mice have as high a frequency of CD3ε^- RORγt$^+$ cells as conventionally housed animals,[42] but also did not provide numbers.

While oral administration of doxycyclin to SPF mice from birth did not alter the number or frequency of group 3 ILCs,[42] treatment with a single antibiotic would not deplete all commensal species, potentially preserving microbial signals necessary for the development of group 3 ILCs. Conversely, others have suggested that the microbiota is necessary for the development of group 3 ILCs. One publication demonstrated that germ-free mice have fewer NK1.1^{int} RORγt^{+} cells than conventionally housed animals, both by frequency and number,[43] indicating that NCR^{+} ILC3s require commensals for their development. This was corroborated by another study which found that germ-free B6 mice have ~8-fold fewer CD3ε^{-} NKp46^{+} IL-7Rα^{+} NK1.1^{-} cells than conventionally housed animals.[44] Thus, while there is no consensus whether all group 3 ILC3s require the microbiota, the data suggesting that commensals are necessary for either the development and/or homeostasis of NCR^{+} ILC3s is more convincing. In addition to a potential role for luminal commensals, recent work has demonstrated that commensals which colonize DCs within intestinal lymphoid tissues promote the accumulation of group 3 ILCs in these tissues.[45] Single-cell RNA-sequencing indicated that the microbiota promotes *Il17a* transcription by multiple subsets of group 3 ILCs.[21]

Microbial metabolites and the modulation of metabolite availability by commensals can impact group 3 ILCs. In addition to RORγt, another transcription factor that is necessary for LTi-like cells and NCR^{+} ILC3s is the ligand-activated aryl hydrocarbon receptor (AhR),[40,46] which binds both endogenous and exogenous molecules, including microbial metabolites. Catabolism of dietary tryptophan by commensals yields the AhR ligand indol-3-carbinol (I3C), which induces IL-22 production by the aforementioned group 3 ILC3s.[46–48] Microbial-derived metabolites, including indole derivatives, are transmitted during nursing and administration of IC3 to pregnant mice was sufficient to increase the number of NCR^{+} ILC3s in the progeny,[8] suggesting that the maternal microbiota can promote the development of some group 3 ILCs. Colonic commensal bacteria generate short-chain fatty acids (SCFAs), including butyrate, which can suppress the number of NCR^{+} ILC3s and their IL-22 production.[49] Commensals can also alter metabolism of the vitamin A metabolite retinoic acid, leading to localized deficiency.[50] Insufficient retinoic acid causes a decrease in group 3 ILCs and a corresponding increase in ILC2s,[51] implying that the microbiota may have the potential to alter the balance between group 2 and 3 ILCs in certain contexts.

The microbiota can also indirectly modulate the functions of group 3 ILCs. Commensal antigens detected by intestinal macrophages cause them to release IL-1β, which activates group 3 ILCs.[52] Upon activation, group 3 ILCs produce colony-stimulating factor 2 (CSF2), which triggers DCs to promote intestinal homeostasis through release of the regulatory cytokine IL-10 and the induction of T regulatory cells (Figure 1).[52]

While neither LTi nor ILC3s have not been shown to produce microbe-specific effector molecules, these populations impact the microbiota in numerous ways. All group 3 ILCs produce IL-22, a cytokine that promotes segregation of luminal microbes from the epithelial barrier by coordinating the release of antimicrobial peptides (AMPs) and increasing mucus production. In the absence of an adaptive immune response, depletion of ILCs or neutralization of IL-22 in *Rag1*$^{-/-}$ mice resulted in increased translocation of commensal bacterial to secondary organs, indicating that IL-22 producing ILCs promote containment of the microbiota.[53] The intestinal commensal segmented filamentous bacteria induces the release of IL-23, which promotes IL-22 production by LTi cells and ILC3s.[54,55] In response to IL-22, epithelial cells serum amyloid A (SAA) produce proteins, which induce IL-17 production by type-1 helper T (Th17) cells,[55] thus allowing group 3 ILCs to promote an adaptive immune response (Figure 1). Furthermore, IL-22 from group 3 ILCs prevents a dysbiotic microbiome that is more permissive to pathogenic colonization, as abrogation of IL-22 production from these populations rendered mice more susceptible to *Citrobacter rodentium* infection in a microbiota-dependent manner.[56] IL-22 production by group 3 ILCs also induces intestinal epithelial cells to express fucosyltransferase 2, resulting in fucosylation of proteins and lipids on the luminal side of the epithelium.[57,58] Fucose is catabolized by commensal bacteria for energy, so IL-22 produced by group 3 ILCs contributes to homeostasis of the microbiota and mice lacking the fucosyltransferase are more susceptible to *S. typhimurium* and *C. rodentium* infections.[57,58]

Although group 3 ILCs are predominantly located in the intestinal lamina propria, the protective effects of IL-22 generated by group 3 ILCs extends to other organs. Antibiotic-mediated ablation of the intestinal microbiota in neonatal mice was recently found to decrease their survival upon intratracheal challenge with *Steptococcus pneumoniae* due to a lack of pulmonary IL-22 producing group 3 ILCs.[59] Commensal antigens detected by DCs promoted ILC3s to up-regulate the lung-homing chemokine receptor CCR4, which enabled an influx of primarily IL-22 producing NCR^{+} ILC3s into the lungs shortly after birth.[59]

Recently, CCR6^{+} LTi cells have also been shown to regulate adaptive immune responses to the microbiota (Figure 1). A portion of both murine and human LTi cells, but not NCR^{+} or NCR^{-} ILC3s, expresses MHC-II and is capable of processing and presenting exogenous bacterial antigens.[60] Selective deletion of MHC-II expression on group 3 ILCs (using *H2-Ab1*$^{f/f}$.*Rorc*-cre mice) caused intestinal inflammation in a microbiota-dependent manner.[60] Intestinal inflammation could be induced in *Rag1*$^{-/-}$ mice by transferring CD4^{+} T cells from *H2-Ab1*$^{f/f}$.*Rorc*-cre mice, but not *H2-Ab1*$^{f/f}$.*Rorc*-cre animals treated with antibiotics,[60] suggesting that the LTi cells were regulating the T response in a microbiota-dependent manner. Further work indicated that LTi cells were inducing apoptosis of commensal-specific CD4^{+} T cells through MHC-II-mediated antigen presentation,[61] likely due to the absence of costimulatory molecules on ILCs.

5 | NATURAL KILLER T CELLS

NKT cells are $\alpha\beta$ T cells, which recognize endogenous and microbial lipids presented by the MHC class Ib molecule, CD1d.[13] On the basis of TCR gene usage, these cells are categorized into type I invariant NKT (iNKT) cells, which express Vα14-Jα18 in mice or the corresponding Vα24-Jα18 pair in humans, or type II noninvariant NKT cells .[13] iNKT cells are classified into type-1 invariant NKT (iNKT1), type-2 invariant NKT (iNKT2), and type-17 invariant NKT (iNKT17) subsets based in their production of Th1-, Th2-, and Th17-associated cytokines,

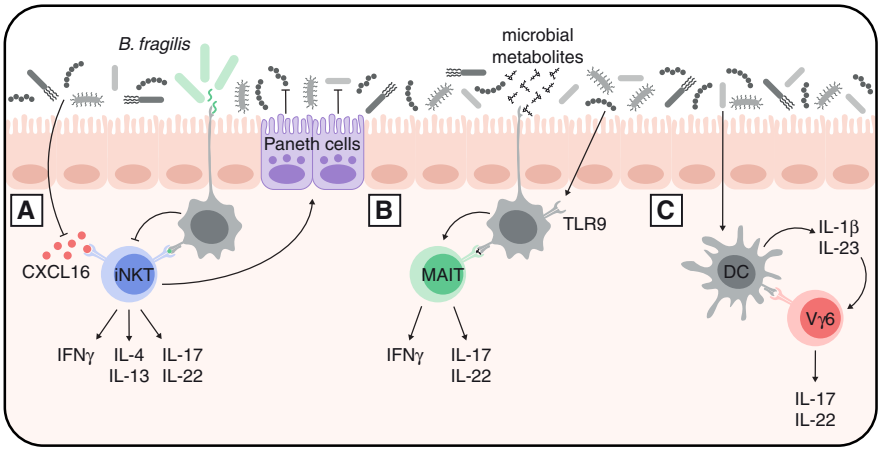

FIGURE 2 **Interactions between innate-like lymphocytes and the microbiota.** (A) Commensals minimize the release of CXCL16, which causes iNKT cells to accumulate in the lamina propria and lungs where they exacerbate colitis and allergic airway responses. Sphingolipids from the commensal *B. fragilis* act as competitive inhibitors that dampen iNKT responses. iNKT cells inhibit microbial colonization though Paneth cells. (B) Microbial metabolites promote the development of MAIT cells and commensal-derived TLR9 ligands up-regulate surface expression of MR1, which increases presentation to MAIT cells. (C) Vγ6$^+$ $\gamma\delta$ T cells respond to the microbiota through cell contact-mediated interactions with CD103$^+$ DCs and the cytokines IL-1β and IL-23

respectively.[13,62] iNKT1 cells have NK receptors and prior to the development of CD1d tetramers, which permits selection of iNKT cells via TCR specificity, where the only subset of iNKT cells that could be identified by expression of an $\alpha\beta$ TCR and NK1.1.[13] iNKT2 cells and a fraction of iNKT1 cells express the CD4 coreceptor, whereas iNKT17 cells express neither CD4 nor CD8.[13] Type II NKT cells have been shown to produce IL-4, IL-13, and IFN-γ.[63]

Multiple studies have reported that iNKT cells develop in the absence of commensals,[64–66] and microbiota does not seem to affect the development of specific iNKT subsets since the frequency of NK1.1$^+$ and CD4$^+$ iNKT cells were comparable between germ-free and SPF animals.[64] In support of these observations, human fetal iNKT cells develop as early as the second trimester, prior to colonization with commensals,[67] though this does not preclude the possibility of maternal transfer of microbial-derived ligands in utero.[8] While iNKT cells can be selected on endogenous ligands in the absence of microbiota, commensals do impact the distribution of iNKT cells. In germ-free mice, iNKT cells accumulate in the colonic lamina propria and lungs due to greater expression of the chemokine CXCL16 by the epithelial cells of these organs (Figure 2), exacerbating colitis and allergic airway responses.[65] While colonization of neonatal germ-free mice prevented CXCL16 release and the subsequent recruitment of iNKT cells, introduction of commensals 5 wk after birth was not sufficient to do so,[65] indicating that exposure to commensals during early life has lasting effects on iNKT cells. Another study found that iNKT cells from germ-free mice were hyporesponsive compared to SPF iNKT, with a lower percentage of cells producing IL-4, IL-13, IFN-γ, and TFN-α in response to TCR stimulation,[66] suggesting that the microbiota promotes iNKT cell function. In response to concanavalin A, fewer leukocytes and DCs express glycolipid/CD1d complexes in germ-free mice compared to SPF animals and this phenotype can be reversed by colonizing the germ-free animals, suggesting that commensals induce iNKT activation.[68] Indeed, there is indication that the intestinal microbiota contains glycolipids that can be recognized by iNKT cells.[68] The intestinal microbiota can also dampen iNKT cell responses. Since some sphingolipids from *Bacteroides fragilis* do not activate iNKT cells, they act as competitive inhibitors of agonists that bind CD1d (Figure 2).[69] Colonization of germ-free neonates with *B. fragilis* minimized the iNKT expansion that typically occurs in the absence of microbes and protected the mice from iNKT-mediated colitis through adulthood.[69] While the microbiota regulates the function of iNKT cells, but not their development, it remains to be determined whether commensals also modulate type II NKT cells. However, type II NKT hybridomas have been shown to recognize lipids derived from *Mycobacterium tuberculosis*, *Corynebacterium glutamicum*, and *Listeria monocytogenes*,[70,71] so the microbiota may influence type II NKT cells.

Intestinal commensal bacteria colonize germ-free *Cd1d*$^{-/-}$ mice more readily than wild-type germ-free animals and activation of NKT cells in SPF mice hinders colonization, suggesting that CD1d-restricted iNKT and/or type II NKT cells regulate the microbiota.[72] Paneth cell granules containing AMPs exhibited morphological defects and degranulation in response to commensal colonization was impaired in germ-free *Cd1d*$^{-/-}$ mice,[72] implying that NKT cells may modulate the microbiota via Paneth cells (Figure 2). Additional work has shown that *Cd1d*$^{-/-}$ mice harbor an altered intestinal microbiome that is proinflammatory in the context of dextran sulfate sodium-induced colitis,[73] suggesting that NKT cells regulate the relative abundance of commensals.

6 | MUCOSAL-ASSOCIATED INVARIANT T CELLS

MAIT cells express semi-invariant T cell receptors (Vα13-Jα33 in mice and Vα7.2-Jα33 in humans) that recognize microbial vitamin B2 (riboflavin) derivatives presented by the MHC class Ib molecule, MR1.[74] MAIT cells are predominantly located at barrier sites and, upon activation, rapidly produce either Th1- or Th17-associated

cytokines.[74] Though present in mice, MAIT cells are more abundant in humans, where they represent approximately 5% of T cells in human peripheral blood, but can reach much higher frequencies in tissues.[75]

Because the riboflavin synthesis pathway is broadly conserved among many species of bacteria and fungi, MAIT cells respond to a wide array of microbes, including known commensals.[76–78] These lymphocytes display heterogeneity in their responses to different microbes,[79] indicating that they may be able to distinguish between pathogens and commensals, perhaps through secondary signals, such as cytokines. Interestingly, MAIT cells are highly dependent on the microbiota, as they are nearly absent from germ-free animals.[80,81] While the number of mature thymic MAIT cells is decreased in germ-free mice, the presence of immature MAIT cells is unaffected, indicating that microbial products are necessary for the development of MAIT cells, but not their selection.[81] Though the presence of functionally mature MAIT cells in second trimester fetuses suggests that that microbial colonization is not necessary for the development of MAIT cells,[82] it is possible that microbial products from the mother's microbiota were transferred in utero,[8] complicating conclusions drawn from this study. Thus, while it remains to be determined whether commensals are required for the developmental acquisition of effector characteristics or the expansion of functionally mature MAIT cells following development, the microbiota is necessary for this population. In addition to providing ligands, they can be recognized by the MAIT cell TCR; the microbiota can also induce surface expression of MR1 through engagement of TLR9 (Figure 2).[83] Though MAIT cells likely promote containment of the microbiota through their production of IL-22, modulation of the microbiota using MAIT cell-deficient animals has yet to be demonstrated.

7 | $\gamma\delta$ T CELLS

Similarly to $\alpha\beta$ T cells, $\gamma\delta$ T cells can be categorized into adaptive and innate-like populations based on the diversity of their TCR repertoire. In mice, the innate-like $\gamma\delta$ T cells include fetal-derived Vγ4$^+$ and Vγ6$^+$ populations (according to the nomenclature of Heilig and Tonegawa[84]) which express IL-17 and a Vγ1$^+$Vδ6.3/4$^+$ subset which produces IL-4 and IFN-γ.[85–87] Though the ligands recognized by these populations have not been identified, other $\gamma\delta$ T cells have been shown to recognize microbial-derived proteins and lipids.[87,88]

While the total number of intraepithelial $\gamma\delta$ T cells is unaffected in the absence of commensals,[89,90] $\gamma\delta$ T cell numbers are significantly decreased in other sites, including the lungs, liver, and oral mucosa.[91–95] This discrepancy is likely due to differences in the composition of the $\gamma\delta$ T cell pool within these tissues, with some subsets more dependent on the microbiota than others. Reports that have assessed the innate-like $\gamma\delta$ T cell populations have found that the Vγ6$^+$ subset requires the microbiota.[93,94] Vγ6$^+$ cells respond to the microbiota through cell contact with CD103$^+$ DCs,[94] though both IL-1 and IL-23 signaling contribute to their activation,[91,93] indicating that cytokines induced during microbial colonization also modulate the Vγ6$^+$ population (Figure 2). $\gamma\delta$ T cells from germ-free mice exhibited decreased lysis of target cells compared to cells isolated from SPF animals,[96] implying that the microbiota can promote $\gamma\delta$ T cell function. While the generation of $\gamma\delta$ T cells can be a benefit of the microbiota in the context of an infection, it can also be deleterious in others. A recent study found that IL-17$^+$ $\gamma\delta$ T cells accumulate in the meninges after a stroke, causing tissue damage, and intestinal dysbiosis minimizes this by reducing the number of $\gamma\delta$ T cells and inducing Tregs.[97] Another demonstrated that antibiotic treatment reduced inflammation in an imiquimod psoriasis model, and this correlated with decreased numbers of IL-22$^+$ Vγ4$^+$ cells.[98] Monocolonization of mice with the intestinal commensal *Escherichia coli* generates hepatic IL-17$^+$ $\gamma\delta$ T cells in a dose-dependent manner,[95] whereas a commensal from the ocular mucosa, *Corynebacterium mastitidis*, induced IL-17-production from conjunctival Vγ4$^+$ T cells,[99] indicating that both the Vγ4$^+$ and Vγ6$^+$ subsets respond to the microbiota. While the impact of commensals on these populations is apparent, how the innate-like subsets of $\gamma\delta$ T cells affect the microbiota has not been established.

8 | OTHER INNATE-LIKE POPULATIONS

$\alpha\beta$ and $\gamma\delta$ T cells within the intestinal epithelium are divided into innate-like "natural" intraepithelial lymphocytes (IELs) and "induced" IELs, which arise from conventional T cells.[100] $\alpha\beta$ IELs are classified based on their coreceptor expression, including the CD8$\alpha\alpha^+$ and CD4$^-$ CD8$^-$ double negative natural IEL populations.[100] Colonization of germ-free rats resulted in an oligoclonal expansion of CD8$\alpha\alpha^+$ IELs, expressing specific TCR Vβ chains.[101] Though the number of $\alpha\beta$ IELs is precipitously decreased in germ-free mice, the frequency of CD8$\alpha\alpha^+$ IELs is increased,[102] suggesting that this population does not require the microbiota for its development. However, CD8$\alpha\alpha^+$ IELs are dependent on AhR signaling for their development and treatment of germ-free mice with an AhR agonist increased the number of CD8$\alpha\alpha^+$ IELs, whereas antibiotic treatment of SPF animals led to a decrease.[103] This implies that the CD8$\alpha\alpha^+$ subset can respond to microbial-derived AhR ligands. The microbiota can also indirectly modulate the CD8$\alpha\alpha^+$ population, as MyD88-deficient mice have fewer CD8$\alpha\alpha^+$ IELs, in part due to a decrease in IL-15 production by intestinal epithelial cells.[104] Although CD4$^+$ CD8$\alpha\alpha^+$ double positive IELs are not considered innate-like lymphocytes, it is of interest that they are induced by an intestinal commensal.[105] Tryptophan derivatives generated by *Lactobacillus reuteri* activate AhR in CD4$^+$ IELs, resulting in the down-regulation of the transcription factor ThPOK, thus enabling CD8$\alpha\alpha$ expression.[105]

B1 and marginal zone (MZ) B cells display characteristics of innate-like lymphocytes, including rapid primary responses and tissue localization, predominately in the peritoneal and pleural cavities or the marginal sinuses of the spleen.[106,107] Numbers of B1 and MZ B cells are unaffected in germ-free mice, suggesting that these cells do not require commensals for their development.[108,109] However, disruption of TLR signaling results in partial deficiencies in B1 and MZ B cells, indicating that the microbiota does modulate innate-like B cell populations to some degree.[108] Additionally, B1 cell-derived IgA is reactive to commensal bacteria, suggesting that these cells may regulate the intestinal microbiome.[35]

TABLE 1 Crosstalk between the microbiota and innate and innate-like lymphocytes

Population	Effector molecules	Predominant location	Effect on the microbiota	Influence of the microbiota
NK cell	IFN-γ, TNF, perforin, granzymes	Spleen, liver	N.D.[a]	Increases cytotoxicity and IFN-γ[16–18]; inhibits long-lived NK cells[20]
ILC1	IFN-γ, TNF, TRAIL	Liver, tonsils	Contribute to intestinal homeostasis by limiting opportunistic pathogens[23]	Promotes *Tbx21* expression in ILC1s [21]
ILC2	IL-4, IL-5, IL-6, IL-9, IL-13, Areg	Lungs, intestines, adipose tissue	Promote microbial containment through IgA[24] and epithelial repair[12]	Indirectly activates ILC2s via IL-25 and TSLP [30,31]; inhibits ILC2s through type-I and II interferons [32]
NCR$^-$ ILC3	IL-17, IL-22, IFN-γ	Intestines	Inhibit microbial translocation through IL-22[53]	Commensal-induced IL-1β and IL-23 stimulate group 3 ILCs[52,54,55];
NCR$^+$ ILC3	IL-22, IFN-γ	Intestines	See NCR$^-$ ILC3	Promotes NCR$^+$ ILC3 development[43,44]; induces IL-22 production through the generation of AhR ligands[46–48]; inhibits NCR$^+$ ILC3s via SCFAs[49]
LTi cell	IL-17, IL-22, LTα, LTβ	Intestines	Induce apoptosis of commensal-specific CD4 T cells[60,61]; promote epithelial fucosylation via IL-22 and LTα[57,58]	See NCR$^-$ ILC3
iNKT1 cell	IFN-γ, (IL-4)[b]	Liver, spleen	Regulate commensal colonization[72,73]; modulate Paneth cell function[72]	Promotes cytokine production [66], though commensal-derived sphingolipids can dampen iNKT responses [69]; prevents accumulation of iNKT cells in intestines and lungs [65]
iNKT2 cell	IL-4, IL-9, IL-13	Lungs, intestines	See iNKT1 cell	See iNKT1 cell
iNKT17 cell	IL-17, IL-22	Lymph nodes, lungs, skin	See iNKT1 cell	See iNKT1 cell
Type II NKT cell	IL-4, IL-13, IFN-γ	Liver, intestines	N.D.	Activated by microbial antigens[70,71]
MAIT cell	IL-17, IL-22 or IFN-γ, TNF, perforin, granzymes	Lungs, liver, intestines	N.D.	Necessary for the development of MAIT cells[80,81]
CD8$\alpha\alpha^+$ T cell	IL-10, TGFβ, IFN-γ	Intestines	N.D.	Induced by commensal-derived AhR ligands[103]
Vγ4$^+$ $\gamma\delta$ T cell	IL-17, IL-22	Lungs, liver, peritoneal cavity, dermis, uterus	Promote release of AMPs[99]	Increases Vγ4$^+$ $\gamma\delta$ T cell numbers and IL-17 production[95,99]
Vγ6$^+$ $\gamma\delta$ T cell	IL-17, IL-22	Lungs, liver, peritoneal cavity, dermis, uterus	N.D.	Increases Vγ6$^+$ $\gamma\delta$ T cell numbers[94] and stimulates IL-17 production via IL-1β and IL-23[91,93]
Vγ1$^+$Vδ6.3/4$^+$	IL-4, IFN-γ	Liver, spleen	N.D.	N.D.
B1 B cell	IL-10, IgM, (IgA)[c]	Peritoneal and pleural cavities	Generate commensal-reactive IgA[35]	Unaffected in germ-free mice [109]
MZ B cell	IL-10, IgM, (IgG), (IgA)[d]	Spleen	N.D.	Unaffected in germ-free mice[108,109]

[a]Not determined.
[b]iNKT1 cells can produce both IFNγ and, to a lesser extent, IL-4.[13]
[c]While typically associated with IgM responses, B1 B cells can generate commensal-specific IgA.[35]
[d]In addition to IgM, MZ B cells can also produce IgG and IgA at steady-state and in response to microbial antigens.[118]

A novel subset of innate-like $\alpha\beta$ T cells that constitutively express IL-4 was described in mice lacking the IL-2-inducible T cell kinase (ITK).[110] While this population developed independently of MHC-I, MHC-II, CD1d, and MR1 molecules, it was dependent on the microbiota, as demonstrated by antibiotic treatment.[110]

9 | CONCLUDING REMARKS

Since the microbiome has been shown to modulate the functions of nearly every innate and innate-like lymphocyte population either directly or indirectly (Table 1), it seems plausible that these cells may be regulated by a common transcriptional program. During their development, iNKT cells, MAIT cells, Vγ1$^+$Vδ6.3/4$^+$ $\gamma\delta$ T cells, ILC1s, ILC2s, and ILC3s, express the transcription factor PLZF (encoded by the gene *Zbtb16*), which imbues these populations with effector characteristics, including their production of effector cytokines, responsiveness to proinflammatory signals, and migration to, and retention within, barrier tissues via chemokines and integrins.[81,111–117] Therefore, PLZF enables many innate and innate-like lymphocyte populations to both respond to commensals and regulate the composition and/or quantity of the microbiota.

While commensal bacteria influence innate and innate-like lymphocytes, the contributions of commensal fungi and viruses on these

populations have hardly been explored. Likewise, how these components of the microbiome are regulated by innate and innate-like lymphocytes remains unknown. Owing to their site-specific colonization and persistence, commensals have therapeutic potential as tissue-directed adjuvants or for localized calibration of the immunologic tone.

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DISCLOSURES

The author has no conflicts of interest to disclose.

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