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Recent advances in gut immunology

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Summary

In recent years, there have been significant advances in our understanding of the mucosal immune system. In addition to unravelling some of the complexities of this system, including the discovery of completely new cells types, further insights into the three-way interactions between mucosal immune cells, the intestinal epithelium and the microbial communities colonizing the GI tract promise to redefine our understanding of how intestinal homeostasis is maintained, but also how dysregulation of these highly integrated interactions conspires to cause disease. In this review, we will discuss major recent advances in the role of key immune players in the gut, including innate lymphoid cells (ILCs), mucosa-associated invariant T cells (MAIT cells) and cells of the mononuclear phagocyte system (MPS), including how these cells interact with the intestinal epithelial and their crosstalk with components of the intestinal microbiota, and how these interactions shape host health.

KEYWORDS

adaptive immunity, Innate immunity, Mucosal immunity

1 | INTRODUCTION

The last 10 years have seen a transformation in the understanding of the mucosal immune system in health and disease. Technological advances in high-throughput sequencing and informatics have allowed the characterization of the microbial communities in the gut and other sites. This has been achieved in a number of ways: firstly, by pyrosequencing 16SrRNA using region-specific primers or secondly using random DNA sequencing to assemble the aggregate of genes in the microbiome—the metagenome. Thirdly, there has been a large increase in the number of gnotobiotic facilities, where mice can be maintained germ-free or with defined microbial communities. This has allowed not only the characterization of how the immune system shapes the microbiota but also how the microbiota shapes the immune system. It is widely and somewhat incorrectly stated that bacterial cells outnumber human cells in our bodies by 10:1 but recent analyses have suggested that the number of bacterial cells and mammalian cells is approximately the same at about 3-4x10^{13} of each.[1] Nonetheless very many studies have now identified clear examples of crosstalk, so the function is more important than the numbers.

Another great advance has been the advent of lineage tracing of immune cells where when a cell commits to a lineage, it becomes permanently marked with a green, yellow or red fluorophore. An excellent example of the power of this technology is the demonstration by fate mapping that CD4 T cells in the gut lamina propria making IL-17 become ex-Th17 cells and become Tregs.[2]

In this review, we would like to give an update on some aspects of mucosal immunology which have developed in the last 10 years.

2 | INNATE LYMPHOID CELLS (ILCS)

2.1 | Innate lymphoid cells—new players at the barrier surfaces

Innate lymphoid cells are a family of mucosal-dwelling innate lymphocytes that are enriched at barrier surfaces. They lack recombined antigen-specific receptors, such as those found on T cells and B cells. Instead these cells respond to other environmental cues in their immediate vicinity, most notably cytokines. Similar to the situation with effector $CD4^+$ T-cell lineages, with which they share important similarities, ILCs are subdivided into different subsets based on the expression (and developmental dependence) of specific transcription factors and the profile of effector cytokines that they produce, which in turn dictates the functional specialization of the different

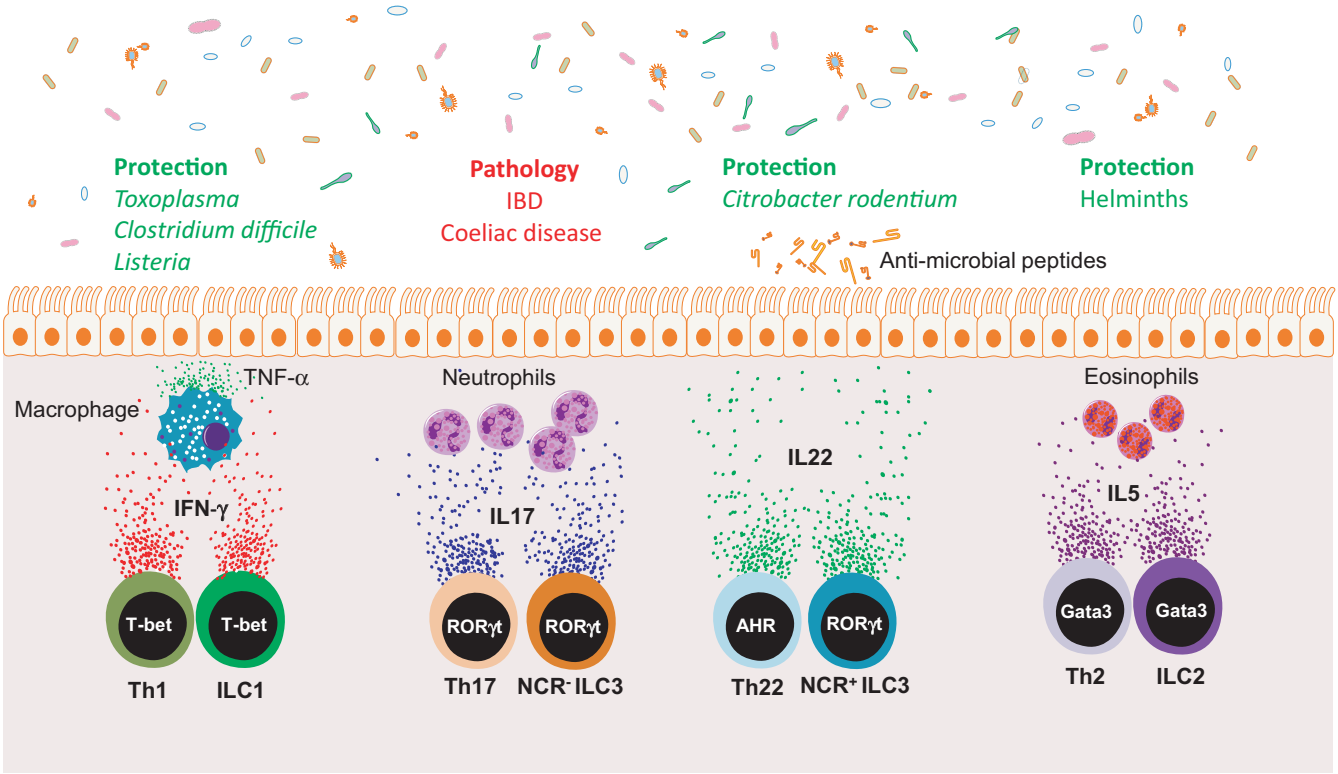

FIGURE 1 Similarities between T-helper cell lineages and innate lymphoid cells (ILC) subsets. ILC1 and Th1 cells make interferon-gamma and are important for immunity to *Listeria monocytogenes*, *Clostridium difficile and Toxoplasma gondii*. Th17 cells and natural cytotoxicity receptor (NCR)-negative ILC3 make IL-17A which attracts neutrophils into the gut and may be important in coeliac disease and IBD. Th22 cells and NCR+ ILC3 make IL-22 which maintains the barrier and protects in the early stages of *Citrobacter rodentium* infection in the colon. Th2 cells and ILC2 make IL-5 which can attract eosinophils into tissues to provide immunity to helminths

lineages and the specificity of their response (Figure 1). ILC1 resemble Th1 cells. They express the transcription factor T-bet and secrete cytokines, such as γ-interferon and TNF-α, and contribute to host defence against intestinal pathogens, including *Toxoplasma gondii*, *Clostridium difficile* and *Listeria monocytogenes*.[3-5] Like Th2 cells, ILC2s express GATA3 and secrete IL-4, IL-5, IL-9 and IL-13, and they are involved in antihelminth immunity.[6] ILC2 are additionally subdivided into "natural" and "inflammatory" subsets (nILC2 and iILC2).[7] In the steady state IL-33 responsive ($ST2^+$) nILC2 predominate. However, in vivo administration of IL-25 results in expansion of $IL\text{-}17RB^+$ iILC2 that do not express ST2 and are unresponsive to IL-33 stimulation.[7]

ILC3 express the transcription factor RORγt, but are further subdivided depending on whether they express natural cytotoxicity receptors (NCRs), such as NKp46 (mouse) or NKp44 (human). NCR^+ ILC3 are involved in protection from acute bacterial infections (eg, *Citrobacter rodentium*) or acute injury to the intestinal epithelium (e.g., short-lived mucositis following transient exposure to chemotherapy).[8-11] They resemble Th22 cells, in that they only produce IL-22, but not IL-17A; however, they express both RORγt and the arylhydrocarbon receptor (AHR), whereas Th22 cells only express AHR. NCR^- ILC3 are the innate counterparts of Th17 cells. These cells both coproduce IL-17 and IL-22 and express the transcription factor RORγt. In additional to their important roles in host defence, ILCs have also been implicated as mediators of immune-mediated diseases in the gut, and most notably IBD. Conventional NK cells with cytotoxic antitumour and antiviral properties can be classified as ILC1 and may be considered the innate counterpart to cytotoxic $CD8^+$ T cells.

2.2 | Innate lymphoid cell differentiation

A detailed review of ILC development and differentiation has been recently published.[12] In short, these innate lymphocytes develop from haematopoietic stem cells (HSCs) via increasingly well-defined intermediaries, under the direction of specific transcription factors, which serve as molecular switches that control cell fate decisions. In mice, ILCs develop from the common lymphoid progenitor (at which stage cells retain the potential to differentiate into T and B cells) into a common "helper" innate lymphoid progenitor (ChILP) that is committed to seed all ILC lineages, but has lost capacity to differentiate into adaptive lymphocytes or NK cells. Early ILC development is dependent on NFIL3, TOX and ID2. Later stages of development are dependent on the lineage defining transcription factors, although GATA3 is required for the development of all ILC lineages.[13] In humans, the developmental pathway is less well defined, although it is recognized that ILCs can be differentiated in vitro from $CD34^+$ HSCs.[14]

2.3 | ILC plasticity

An increasingly important concept is the notion of plasticity, where apparently terminally differentiated lymphocyte lineages transdifferentiate into other lineages, with an attendant shift in function. Perhaps the best example of this phenomenon is the differentiation of "established" Th17 $CD4^+$ T cells into Th1 cells.[15,16] Plasticity is directed by microenvironmental conditions, and most notably through changes in the local cytokine milieu. ILC2 transdifferentiate to ILC1-like cells (termed "ex-ILC2" ILC1 cells) under the influence of IL-1β, IL-12 and IL-18.[17] IL-1β triggers production of classical ILC2 cytokines, such as IL-5 and IL-13 by increasing the expression of IL-25R and IL-33.[18] However, IL-1β also induces expression of IL-12Rβ2 chain and the ILC1 transcription factor T-bet in ILC2, thus priming ILC2 to be responsive to local IL-12 production and transition to an ILC1 phenotype.[19] ILC2 from patients unable to respond to IL-12 by virtue of IL-12Rβ1 deficiency are unable to transdifferentiate to ILC1.[20] T-bet is recognized to directly transactivate numerous ILC1/Th1 genes, including *IFNG* and components of the IL-18 receptor (eg, *IL-18RAP*)[21] and as might be expected, ILC2 conversion to "ex-ILC2." ILC1 cells can also be supported by IL-18.[17] In mucosal diseases, such as chronic obstructive pulmonary disease (COPD) where IL-1β, IL-12 and IL-18 are abundantly expressed, there is marked contraction of tissue ILC2 and a reciprocal expansion of ILC1 cells, consistent with the possibility that this ILC lineage transition may be in operation in human disease.[22] Indeed, the degree of ILC1 expansion in the lung of COPD patients correlates with disease severity and risk of disease exacerbation.[22] There is also plasticity between the ILC2 subsets. In short-term in vitro culture or following adoptive transfer into mice lacking endogenous ILCs (*Rag2*$^{-/-}$ *Il2rg*$^{-/-}$), iILC2 transition into nILC2 cells with acquisition of IL-33 receptor expression and loss of IL-25 receptor expression.[7] Under Th17 permissive conditions (IL-6 and TGF-β exposure), iILC2 also switch on IL-17A production, which is mostly co-expressed with IL-13.[7]

Bidirectional plasticity between ILC1 and ILC3 is also recognized and is once again directed by cytokines. In the gut, IL-12 produced by $CD14^+$ mononuclear phagocytes guides the transdifferentiation of ILC3 into to ILC1.[8] Conversely, $CD14^-$ mononuclear phagocytes direct IL-23- and IL-1β-dependent transition of ILC1 into IL-22-producing ILC3, which is additionally augmented by retinoic acid.[8]

Plasticity of cytokine production profiles has also been described in human ILC3 following ligation of the natural cytotoxicity receptor NKp44. Stimulation of NKp44 results in diminished IL-22 production and functional switching to TNF-α production,[9] which might also be construed as transitioning from an immunoregulatory phenotype to an inflammatory phenotype. It is likely that evolutionary pressures have favoured the development of ILC plasticity to enable rapid adaptation to changing environmental conditions, including induction of different host immune responses at different times. Indeed, rapid deployment of antihelminthic responses is likely to be of benefit following exposure; however, there are obvious benefits for the host if ILC2 can switch to an γ-interferon producing phenotype to combat bacterial and viral infections.

2.4 | Crosstalk between ILCs and T cells regulates mucosal homeostasis

Dialogue between ILCs and T cells plays an important role in the initiation and evolution of mucosal immune responses, which simultaneously impacts both ILCs and T cells. There are convincing data showing that both ILC2 and NCR^- ILC3 serve as antigen-presenting cells (APCs). In the steady state NCR^- ILC3 express low levels of MHC II and negligible expression of key costimulatory molecules required to drive T-cell activation. However, upon activation (IL-1β), NCR^- ILC3 significantly upregulate MHC II expression and costimulatory molecules, including CD80, CD86 and CD40.[23] They can also uptake antigen and present antigenic peptides to drive potent activation of T cells.[23] However, induction of MHC II and costimulatory molecules in ILC3 is regulated in a tissue-specific manner. While splenic ILC3 can upregulate MHC II and costimulatory molecules after exposure to inflammatory stimuli, ILC3 from the small intestine fail to upregulate costimulatory molecules.[24] Indeed, intestinal ILC3 are unable to trigger antigen-specific $CD4^+$ T-cell proliferative or cytokine responses. Selective genetic deletion of MHC II in ILC3 (RORγt-cre MHC $II^{fl/fl}$ mice) results in exaggerated T-cell activation and spontaneous intestinal inflammation,[24] consistent with the possibility that MHC II expressing ILC3 are physiologically relevant regulators of T-cell activation, presumably through induction of anergy as a consequence of antigen being presented in the absence of appropriate costimulation.

ILC2 can also prime antigen-specific naïve T-cell responses, including induction of proliferation and cytokine production.[25] Although ILC2 are less effective at driving T-cell activation than professional APCs, such as DC, they have comparable efficiency to B cells.[25] In addition to their role as direct primers of antigen-specific $CD4^+$ T cells, ILC2 also indirectly impact memory $CD4^+$ T-cell recruitment to the tissues through crosstalk with DCs. In models of allergen-driven memory Th2 cell-dependent eosinophilic lung inflammation, ILC2 accumulate in the tissue at early time points prior to the arrival of memory Th2 T cells.[26] Selective depletion of ILC2 in this context results in impaired recruitment of memory Th2 cells resulting from loss of early IL-13 by tissue ILC2 which is responsible for triggering upregulation of the chemokine CCL17 by $IRF4^+$ $CD11b^+$ $CD103^-$ mononuclear phagocytes, which attracts CCR4 expressing Th2 memory T cells.[26] The cognate interaction between T cells and ILCs reciprocally activates ILCs, through T-cell production of the key ILC2 growth factor, IL-2.

2.5 | Innate lymphoid cells regulate barrier protection

ILCs are important early responders to pathogen invasion, and experiments in animal models indicate that ILCs play a key functional role in host defence. Different ILC subsets have been shown to provide protective immunity from a broad range of pathogens. ILC1 are involved in host resistance to *Clostridium difficile*, *Listeria monocytogenes* and *Toxoplasma gondii*.[3-5] ILC3 contribute to host defence against bacterial pathogens, such as *Citrobacter rodentium*.[7,8] The mechanism of protection is multifactorial, but at least in part depends on IL-22, which

is responsible for inducing antimicrobial peptides, such as Reg family peptides, lipocalin-2 and β-defensins, by intestinal epithelial cells, and especially by Paneth cells in the small intestine.[9] IL-22 also directly shapes glycosylation patterns of intestinal epithelial cells by regulating the expression of the enzyme fucosyltransferase 2.[27,28] Perturbation of IL-22-dependent epithelial fucosylation disrupts the composition of the bacterial communities colonizing the mucus layer overlying the epithelium. Subtle alteration of this environmental niche discourages colonization with mutualistic bacteria and instead favours growth of potentially pathogenic species, which render the host more susceptible to inflammation and infection.[27,28] In addition to its antimicrobial properties, IL-22 produced by ILC3 plays an important role in tissue regeneration and restoration of a healthy, functional epithelial barrier following infection or acute tissue injury. These trophic effects of IL-22 are centred on Lgr5$^+$ intestinal stem cells, which express the highest levels of IL-22R. IL-22 drives epithelial stem cell proliferation,[29] and overall this pathway is crucial to host defence and tissue recovery following infection or injury.

2.6 | ILCs and inflammatory bowel disease

In contrast to their role in host immunity and maintenance of intestinal homeostasis, the inflammatory functions of ILCs can also be inappropriately mobilized to orchestrate tissue injury in chronic intestinal inflammation. ILCs play an indispensable role in some preclinical models of IBD and are expanded in inflammatory lesions in patients with both Crohn's disease and ulcerative colitis.[30-36] It is important to recognize that pathological roles for ILCs have mostly been described in mice lacking T cells and B cells. Although this approach allows selective scrutiny of ILCs without confounding influence from effector functions mediated by adaptive lymphocytes, it could also be argued that this artificial system lacks physiological relevance, since in mice replete with adaptive lymphocytes T cells far outnumber ILCs. Nevertheless, in multiple different experimental models of colitis performed in mice with genetic disruption of recombinase activating genes (*Rag1*$^{-/-}$ or *Rag2*$^{-/-}$), ILCs play nonredundant pathogenic roles. The *Helicobacter hepaticus*-induced model of chronic intestinal inflammation is dependent on IL-17A and γ-interferon producing ILCs.[27] TRUC mice (*Tbx21*$^{-/-}$ *Rag2*$^{-/-}$ Ulcerative Colitis) mice develop a progressive, microbiota-dependent distal colitis which is mediated by NCR$^-$ ILC3. These cells produce IL-17A and IL-22 which is triggered by and is dependent on IL-23, IL-1β and IL-6.[30,31] The importance of IL-23 driven intestinal inflammation is of central importance in IBD. Polymorphisms at loci encoding multiple components of the IL-23 pathway alter IBD susceptibility, including one of its subunits (*IL-12A*, which encodes IL-12p40, a shared common subunit for both IL-12 and IL-23), the *IL-23R* and signalling components, such as *STAT3*, *JAK2* (and to a lesser extent *STAT4* and *TYK2*).[37] Following successful phase III clinical trials, antibodies targeting IL-12p40 have been approved for Crohn's disease.[38] Crucially, studies using IL-23R-GFP reporter mice show that ILCs are the main population of immune cells expressing IL-23R in the gut,[39] significantly outnumbering CD4$^+$ T cells and γδ T cells, which emphasizes the potential importance of intestinal ILCs in IL-23 driven inflammation.

2.7 | The role of ILCs in antihelminth immunity

In the very first report of ILC2, by Andrew McKenzie's group, it was shown that ILC2 (initially termed nuocytes) were a major early source of IL-13 in *Nippostrongylus brasiliensis* infection.[6] In the absence of IL-25 or IL-33, ILC2 failed to expand, resulting in delayed worm expulsion. Importantly, restoration of worm expulsion kinetics occurred following adoptive transfer of WT ILC2, but not *Il13*$^{-/-}$ ILC2.[40] Since then models of parasitic worm infection have become the staple experimental tools for probing ILC2 function, and there is much ongoing work investigating the role of these cells in host resistance.[41]

A key proximal activating signal for ILC2 is intestinal epithelial-derived IL-25 and epithelial-derived IL-33. Recently, it has been shown that a rare population of small intestinal epithelial cells called tuft cells (<1% of small intestinal epithelial cells) are the principle cellular source of IL-25 following infection with different parasitic worms, including *Nippostrongylus brasiliensis*, *Heligmosomoides polygyrus* and *Trichinella spiralis*.[42-44] By day seven post-infection, tuft cells undergo rapid hyperplasia throughout the small intestine (15-fold increase), seeded by Lgr5$^+$ stem cells. Interestingly, a positive feedback circuit between tuft cells and IL-13-producing ILC2 plays a central role in worm expulsion. IL-25 produced by tuft cells drives activation of ILC2, including induction of IL-13 production, which in turn supports tuft cell expansion, which is dependent on IL-13 and epithelial STAT6.[42-44] Although tuft cells are the primary source of IL-25 in the gut (and trachea and gall bladder), other non-tuft cell epithelial cells are the primary source of other ILC2 activating cytokines, including IL-33 and TSLP.[44] Although one interpretation of these data is that local interactions between ILC2 and the intestinal epithelium may be sufficient to initiate helminth expulsion without the need for priming in the draining mesenteric lymph nodes, there is mounting evidence supporting a role for ILC2 in driving T-cell activation in *N. brasiliensis* infection. Selective depletion of ILC2 results in significant impairment of Th2 differentiation, reduced production of T-cell-derived IL-5 and IL-13, and delayed expulsion of *N. brasiliensis*.[25] Crucially, adoptive transfer of WT ILC2 can restore Th2 activation and worm expulsion; however, this phenotype cannot be rescued in ILC2 lacking MHCII, highlighting the importance of ILC2/T-cell interactions in host immunity to worms.

Important outstanding questions include the relative contributions of ILC2s and Th2 CD4$^+$ T cells in host immunity to different helminths at different phases of disease, as well as the overall interpretation of results in some experimental systems.[41] It is well established that different mouse strains vary in their capacity to mount different arms of host immunity, including type 2 responses,[45] which in turn impacts on strain susceptibility to worm infection. Indeed, it could be argued that investigating host immunity, including ILC2 responses, in inbred strains with comparatively weak (if not overtly defective) Th2 responses, might artificially inflate contributions made by other cell types, including ILC2. Consequently, caution should be exercised in extrapolating experimental data reported in contrived model systems selected because they exhibit heightened disease susceptibility. Most studies investigating host immunity to *N. brasiliensis* are reported in C57/B6 mice that mount poor Th2 responses. Consequently, the

important early role attributed to ILC2 activity in this infection may merely represent an appropriate compensatory ILC2 response when Th2 responses are suboptimally generated.

3 | THE INTESTINAL MICROBIOTA

Culture-independent technologies, including next generation sequencing, have revolutionized our understanding of the microbial colonies populating the gastrointestinal tract and the functional contribution of these communities to host health. The metagenome (genes carried by our colonizing microbes) profoundly impacts wide-ranging aspects of the host phenotype, including metabolic, endocrine, neurological and immunological factors. Remarkably, cumulatively as a species, humans possess an additional 10 million genes contributed by intestinal bacteria.[46] Intestinal microbial communities are diverse and although there are hundreds of different bacterial species colonizing the human gut, the community structure differs across human populations according to age, diet, geographic distribution and host genetics.[46] Crucially, significant perturbation of the community structure of intestinal bacteria, or dysbiosis, is linked to important alterations in host immunity and susceptibility to immune-mediated diseases both within and beyond the gut.

3.1 | The intestinal microbiota profoundly influence mucosal and systemic T-cell responses

In recent years, important advances have been made in our understanding about how specific components of the intestinal microbiota directly shape specific aspects of host immune responses (Figure 2). Unless mice are colonized with an unculturable Clostridia-like bacterium called segmented filamentous bacteria (SFB), they are unable to mount effective Th17 responses.[47] SFB colonize the small intestine and are found in highest numbers in the terminal ileum, a key inductive site for T cells, and to a lesser extent the proximal small intestine and colon.[47] As well as impacting on local intestinal Th17 responses, intestinal colonization with SFB is also required to generate Th17 responses at sites remote from the gut, which in turn impacts on the susceptibility of the host to systemic inflammation. Germ-free mice have significantly impaired mucosal and systemic Th17 responses, fail to generate effective antibody responses and are protected from extra-intestinal autoimmune diseases, including experimental models of rheumatoid arthritis and multiple sclerosis.[48,49] However, monoassociation with SFB is sufficient to reinstate systemic Th17 responses and restore susceptibility to disease induction.[48,49] SFB instructed Th17 induction depend on close contact between SFB and intestinal epithelial cells, where they can observe penetrating epithelial cell membranes with their long filaments. Indeed, other bacterial species that can form intimate interactions with intestinal epithelial cells, including *E.coli O157* and *Citrobacter rodentium* also induce Th17 responses.[50] Indeed, monoassociation of mutant forms of *C. rodentium* or *E. coli O157* that are genetically deficient for intimin, a protein required for epithelial adhesion, lose the capacity to tightly adhere to ileal epithelial cells in vivo and fail to trigger Th17 responses.[50]

The molecular determinants of SFB-induced Th17 responses are just starting to be understood. SFB, and other Th17-inducing bacteria, strongly induce the expression of serum amyloid A protein 1 and 2 (SAA1/2) by ileal epithelial cells.[51] Naïve T-cell differentiation towards the Th17 lineage is significantly enhanced in the presence of SAA1 in vitro[51] and there is significant, albeit only partial, impairment of in vivo Th17 differentiation in $Saa1^{-/-}$ $Saa2^{-/-}$ double knockout mice.[51] SFB-induced expression of SAA1 and SAA2 is dependent on IL-22 production by group 3 ILCs and STAT3 signalling in the intestinal epithelium.[51]

Segmented filamentous bacteria do not colonize the human intestine; however, it is possible that bacteria with similar characteristics, such as the capacity to tightly adhere to the intestinal epithelium, may be permissive for Th17 responses in man.

3.2 | The intestinal microbiota control regulatory T-cell (Treg) development

Selective bacteria can also promote the differentiation of Tregs. Inoculation of a consortium of seventeen Clostridia strains isolated from healthy human colon into germ-free mice triggers mucosal production of TGF-β, which results in expansion of IL-10-producing Tregs.[52] Adoptive transfer of this Clostridia consortium attenuates disease in models of colitis. These clostridia clusters are prominent producers of short-chain fatty acids (SCFAs), such as butyrate, which can support the differentiation of Tregs in vitro and in vivo. Another single species of bacteria, called *Bacteroides fragilis*, also supports Treg differentiation, a property that can be localized to a single molecule expressed by this bacterium called polysaccharide A (PsA).[53] Unlike other polysaccharides, PsA is processed by dendritic cells and presented to T cells. *B. fragilis* ameliorates models of colitis, although this immunomodulatory action is lost when *B. fragilis* lacks PsA. Furthermore, administration of purified PsA induces IL-10-producing Tregs in vivo, which inhibits pathogenic Th1 and Th17 responses and limits disease in experimental models of IBD.[54] Mechanistic insights suggest that PsA is packaged in to outer membrane vesicles (OMVs) extruded from the surface of B. *fragilis* to communicate with immune cells and favour Treg generation.[55] Specifically, PsA-containing OMVs secreted by *B. fragilis* are sensed by intestinal dendritic cells by TLR2, which subsequently induce IL-10-producing Tregs. This immunomodulatory property of DCs is dependent on intact autophagy, and selective genetic disruption of the key autophagy gene Atg16l1 in dendritic cells (*CD11c cre* $Atg16l1^{fl/fl}$ mice) results in impaired Treg generation and unhampered expansion of Th1 and Th17 cells, and more severe experimental colitis.[55] Single nucleotide polymorphisms at the *ATG16L1* locus are associated with increased susceptibility to Crohn's disease, and crucially dendritic cells from patients harbouring homozygous risk alleles at the *ATG16L1* locus (A300) exhibit defective promotion of Tregs when pulsed with OMVs or PsA.[56]

3.3 | The intestinal microbiota in helminth infection

As well as directly impacting host immunity, worm infections alter the composition of the bacterial communities colonizing the

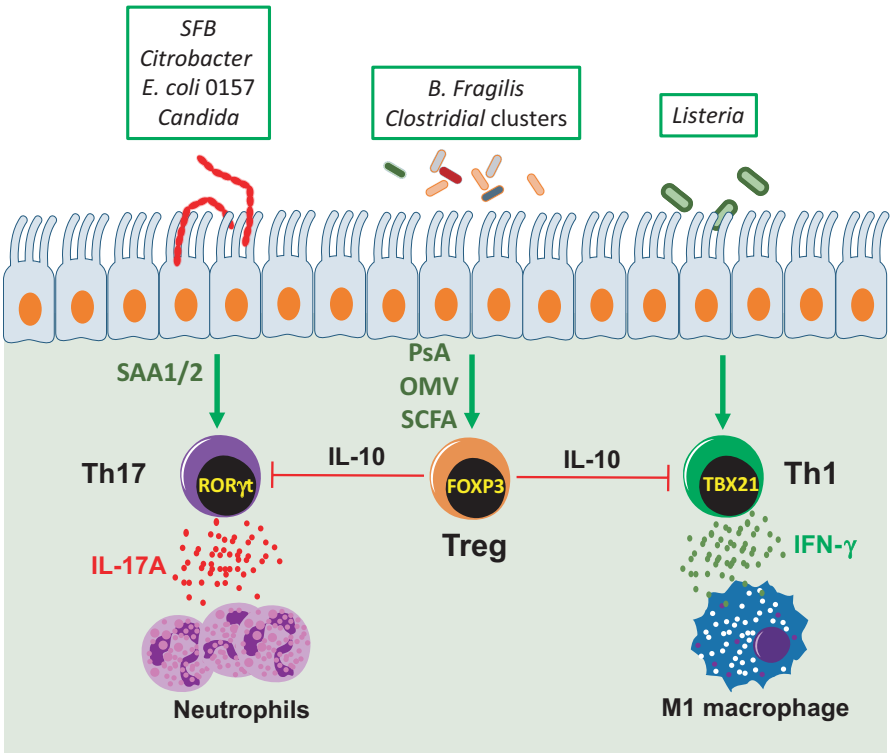

FIGURE 2 Gut bacteria can control T-cell responses. The segmented filamentous bacterium in mouse gut, as well as other bacteria can drive Th17 cell development, perhaps by driving epithelial production of serum amyloid A. *Bacteroides fragilis* polysaccharide-specific antigen (PsA), outer membrane vesicles of *B. fragilis*, Clostridia and short-chain fatty acids (SCFA) have been shown to induce Tregs in the gut. *Listeria monocytogenes* provokes strong Th1 responses

mammalian gastrointestinal tract, which in turn modulates host immune responses. Several studies have shown, often striking, shifts in the community structure of intestinal bacteria following helminth infection in different mammals including mice, rats, pigs and humans.[57-62] These changes often occur in a dose-dependent manner (i.e., the greater the worm burden the more pronounced the changes) and are observed in naturally infected animals and humans, and not just in experimental models.[62,63] Mostly worm-induced alterations in the intestinal microbial community structure lack specificity, a few studies have shown an association with increased bacterial diversity and expansion of some bacterial families, such as *Lactobacillaceae* and *Enterobacteriaceae*. It should also be noted that the majority of these studies are correlative and the relevance and direction of the association uncertain. Diarrhoea by itself, even in the absence of pathology, can induce profound changes in the intestinal microbiota. Indeed, administration of osmotic laxatives to healthy humans induces diarrhoea and striking alterations the intestinal bacterial community profile.[64] Accordingly, it could be argued that physiological changes induced by worm infection, such as increased mucus production and intestinal smooth muscle hyper-contractibility (the so called weep and sweep response) could indirectly induce changes to the intestinal microbiota by altering transit time and other physiological parameters that are key to supporting this unique environmental niche. Notably, helminth-associated disruption of bacterial community structures reverts back to normal following worm expulsion.[62] Downstream modulation of host immune responses, and indeed other aspects of host phenotype, including metabolism, as a consequence of helminth-induced intestinal bacterial community shifts has recently been comprehensively reviewed.[65]

4 | INTESTINAL MACROPHAGES

4.1 | Origin of tissue macrophages

For many years, it was considered that tissue macrophages such as Kupffer cells in the liver, microglia in the brain, and Langerhans cells in the skin were tissue-resident cells constantly being replaced by monocytes from the blood, themselves derived from hematopoietic stem cells in the bone marrow. However, in 2010 using bone-marrow chimeras, parabiotic mice, fate mapping and macrophage colony-stimulating factor 1 (CSF-1) and CSF-R null mice it was discovered that microglia in the brain, but not lung macrophages, were not bone-marrow derived, but were derived from the foetal yolk sac at 9.5 days post-conception.[66] This was confirmed - in 2012 when it was shown that Langerhans cells in the skin and Kupffer cells in the liver were also derived from the yolk sac and not hematopoietic stem cells in the marrow. In tissues, these yolk sac-derived macrophages are self-renewing.[67] However, when many of the same methodologies used by Ginhoux et al. were used to study intestinal macrophages, it was discovered that although macrophages from the yolk sac entered the foetal gut, they did not persist into adulthood.[68] In fact after weaning, virtually all macrophages in the mouse gut are derived from hematopoietic stem cells. Their presence also depends on the microbiota.[68]

4.2 | Novel pathways for antigen sampling by macrophages in the gut

It had also traditionally been considered that the way in which the immune system sampled luminal antigens was via the phagocytic

activity of M cells on the domes of Peyer's patches and isolated lymphoid follicles in the small intestine and colon. However, using CX3CR1-GFP reporter mice (the fractalkine receptor), it was shown in the mouse ileum that CX3CR1-GFP+ dendritic cells send processes between epithelial cells to sample bacteria directly from the gut lumen and then withdraw the dendrites back to the cell body in the lamina propria.[69] Further studies have shown that the cells sending dendrites through the epithelium are not dendritic cells, but macrophages, because they are highly phagocytic and express F4/80, CD64 and CD11b.[70] Other studies showed that a subset of dendritic cells expressing lysozyme in the subepithelial dome of Peyer's patches sent dendrites through pores in M cells to sample gut antigens and then retract back into the tissue.[71] It is clear therefore that there is an active dialogue between the microbiota, dendritic cells and macrophages across the gut epithelium. It is tempting to speculate that this crosstalk is important in maintaining homeostasis in the healthy gut which is densely infiltrated with immune cells but not inflamed.

4.3 | Function of intestinal macrophages

In contrast to an extensive literature on fate mapping and phenotype of intestinal macrophages, functional studies on gut macrophages are still relatively few. It is important also to appreciate that there may be major differences between the small bowel and the colon and between species. In the small bowel, the macrophages are in the cores of the villi but in the colon, they form a layer immediately below the epithelium. A final issue is that it is rather difficult to isolate live macrophages from the gut for functional studies because of their low numbers.

Nonetheless, small intestinal macrophages from healthy human gut are highly unusual cells. They mostly lack innate receptors such as CD14 and therefore do not respond to lipopolysaccharide. In fact they are profoundly anergic to stimuli that evoke rapid cytokine production in monocytes. In contrast, they are highly phagocytic and bacteriocidal.[72] In mouse colon, there is a similar population of cells which may also make IL-10,[73] but there is no evidence that macrophages in healthy human gut make IL-10. It would appear therefore that in normal gut the function of most lamina propria macrophages is to phagocytose bacteria (both commensals and pathogens) crossing the epithelium, without evoking a strong inflammatory response. It is thought that transforming growth factor-β production by stromal cells is responsible for rendering intestinal macrophages anergic.[72] However, there are small numbers of CD14+ macrophages in normal human gut (0.5% of CD33+ cells), presumably recent immigrants from blood. These cells express TLR4 and CD40 as well as costimulatory molecules and make IL-12, IL-23, IL-6, TNF-α and IL-10 when activated in vitro with Gram-negative gut bacteria.[74] These cells are also potent antigen-presenting cells and when isolated from normal gut drive Th1 activation.[75]

One of the features of chronic inflammatory bowel disease in man, especially Crohn's disease is a dramatic increase in macrophages derived from blood monocytes. These cells express CD14 and produce large amounts of pro-inflammatory cytokines such as TNF-α, IL-12 and IL-6.[76]

A feature of Th2 responses in the peritoneum, pleural cavity and liver is that IL-4 drives local macrophage proliferation.[77] As described above, there is crosstalk between ILC2 and conventional T cells in the gut, but it is not known whether in Th2 responses driven by intestinal parasites, impacts local division of macrophages.

5 | RETINOIC ACID

One of the most important discoveries in mucosal immunology in recent years has been the discovery that gut-specific homing of T and B cells from gut-associated lymph tissue (GALT) to the lamina propria is driven by dietary vitamin A.[78] In meat products, vitamin A is found in the form of retinyl palmitate which is converted into retinol in the small intestine. After absorption, retinol is converted into all-trans retinoic acid by retinol dehydrogenase and retinaldehyde dehydrogenase (RALDH). Interestingly, dendritic cells in GALT express RALDH so when presenting peptides to T cells, they secrete retinoic acid which functions as a signalling molecule by binding to retinoic acid receptors and regulating transcription. In particular, two genes are upregulated, CCR9 and the α 4 β 7 integrin. When cells leave GALT and enter blood, they can migrate back to the lamina propria because intestinal endothelial cells express the α 4 β 7 ligand, MAdCam1, and CCR9 is the surface receptor for CCL25 made by gut epithelial cells.

Retinoic acid also has other functions in the gut. Recently it has been shown that retinoic acid secreted by macrophages is pro-inflammatory in the gut as it drives the differentiation of TNF-α secreting macrophages.[79] Retinoic acid secreted by lamina propria DCs and transforming growth factor-β also are highly effective at generating Tregs.[80] It has also been demonstrated that retinoic acid inhibits Th17 cell development and also drives T cells to become Tregs.[81] The situation is further complicated by the observation that retinoic acid is needed to maintain Th1 stability and inhibits their ability to become Th17 cells.[82] Further studies are needed to unravel the apparently contradictory roles of retinoic acid in the gut.

6 | MUCOSA-ASSOCIATED INVARIANT T CELLS (MAIT CELLS)

In 2003, a new population of cells was identified in human gut expressing a semi-invariant TcR using TRAV1-2-TRAJ33 and TRBV6 and TRBV20. In mouse gut, TcR usage of MAIT cells is TRAV19, and TRAJ33.[83] At the same time, it was discovered that these unusual T cells were restricted by MR1, a highly conserved monomorphic MHCI-like molecule.[83] These cells remained quite understudied, but a major advance was achieved when it was shown that the ligands for MR1 are bacterially derived metabolites of vitamin B, pathways not present in vertebrates.[84] Thus MAIT cells are in many ways similar to natural killer T cells in that they both respond to bacterial ligands (MAIT cells respond to bacterial vitamin B metabolites,

and NK T cells to bacterial phospholipids) using T-cell receptors of limited variability. MAIT cells are relatively uncommon in mice, but in humans make up 5-50% of T cells in tissues and blood.[85] CD4+ MAIT cells are uncommon, the majority either being CD8+ or CD4-, 8-. In terms of transcription factors and other surface markers, they resemble Th17 cells in being RORγt+, CD161+ and IL-23R+ but some also express T-bet.[85] When activated, they can secrete IL-17A, TNF-α and interferon-gamma.[85]

Mucosa-associated invariant T cells cells were difficult to study although transgenic mice expressing their invariant T-cell receptors gave some insight. But technical advances have made them more amenable for investigation. First was the development of MR1 tetramers to unequivocally identify MAIT cells.[86] Recently, this technology has been used to analyse MAIT development in the thymus.[87] This occurs in two phases, with Stage 1 and Stage 2 cells predominating at this site, while Stage 3 MAIT cells expand in the periphery with age.[71] Expansion of Stage 3 MAIT cells depends on the gut microbiota, and the transcription factor promyelocytic leukaemia zinc finger protein.[87] The second advance was the discovery that an inbred mouse derived from wild mice in Thailand, the CAST/EiJ mouse, had a 20-fold increase in MAIT cells.[88] Mapping of the locus responsible for the increase in MAIT cells identified a region in the 3′ end of the Vα locus, TCR-δ, Jα and Cα segments. This then allowed the generation of a congenic mouse on a C57BL6 background. This mouse was then crossed with a RORCγt-GFP reporter mouse to generate a mouse where MAIT cells could be identified by green fluorescence.[88]

There still remains relatively few publications on the function of MAIT cells. MAIT cells can be activated by cells infected with bacteria or fungi, but not viruses.[89] They may also protect against urinary tract infections.[88] MAIT cells can kill epithelial MR1+ cells infected with *Shigella flexneri*.[89] MAIT cells are also activated in vivo in humans infected with dengue, hepatitis or influenza,[90,91] and in nonhuman primates infected with *Mycobacterium tuberculosis*.[92] Their role in inflammatory bowel disease is unclear as it has been reported that MAIT cells are decreased in IBD[93] while at the same time, it has been reported that they are activated in IBD.[94] Strikingly, a literature search in PubMed of MAIT cells and helminths reveals no publications. Table 1 summarized the main features of MAIT cells.

TABLE 1 Characteristics of mucosa-associated invariant T cells

T-cell receptor usage	TRAV1-2- TRAJ33 and TRBV6 and TRBV20 in man and TRAV19 and TRAJ33 in mice
Restriction element	The monomorphic MHC1-like molecule MR1
Antigenic specificity	Bacterial and fungal vitamin B metabolites
Phenotype	CD8+ or CD4-8-
Cytokine secretion	IL-17A, TNF-α and interferon-gamma
Frequency	Uncommon in most mouse strains, 5-50% of all T cells in humans
Transcription factors	Promyelocytic leukaemia zinc finger protein, RORγt+, but some also express T-bet
Development	In the thymus but numbers are dramatically decreased in mice lacking gut bacteria

7 | CONCLUSIONS

It is fascinating to consider that ILCs were only identified in 2010, many years after the lymphoid family was thought to be complete. There has been an explosion of publications since then. The challenge remains however to identify their role in a lymphocyte-replete animal as compared to a lymphopenic animal where they expand in huge numbers in animals with gut bacterial infections. They may end up like γ/δ T cells, extremely interesting, but their exploitation for human health has not materialized. Another paradigm over-turned in the last few years is the notion that tissue macrophages derive from hematopoietic stem cells and do not divide in tissues. It is now clear that for some tissues such as skin, macrophages are yolk sac derived and are maintained by local division. However, this is not the case for the gut, where after weaning, all macrophages are derived from circulating monocytes. It has also been discovered that retinoic acid controls cell homing to the gut and also has major effects on Th1, Th17 and Treg differentiation and function. Finally for MAIT cells, tools and reagents to probe their function are now available and we expect an explosion of interest in the next few years.

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CONFLICT OF INTEREST

The authors have no conflict of interests to declare.

REFERENCES

1. Sender R, Fichs S, Milo R. Revised estimates for the number of human and bacterial cells in the body. *PLoS Biol*. 2016;14:e1002533.
2. Gagliani N, Amezcua Vesely C, Iseppon A, et al. Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature*. 2015;523:221-225.
3. Abt MC, Lewis BB, Caballero S, et al. Innate immune defenses mediated by two ILC subsets are critical for protection against acute Clostridium difficile infection. *Cell Host Microbe*. 2015;18:27-37.
4. Klose CS, Flach M, Mohle L, et al. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. *Cell*. 2014;157:340-356.
5. Reynders A, Yessaad N, Vu Manh TP, et al. Identity, regulation and in vivo function of gut NKp46+RORgammat+ and NKp46+RORgammat- lymphoid cells. *EMBO J*. 2011;2011:2934-2947.
6. Neill DR, Wong SH, Bellosi A, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature*. 2010;464:1367-1370.

7. Huang Y, Guo L, Qiu J, et al. IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential 'inflammatory' type 2 innate lymphoid cells. *Nat Immunol*. 2015;16:161-169.
8. Sanos SL, Bui VL, Mortha A, et al. RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. *Nature Immunol*. 2009;10:83-91.
9. Glatzer T, Killig M, Meisig J, et al. RORgammat(+) innate lymphoid cells acquire a proinflammatory program upon engagement of the activating receptor NKp44. *Immunity*. 2013;38:1223-1235.
10. Satoh-Takayama N, Vosshenrich CA, Lesjean-Pottier S, et al. Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. *Immunity*. 2008;29:958-970.
11. Zheng Y, Valdez PA, Danilenko DM, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat Med*. 2008;14:282-289.
12. Zook EC, Kee BL. Development of innate lymphoid cells. *Nature Immunol*. 2016;17:775-782.
13. Yagi R, Zhong C, Northrup DL, et al. The transcription factor GATA3 is critical for the development of all IL-7Rα-expressing innate lymphoid cells. *Immunity*. 2014;40:378-388.
14. Moretta F, Petronelli F, Lucarelli B, et al. The generation of human innate lymphoid cells is influenced by the source of hematopoietic stem cells and by the use of G-CSF. *Eur J Immunol*. 2016;46:1271-1278.
15. Harbour SN, Maynard CL, Zindl CL, Schoeb TR, Weaver CT. Th17 cells give rise to Th1 cells that are required for the pathogenesis of colitis. *Proc Natl Acad Sci USA*. 2015;112:7061-7066.
16. Hirota K, Duarte JH, Veldhoen M, et al. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nature Immunol*. 2011;12:255-263.
17. Bal SM, Bernin JH, Nagasawa M, et al. IL-1beta, IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs. *Nature Immunol*. 2016;17:636-645.
18. Ohne Y, Silver JS, Thompson-Snipes L, et al. IL-1 is a critical regulator of group 2 innate lymphoid cell function and plasticity. *Nature Immunol*. 2016;17:646-655.
19. Bernink JH, Krabbendam L, Germar K, et al. Interleukin-12 and -23 control plasticity of CD127(+) group 1 and group 3 innate lymphoid cells in the intestinal lamina propria. *Immunity*. 2015;43:146-160.
20. Lim AI, Menegatti S, Bustamante J, et al. IL-12 drives functional plasticity of human group 2 innate lymphoid cells. *J Exp Med*. 2016;213:569-583.
21. Jenner RG, Townsend MJ, Jackson I, et al. The transcription factors T-bet and GATA-3 control alternative pathways of T-cell differentiation through a shared set of target genes. *Proc Natl Acad Sci USA*. 2009;106:17876-17881.
22. Silver JS, Kearley J, Copenhaver AM, et al. Inflammatory triggers associated with exacerbations of COPD orchestrate plasticity of group 2 innate lymphoid cells in the lungs. *Nature Immunol*. 2016;17:626-635.
23. von Burg N, Chappaz S, Baerenwaldt A, et al. Activated group 3 innate lymphoid cells promote T-cell-mediated immune responses. *Proc Natl Acad Sci USA*. 2014;111:12835-12840.
24. Hepworth MR, Monticelli LA, Fung TC, et al. Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. *Nature*. 2013;498:113-117.
25. Oliphant CJ, Hwang YY, Walker JA, et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity*. 2014;41:283-295.
26. Halim TY, Hwang YY, Scanlon ST, et al. Group 2 innate lymphoid cells license dendritic cells to potentiate memory TH2 cell responses. *Nature Immunol*. 2016;17:57-64.
27. Goto Y, Obata T, Kunisawa J, et al. Innate lymphoid cells regulate intestinal epithelial cell glycosylation. *Science*. 2014;345:1254009.
28. Pham TA, Clare S, Goulding D, et al. Epithelial IL-22RA1-mediated fucosylation promotes intestinal colonization resistance to an opportunistic pathogen. *Cell Host Microbe*. 2014;16:504-516.
29. Lindemans CA, Calafiore M, Mertelsmann AM, et al. Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature*. 2015;528:560-564.
30. Buonocore S, Ahern PP, Uhlig HH, et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature*. 2010;464:1371-1375.
31. Powell N, Walker AW, Stolarczyk E, et al. The transcription factor T-bet regulates intestinal inflammation mediated by interleukin-7 receptor(+) innate lymphoid cells. *Immunity*. 2012;37:674-684.
32. Powell N, Lo JW, Biancheri P, et al. Interleukin 6 increases production of cytokines by colonic innate lymphoid cells in mice and patients with chronic intestinal inflammation. *Gastro*. 2015;149:456-467.
33. Ermann J, Staton T, Glickman JN, et al. Nod/Ripk2 signaling in dendritic cells activates IL-17A-secreting innate lymphoid cells and drives colitis in T-bet-/-.Rag2-/- (TRUC) mice. *Proc Natl Acad Sci USA*. 2014;111: E2559-E2566.
34. Geremia A, Arancibia-Carcamo CV, Fleming MP, et al. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. *J Exp Med*. 2011;208:1127-1133.
35. Mizuno S, Mikami Y, Kamada N, et al. Cross-talk between RORgammat+ innate lymphoid cells and intestinal macrophages induces mucosal IL-22 production in Crohn's disease. *Inflamm Bowel Dis*. 2014;20:1426-1434.
36. Song C, Lee JS, Gilfillan S, et al. Unique and redundant functions of NKp46+ ILC3s in models of intestinal inflammation. *J Exp Med*. 2015;212:1869-1882.
37. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*. 2012;491:119-124.
38. Feagan BG, Sandborn WJ, Gasink C, et al. Ustekinumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med*. 2016;375:1946-1960.
39. Awasthi A, Riol-Blanco L, Jager A, et al. Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. *J Immunol*. 2009;182:5904-5908.
40. Saenz SA, Siracusa MC, Perrigoue JG, et al. IL25 elicits a multipotent progenitor cell population that promotes T(H)2 cytokine responses. *Nature*. 2010;464:1362-1366.
41. Grencis RK, Humphreys NE, Bancroft AJ. Immunity to gastrointestinal nematodes: mechanisms and myths. *Immunol Rev*. 2014;260:183-205.
42. Gerbe F, Sidot E, Smyth DJ, et al. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature*. 2016;529:226-230.
43. von Moltke J, Ji M, Liang HE, Locksley RM. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature*. 2016;529:221-225.
44. Howitt MR, Lavoie S, Michaud M, et al. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science*. 2016;351:1329-1333.
45. Hsieh CS, Macatonia SE, O'Garra A, Murphy KM. T cell genetic background determines default T helper phenotype development in vitro. *J Exp Med*. 1995;181:713-721.
46. Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. *New Engl J Med*. 2016;375:2369-2379.
47. Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009;139:485-498.
48. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA*. 2011;108(Suppl 1):4615-4622.

49. Wu HJ, Ivanov II, Darce J, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity*. 2010;32:815-827.
50. Atarashi K, Tanoue T, Ando M, et al. Th17 cell induction by adhesion of microbes to intestinal epithelial cells. *Cell*. 2015;163:367-380.
51. Sano T, Huang W, Hall JA, et al. An IL-23R/IL-22 circuit regulates epithelial serum amyloid A to promote local effector Th17 Responses. *Cell*. 2015;163:381-393.
52. Atarashi K, Tanoue T, Oshima K, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*. 2013;500:232-236.
53. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*. 2008;453:620-625.
54. Round JL. Mazmanian SK Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci USA*. 2010;107:12204-12209.
55. Shen Y, Giardino Torchia ML, Lawson GW, et al. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. *Cell Host Microbe*. 2012;12:509-520.
56. Chu H, Khosravi A, Kusumawardhani IP, et al. Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. *Science*. 2016;352:1116-1120.
57. Holm JB, Sorobetea D, Kiilerich P, et al. Chronic Trichuris muris infection decreases diversity of the intestinal microbiota and concomitantly increases the abundance of lactobacilli. *PLoS One*. 2015;10:e0125495.
58. Walk ST, Blum AM, Ewing SA, et al. Alteration of the murine gut microbiota during infection with the parasitic helminth Heligmosomoides polygyrus. *Inflamm Bowel Dis*. 2010;16:1841-1849.
59. Rausch S, Held J, Fischer A, et al. Small intestinal nematode infection of mice is associated with increased enterobacterial loads alongside the intestinal tract. *PLoS One*. 2013 Sep 10;8:e74026.
60. McKenney EA, Williamson L, Yoder AD, et al. Alteration of the rat cecal microbiome during colonization with the helminth Hymenolepis diminuta. *Gut Microbes*. 2015;6:182-193.
61. Kay GL, Millard A, Sergeant MJ, et al. Differences in the faecal microbiome in *Schistosoma haematobium* infected children vs. uninfected Children. *PLoS Negl Trop Dis*. 2015;9:e0003861.
62. Li RW, Wu S, Li W, et al. Alterations in the porcine colon microbiota induced by the gastrointestinal nematode *Trichuris suis*. *Infect Immun*. 2012;80:2150-2157.
63. Kreisinger J, Bastien G, Hauffe HC, Marchesi J, Perkins SE. Interactions between multiple helminths and the gut microbiota in wild rodents. *Philos Trans R Soc Lond B Biol Sci*. 2015; 370: 20140295.
64. Gorkiewicz G, Thallinger GG, Trajanoski S, et al. Alterations in the colonic microbiota in response to osmotic diarrhea. *PLoS One*. 2013;8:e55817.
65. Reynolds LA, Finlay BB. Worming their way into the picture: microbiota help helminths modulate host immunity. *Immunity*. 2015;43: 840-842.
66. Ginhoux F, Greter M, Leboeuf M, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science*. 2010;330:841-845.
67. Schulz C, Perdiguero EG, Chorro L, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science*. 2012;336:86-90.
68. Bain CC, Bravo-Blas A, Scott CL, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol*. 2014;15:929-937.
69. Niess JH, Brand S, Gu X, et al. A CX3CR1- mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science*. 2005;307:254-258.
70. Cerovic V, Bain CC, Mowat AM, Milling SWF. Intestinal macrophages and dendritic cells: what's the difference. *Trends Immunol*. 2014;35:270-77.
71. Lelouard H, Fallet M, de Bovis B, et al. Peyer's patch dendritic cells sample antigens by extending dendrites through M-cell specific transcellular pores. *Gastro*. 2012;142:592-601.
72. Smythies LE, Sellers M, Clements RH, et al. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. *J Clin Invest*. 2005;115:66-75.
73. Bain CC, Scott CL, Uronen-Hansson U, et al. Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors. *Mucosal Immunol*. 2013;6:498-510.
74. Kamada N, Hisamatsu T, Okamoto S, et al. Unique CD14+ intestinal macrophages contribute to the pathogenesis of Crohn's disease via IL-23/IFN-gamma axis. *J Clin Invest*. 2008;118:2269-2280.
75. Kamada N, Hisamitsu T, Honda H, et al. Human CD14+ macrophages in intestinal lamina propria exhibit potent antigen-presenting ability. *J Immunol*. 2009;183:1724-1731.
76. Rugtveit J, Nilsen EM, Bakka A, et al. Cytokine profiles differ in newly recruited and resident subsets of mucosal macrophages from inflammatory bowel disease. *Gastro*. 1997;112:1493-1505.
77. Jenkins SJ, Ruckerl D, Cook PC, et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. *Science*. 2011;10:1284-1288.
78. Iwata M. Retinoic acid production by intestinal dendritic cells and its role in T-cell trafficking. *Semin Immunol*. 2009;21:8-13.
79. Sanders TJ, McCarthy NE, Giles EM, et al. Increased production of retinoic acid by intestinal macrophages contributes to their inflammatory phenotype in patients with Crohn's disease. *Gastro*. 2014;146:1278-1288.
80. Sun CM, Hall JA, Blank RB, et al. Small intestine lamina propria dendritic cells promote de nove generation of Focp3 T reg cells via retinoic acid. *J Exp Med*. 2007;204:1775-1785.
81. Mucida D, Park Y, Gisen K, et al. Reciprocal Th17 and regulatory T cell differentiation mediated by retinoic acid. *Science*. 2007;317:256-260.
82. Brown CC, Esterhazy D, Sarde A, et al. Retinoic acid is essential for Th1 lineage stability and prevents transition to a Th17 program. *Immunity*. 2015;42:499-511.
83. Treiner E, Duban L, Bahram S, et al. Selection of evolutionary conserved mucosal-associated invariant T cells by MR1. *Nature*. 2003;422:164-169.
84. Kjer-Nielsen L, Patel O, Corbett AO, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature*. 2012;491:717-723.
85. Godfrey DI, Uldrich AP, McCluskey J, et al. The burgeoning family of unconventional T cells. *Nature Immunol*. 2015;16:1114-1123.
86. Reantragoon R, Corbett AJ, Salaka IG, et al. Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. *J Exp Med*. 2013;210:2305-2320.
87. Koay H-F, Gherardin NA, Enders A, et al. A three-stage intrathymic developmental pathway for the mucosal-associated invariant T cell lineage. *Nat Immunol*. 2016;17:1300-1311.
88. Cui Y, Franciszkiewicz K, Mburu YK, et al. Mucosal associated invariant T cell-rich congenic mouse strain allows functional evaluation. *J Clin Invest*. 2015;125:4171-4185.
89. Le Bourhis L, Martin E, Peguillet I, et al. Antimicrobial activity of mucosal-associated invariant T cells. *Nat Immunol*. 2010;11:701-708.
90. Le Bourhis L, Dusseaux M, Bohineust A, et al. MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. *PLoS Pathog*. 2013;9:e1003681.
91. Van Wigenburg B, Scherwitzl I, Hutchinson EC, et al. MAIT cells are activated during human viral infections. *Nat Commun*. 2016;6:11653.
92. Greene JM, Dash P, Roy S, et al. MR-1 restricted mucosal-associated invariant T (MAIT) cells respond to mycobacterial vaccination and infection in nonhuman primates. *Mucosal Immunol*. 2016;10:802-813.
93. Heijima E, Kawai T, Nakase H, et al. Reduced numbers and proapoptotic features of mucosal-associated invariant T cells as a characteristic

finding in patients with inflammatory bowel disease. *Inflamm Bowel Dis*. 2015;21:1529-1540.

94. Serriari NE, Eoche M, Lamotte L, et al. Innate mucosal-associated invariant T (MAIT) cells are activated in inflammatory bowel disease. *Clin Exp Immunol*. 2014;176:266-274.

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