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REVIEW

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The thin line between conventional dendritic cells (cDCs) and group 3 innate lymphoid cells (ILC3s) in the gut

Alina Ulezko Antonova*, José Luís Fachi*,, Susan Gilfillan and **Marco Colonna**

Department of Pathology and Immunology, Washington University School of Medicine, Saint Louis, MO 63108, USA

Correspondence to: J.L. Fachi; E-mail: fachijl@wustl.edu or M. Colonna; E-mail: mcolonna@wustl.edu

*These authors contributed equally.

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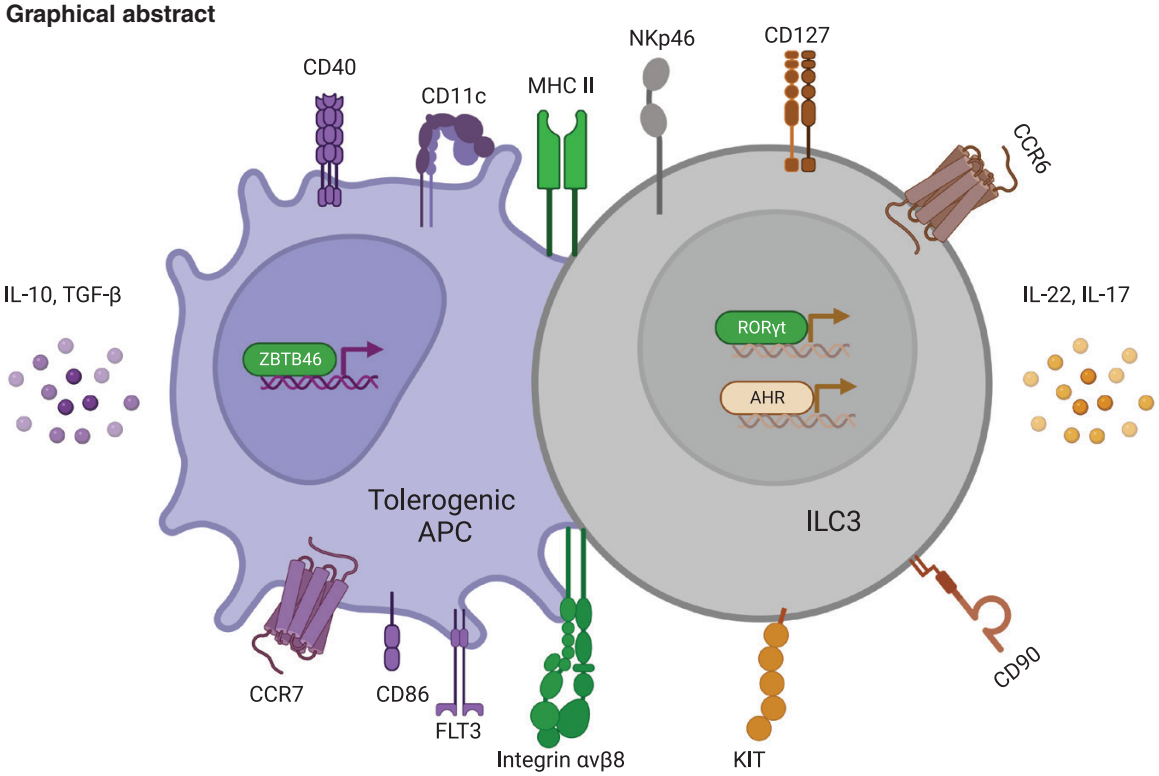
Abstract

Dendritic cells (DCs) express major histocompatibility complex class II (MHC-II) and are best known for proficiently presenting antigens to T cells, thereby eliciting specific adaptive T cell responses. Moreover, conventional DCs (cDCs) are specifically adept at handling intestinal antigens. Relatively recent discoveries and investigations have proven the existence of a new group of innate lymphocytes that reside in tissues like the intestine. They lack specific antigen receptors and can express MHC-II. These group 3 innate lymphoid cells (ILC3s) comprise a subset of heterogeneous innate lymphocytes that mirror the phenotype and functions of T-helper cells and act in the first line of defense. Considering that ILC3s are crucial for maintaining homeostasis of the intestinal mucosa and are found in niches alongside DCs, we herein describe the roles played by cDCs and ILC3s in the gut, highlighting the most recent studies. We discuss how these cells are alike and differ, constantly pointing out the thin, blurry line that separates cDCs and ILC3s.

Graphical abstract



Keywords: antigen presentation, colitis, microbiota

Introduction

Immune cells are divided into two categories, innate and adaptive, based on their ability to recognize specific antigens. Both cell categories may have emerged simultaneously in evolution around 500 million years ago in vertebrate ancestors, with high functional specialization and population divergence in mammals and birds (1, 2). Adaptive lymphocytes, i.e. T and B cells, recognize specific antigens through their unique antigen receptors, the TCR and BCR respectively, that are dependent on recombination-activating genes (*Rag1* and *Rag2*) for their assembly and acquisition of specificity. On the other hand, innate cells do not have specific antigen receptors and can be activated by recognition of pathogen-associated molecular patterns (PAMPs)—molecules with conserved motifs that serve as ligands for host pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs).

At the interphase between innate and adaptive immunity, there is also a group of cells that present antigens to adaptive immune cells and are dubbed 'dendritic cells' (DCs) for their pseudopod-arranged cytoplasm and large size (3). These are professional antigen-presenting cells (APCs) that express major histocompatibility complex class II (MHC-II) and are most known for their ability to generate specific T cell responses in three well-described steps: (i) presentation of antigen as a peptide–MHC complex that is recognized and bound by the TCR, (ii) additional molecular interactions between the conventional DC (cDC) and T cell that co-stimulate the T cell and (iii) cytokine-mediated T-cell differentiation and expansion (4).

However, recent investigations and discoveries have revealed the existence of a new group of lymphoid cells that are highly reminiscent of T-helper (Th) cells in phenotype, function and development, but lack rearranged antigen-specific receptors and are not antigen-specific, so are classified as 'innate lymphoid cells' (ILCs) (5–7). These cells do not recognize antigens, and their activation depends mainly on the binding of cytokines and alarmins produced by other cells present in the tissue (2, 6, 8). Nonetheless, two subsets of these cells, group 2 and group 3 ILCs (ILC2s and ILC3s), can also express MHC-II and process antigens, regulating antigen-specific T-cell mediated responses (9–13).

More intensively studied in this context, ILC3s have been shown to induce tolerance to commensal bacteria through both T and B cell responses (12, 14), and mice lacking MHC-II in ILC3s develop spontaneous colitis (15). However, the role of ILC3s in antigen presentation and how this influences the generation of intestinal immunity is not fully understood. Furthermore, it is not yet known how or when ILC3s and cDCs may functionally overlap and hence may be redundant in some situations. Here, we describe the roles played by these two types of cells in the gut; this article is based on the most recent studies and highlights the differences, similarities and interactions between them.

Dendritic cells

The field of DC biology began with their discovery by Steinman in 1973 (16). He identified them in the mouse semi-adherent splenocyte fraction as a cell type distinct from lymphocytes, granulocytes and mononuclear phagocytes (16). As this era was contemporaneous with the discovery of antigen processing in macrophages (17) and 'cross-presenting' exogenous antigens that are presented by the host MHC-I (18), it was hotly debated whether the cells best at antigen presentation to T cells were DCs or macrophages.

Steinman *et al.* noted that DCs differed from macrophages by their lack of endocytic potential in the spleen and fewer lysosomes (16), which was later attributed to their mature state. In 1989 it was shown that DCs capture antigens as immature cells and lose this capacity as they mature and encounter the T cell, which confines the processing and presentation of antigens to distinct temporal windows of the DC lifespan (19, 20). Steinman's group later showed that DCs were the most potent stimulators of T cells in mixed lymphocyte reactions in both mice (21) and humans (22). In his original 1973 paper, Steinman also detected DCs in the mesenteric lymph nodes (mLNs), axillary LNs and cervical LNs and in the Peyer's patches (PPs) of mice (3).

To date, DCs have been identified in most organs, in both humans and mice, in steady state and during inflammation In this section, we will focus on intestinal cDCs as, in this antigen-loaded niche, they are quite specialized. Like in other anatomic locations, intestinal cDCs can migrate from primary lymphoid organs to secondary lymphoid tissues by following chemokine gradients, though the exact mechanisms underlying this unique ability and why macrophages do not have it are yet unknown. cDCs can also be either activating or tolerogenic depending on the signals that they receive and their niche; for instance, thymic cDCs have roles in the negative selection of T cells, whereas cDCs in the skin sample skin-derived antigens (4).

Historically, DCs have been subdivided into two main subtypes: cDCs and plasmacytoid DCs (pDCs). The name 'dendritic' reflects purely morphological etymology. pDCs are an innate cell type specialized in rapid secretion of type I interferons (type I IFNs) upon encounter with the appropriate stimuli (23, 24). Although several studies have shown that pDCs can present and cross-present antigens (25–28), they are greatly inferior to cDCs in this function (28). Moreover, pDCs are mostly derived from a common lymphoid progenitor (CLP) (Fig. 1), unlike cDCs, which are thought to derive exclusively from a common DC progenitor (CDP) (29, 30). In addition, all cDCs express the transcription factor ZBTB46, and it is considered a selective marker for this cell type (31).

Since 1973, a multitude of studies have redefined the 'dendritic cell' nomenclature to categorize cDCs only, because only they are dedicated antigen presenters. Although antigen presentation is a phenomenon carried out by multiple cells, as virtually any cell type can act as an antigen presenter and a T-cell inducer in the right context, we will hereafter focus on cDCs in the intestine as they are cell types best suited for this function.

Peripheral cDCs seed tissues postnatally, where they reside in an immature state and migrate to LNs after their maturation (32, 33). They originate from a hematopoietic stem cell (HSC) in the bone marrow, which transitions through a series of developmental stages. Most DCs develop from the common DC progenitor, which emerges from a monocyte and

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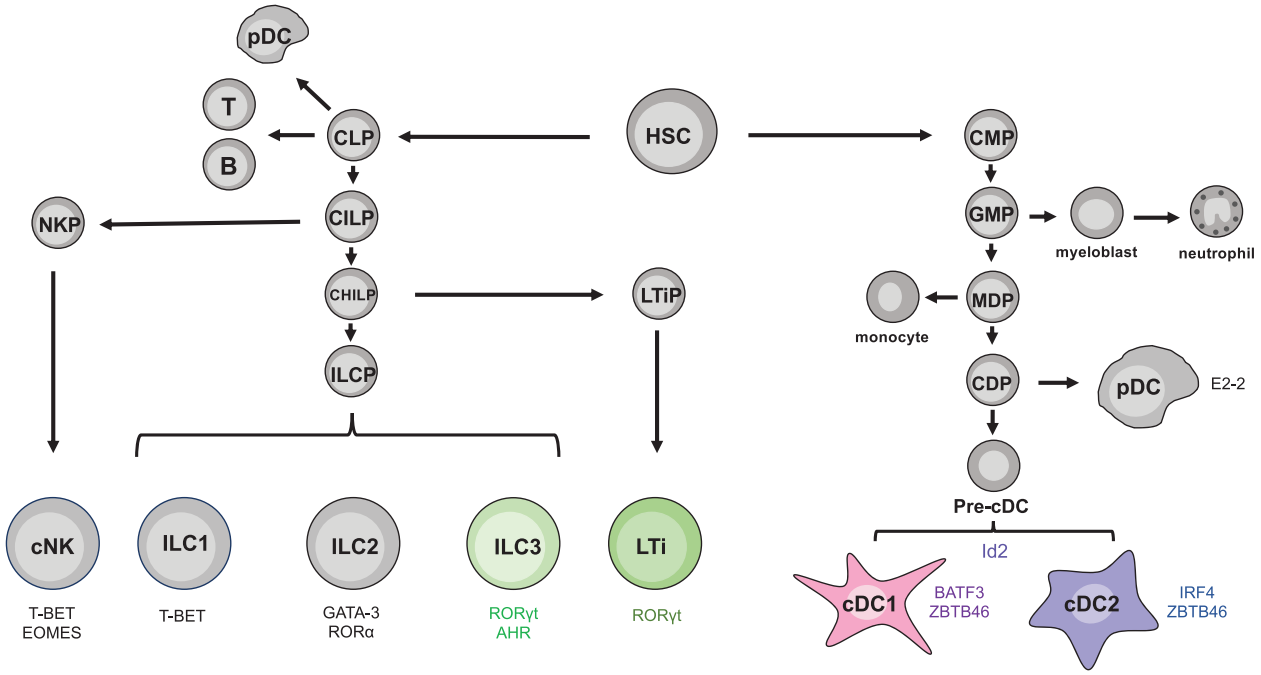

Fig. 1. The hematopoietic origin of ILC and DC subsets. Immune cells originate from a hematopoietic stem cell in the bone marrow, which transitions through a series of developmental stages. In particular, cDCs and ILCs originate from the differentiation of distinct hematopoietic progenitors. Whereas most pDCs derive from a common lymphoid progenitor, all cDCs develop from the common DC progenitor, which emerges from the monocyte-dendritic cell progenitor—a process dependent on IRF8. In contrast, the ILC-restricted progenitor (common innate lymphoid progenitor) derives from the CLP, giving rise to two main lineages of ILCs, the killer ILC progenitor [natural killer progenitor (NKP)] and the helper-like progenitor (CHILP). Group 3 ILCs, as well as groups 1 and 2 ILCs, are then derived from the ILC progenitor (ILCP). However, CCR6$^+$ ILC3s (LTi) are derived from a distinct progenitor called LTiP. Characteristic transcription factors found in ILC and cDC subsets are also shown. cNK, conventional natural killer; CMP, common myeloid progenitor; GMP, granulocyte-monocyte progenitor.

DC progenitor (MDP) (Fig. 1). This transition is largely mediated through upregulation of interferon response factor 8 (IRF8). A committed DC precursor, the 'pre-cDC', exits the bone marrow and travels through the blood to seed the intestine through high endothelial venules (HEVs) (34).

Once integrated into the intestinal cDC network, cDCs further divide and develop, mainly through the cytokine FLT3L and its receptor FLT3 (34–36). Moreover, it has been shown that vitamin A-derived retinoic acid (RA) in the intestine binds to the gut RA receptor α (RARα). Pre-cDCs, under the influence of RA, upregulate the trafficking receptor α4β7 and home to the intestine (37). In the intestine, cDCs have two ways of capturing luminal antigens: either via discharge of intestinal contents through M cells in the PPs (38) or by extending their dendrites through the epithelial connections to sample luminal antigens directly, preserving the barrier by expressing tight-junction proteins (39).

It is well accepted, based on the type of immune response that they drive, that there are two main types of cDCs: cDC1s, which are specialized cross-presenting cells that mediate type 1 immune responses, and cDC2s, which are a heterogeneous group of cells that present antigen to T cells via MHC-II and mediate type 2 and type 3 immune responses. Type 1 immune responses are directed at intracellular pathogens, such as viruses, and are characterized by the production of type I IFNs, primarily in response to IL-12. In contrast, type 2 responses dominate in allergies and often culminate in the elimination of large extracellular parasites, such as helminths, through IL-4, IL-5 and IL-13 induced by IL-25, IL-33 and TSLP. Type 3 responses combat extracellular microbes, such as bacteria and fungi and are characterized by the production of effector cytokines IL-17 and IL-22 in response to IL-23 and IL-1 (5, 40–42).

cDC1s (BATF3-dependent cells, or CD103$^+$CD11b$^-$)

cDC1s commit to their lineage through an interplay between basic leucine zipper ATF-like transcription factor 3 (BATF3) and IRF8, transitioning through a BATF3-independent stage to a BATF3-dependent stage (43, 44). The latter is fully committed to the cDC1 fate through a specific enhancer located +32 kb downstream of the transcriptional start site of IRF8, which mediates binding of AICE-family proteins to the BATF3–IRF8 complex (43, 44). Other transcription factors have been implicated in commitment to the cDC1 lineage, including NFIL3 (45) and Id2 (46, 47).

Phenotypically, cDC1s express X-C motif chemokine receptor 1 (XCR1) in both humans and mice, and mature cells specifically express CD24 in mice and CADM1, CLEC9A and CD141 in humans (48). In the intestine, cDC1s can be found in the intestinal lamina propria (LP) and as migratory cells of the mLNs, or in gut-associated lymphoid tissues (GALT), like PPs and the resident compartment of the mLNs. They are particularly abundant in GALT. In fact, it has been proposed that the main cell types in isolated lymphoid follicles of the intestine are cDC1s and pDCs; they express CXCL13,

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potentially to recruit B cells and support the development of germinal centers (49). In GALT, such as PPs, cDC1s are specifically located within T-cell rich areas (50) and are presumed to travel deep into the T-cell zone following a CCL19/CCL21 gradient (51).

In both primary intestinal locations and GALT, cDC1s depend on the IRF8–BATF3 interaction (52). In the LP, cDC1s are Lin$^-$ (i.e. negative for B220, CD3, CD14, CD16 and F4/80) and lack the integrin CD11b but they specifically express the integrin αEβ7 (CD103). cDC1s in the LP are mainly found in the colon rather than the small intestine and are the main CD103$^+$ cell subtype there; however, deletion of cDC1s results in reduced numbers of T cells only in the small intestine but not the large intestine (53). In GALT, cDC1s are also defined by the expression of the classical cDC1 marker XCR1, as well as being Lin$^-$, CD8α$^+$ and CD4$^-$ (50).

Functionally, intestinal cDC1s can open tight-junctions between epithelial cells to send dendrites to the intestinal lumen and sample antigens without disrupting the epithelial barrier (39, 54, 55). Specifically, they have the unique ability to cross-prime intestinal antigens both in lymphoid and non-lymphoid intestinal locations, which was shown in a model in which epithelial intestinal cells were loaded with ovalbumin (OVA) and cDC1s were the only cells that were able to cross-prime and induce OVA-specific T-cell polarization (56). Through this process, they can prime antigen-specific CD8$^+$ cytotoxic T lymphocyte (CTL) responses and T-helper 1 (Th1) responses (53).

cDC1s have been proposed to be the main cell type that enables the development of peripherally (extrathymic) induced regulatory T cells (iTregs) in the intestine (57). Although cDC2s have also been suggested to be important for the generation of iTregs, IRF8-targetted deletion of intestinal cDC1s in the mLNs, LP and spleen showed that these cDCs were superior to cDC2s in polarizing naive T cells into iTregs, though their deletion did not interfere with oral tolerance (57). Similarly, a recent study showed that XCR1$^+$ cDC1s specifically induce Tregs in the colonic mucosa and that mice lacking these cells have more Th1/Th17 cells and fewer iTregs, which was associated with exacerbated colitis in two distinct models (58).

However, this long-accepted notion has recently been challenged by three groups in parallel, who have discovered that peripheral induction of Tregs, which express the transcription factor retinoid-related orphan receptor γt (RORγt), is mediated by a subset of APCs that also express RORγt (12, 59, 60). These findings will be discussed in more detail in 'The thin line between intestinal cDCs and ILC3s' section, although more work is needed to elucidate the effectiveness of cDC1s and/or integrin αvβ8$^+$ cells to prime Tregs in either the early life Treg-seeding window or in response to infections. Finally, whatever the mechanism, the induction of peripheral Tregs has long been proposed to be mediated by microbiota (61).

cDC2s (IRF4-dependent cells or CD11b$^+$)

cDC2s require IRF4 for normal function and development (62), but other transcription factors are involved, including RelB, Traf6, Notch2, Irf2, Id2 (63) and Klf4 (64). There is evidence that suggests that IRF4-dependent cells are functionally heterogeneous. Whereas Klf4-dependent cDCs initiate and maintain type 2 responses (64), Notch2 is needed for cDC2s to sustain type 3 responses (65). Specifically, Notch2 deletion in CD11c-expressing cells leads to complete loss of CD11b$^+$CD103$^+$ cDCs in the intestinal LP and a consequential reduction of Th17 cells in the intestine (65, 66). Notch2-dependent cDC2s, but not Klf4-dependent cDC2s, are uniquely required in promoting the formation of follicular-helper T cells (Tfh cells) and germinal center B cells, making this subset of cDC2s uniquely equipped to support humoral immune responses (65). Finally, more recently it was shown that pre-cDC2 development to mature cDC2 is tightly controlled by competitive binding of NFIL3 with C/EBPα and C/EBPβto the −165 kb Zeb2 enhancer (67).

The cDC2 phenotype is phenotypically distinct from cDC1s. Mouse cDC2s were first identified by their expression of CD4 and it is now known that most cDC2s express Sirpα and CD11b. Like cDC1s, some intestinal cDC2s express the integrin CD103 and it is the only organ in which they express it (4). In fact, intestinal cDC2s can be subdivided into CD103$^+$CD11b$^+$ and CD103$^-$CD11b$^+$ cDCs, making CD11b in mice and SIRPα in humans the most specific markers for both subtypes of intestinal cDC2s. Interestingly, the identity of CD103$^-$CD11b$^+$ cells has been a matter of debate because they have been suggested to be more reminiscent of macrophages, since they develop in an FLT3L-independent manner, suggesting that they could in fact be macrophages rather than cDCs, although evidence for both exists (4).

In PPs, cDC2s are found in the follicle-associated epithelium, specifically in the subepithelial dome (50). Whereas cDC2s in the mLNs are mostly Esam$^+$ and CD24^{high} (68), some small-intestinal cDC2s are Clec12a$^+$ and Esam$^-$. It has been observed that cDC2s have lower levels of CCR7 than cDC1s, which could explain their border location and preferential interaction with naive CD4 T cells (68). PD-L1$^+$ cDCs are mainly found along the small-intestinal LP and have been proposed to be the regulatory subset of cDC2s in the gut, as depletion of these subsets resulted in an inflammatory gut environment (58). However, as discussed later in 'The thin line between intestinal ILC3s and cDCs' section, other cells have been found to play a role in this process.

In mLNs, both cDC1s and cDC2s can prime Th1 responses, but cDC2s are uniquely required for Th2 and Th17 responses. Specifically, the Th2 priming that occurs in response to *Trichuris muris* worms and *Schistosoma mansoni* eggs was shown to be entirely mediated by IRF4-dependent cDCs (69). The same study also showed that CD103$^+$CD11b$^+$ cDCs induce Th2 polarization in the small intestine, whereas CD103−CD11b$^+$ cDCs had this role in the colon (69). This cDC2-driven response is tightly controlled by IL-12 production from cDC1s, which suppresses Th2 response development (70). A recent paper showed that CD103$^+$CD11b$^+$ migratory cDC2s that produce IL-6 and express CD40 and CCL17/CCL21 can prime naive T cells into a Th17 phenotype in the mLNs; thereafter, Th17s migrate to the intestine where final Th17 differentiation and maintenance occur and are likely supported by intestinal macrophages (68).

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Group 3 innate lymphoid cells

Recently, in 2008—40 years after the discovery of T and B cells—12 independent groups around the world reported a new group of cells, present in both humans and mice, that were identified as innate lymphocytes. These cells were shown to be mostly barrier-resident and are now considered the innate counterparts of the helper and/or cytotoxic T-cell subtypes because of the pattern of transcription factors they express and the effector cytokines they release. According to currently accepted nomenclature (7), they are divided into five subsets: natural killer (NK) cells, ILC1s, ILC2s, ILC3s and LTi (lymphoid tissue inducer) cells. It is also important to mention that some unconventional T and B cells are considered to be part of the innate lymphocyte group and are termed 'innate-like'. They are enriched within the mucosa and are characterized by limited clonal diversity and memory, lack of antigen-specific receptor rearrangement, and rapid responses to stimuli (71). These cells are divided into three main groups, namely: (i) unconventional innate-like T cells, which include NKT, mucosal-associated invariant T (MAIT), γδ T and natural CD8αα$^+$TCRαβ$^+$ cells, (ii) conventional innate-like T cells, represented by innate and virtual memory CD8$^+$ T cells; and (iii) innate B cells, which include B-1 and marginal zone B cells (72). Here, we aim to describe only the ILCs, with greater emphasis on the intestinal ILC3.

Though both NK cells and ILC1s express the transcription factor T-BET and secrete IFN-γ, NK cells are dedicated cytotoxic cells in the bloodstream whereas ILC1s are mainly tissue-resident and secrete high levels of IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF); together their responses mirror those of CD8$^+$ and Th1 cells. ILC2s express GATA3, secrete IL-4, IL-5 and IL-13 and resemble Th2 cells; ILC3s express the transcription factors RORγt and AHR (Aryl hydrocarbon receptor), secrete mainly IL-22 and IL-17 and resemble Th17 cells. Finally, tissue-resident LTi cells, another subset of group 3 ILCs, express RORγt and produce IL-17, IL-22, lymphotoxin and TNF-α (73).

Similar to T and B lymphocytes, immature ILCs originate in the bone marrow through a CLP. However, an ILC-restricted progenitor (the common innate lymphoid progenitor, CILP) appears, giving rise to two main lineages of ILCs: the NK progenitor (NKP) and the common helper-like ILC progenitor (CHILP) (Fig. 1) (74). Whereas helper-like ILCs express IL-7Rα and require GATA-3 for their differentiation, killer ILCs do not express IL-7Rα and are independent of GATA-3 for maturation (75). These IL-7Rα$^+$ cells tend to migrate to barriers and mucosal tissues, mainly the intestine, lung and skin, although some go to lymphoid tissues, such as the spleen and lymph nodes as well as non-lymphoid organs like the liver, brain and pancreas (76).

Specifically, ILC3s play a key role in intestinal homeostasis and maintenance of epithelial barriers (77). These cells, which are abundant in the gut LP—mainly in the small intestine—are characterized by two key features: (i) expression of the transcription factor RORγt in which the 't' stands for thymus as this isoform of the *Rorc* gene was originally identified in this organ and was long thought to function solely in Th17 cells and (ii) production of IL-17A, IL-22, TNF-α, IFN-γ and GM-CSF in response to signals chiefly released by resident cells, such as IL-1β and IL-23 (78).

ILC3s constitute a heterogeneous population of cells with some distinct and non-redundant functions. Expression of the surface protein NKp46—also known as natural cytotoxicity receptor 1 (NCR1)—distinguishes the main two subpopulations of ILC3s (NCR$^+$ or NCR$^-$). One of the NCR$^-$ iLC3 subsets, LTi cells, express CD4 and the chemokine receptor CCR6, originate from fetal liver and are present in the lymphoid organs and colon, whereas the NCR$^-$CCR6$^-$ ILC3s are more broadly distributed along the whole intestinal LP (79–81). The NCR$^-$CCR6$^+$ LTi cells produce lymphotoxin and TNF-α and stimulate the production of chemokines and expression of adhesion molecules by mesenchymal cells and participate in the formation of lymphoid organs (73). On the other hand, NCR$^-$CCR6$^-$ ILC3s are a substantial source of IL-22 and IL-17, mainly acting on epithelial and stromal cells (82).

Emerging RORγt$^+$CCR6$^-$ ILC3s acquire expression of T-BET for subsequent expression of NCR, manifesting an ILC1-like phenotype marked by production of TNF-α and IFN-γ, in addition to some IL-22 and relatively little IL-17 (81, 83, 84). In general, CCR6$^-$ ILC3s directly participate in the homeostatic regulation of the interaction between host and the intestinal microbiota and contribute to the response during infectious conditions, such as those caused by *Citrobacter rodentium* and *Clostridium difficile*, and are essential in T-cell deficient mice (85–90).

The thin line between intestinal cDCs and ILC3s

Having reviewed the major function of these cells, it is now clear that ILC3s and cDCs have distinct roles in the intestine. However, there is increasing evidence that the boundaries delimiting these two cell types are blurred in some cases. One very clear area of current interest is the identity of the cell responsible for presenting intestinal antigens such that tolerance to commensal microbiota is established through the generation of iTregs, which express RORγt in the gut and suppress effector Th17 responses. Importantly, as mentioned above and detailed below, multiple groups have suggested that the cell that does this job expresses the transcription factor RORγt.

From a developmental point of view, although cDCs and ILCs arise from different cell lineages (Fig. 1), there are many similarities between the development of the two cell types. For instance, a publication from 2019 (91), described a classification of mouse type 2 DCs that is very reminiscent of the classification of ILCs and T cells: cDC2As, which are T-bet and Runx3-dependent cells; and cDC2Bs, which are RORγt and C/EBPα-dependent. This antagonism between T-bet and RORγt echoes that seen in the development of ILCs and T cells. Moreover, recently a group identified a subset of ZBTB46-expressing ILC3s—the transcription factor that was thought to be most specific to DCs (92). These ILC3s were of the CCR6$^+$ LTi type.

An important field was opened in 2021, with the discovery of a group of cells in the mouse that express RORγt in addition to the autoimmune regulator (AIRE) (93). Although extrathymic AIRE-expressing cells (eTACs) have long been hypothesized to exist in secondary lymphoid organs (94–96),

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it was first established that eTACs comprise two similar cell types in this study. The first eTAC subtype expresses CCR7 and is a migratory cDC subset, whereas the second one is an AIRE^{hi} population that co-expresses AIRE and RORγt, which the Gardner group named Janus cells (93). Both eTAC subtypes were found to highly resemble CCR7^{+} migratory cDCs and established AIRE expression via RANKL–RANK interactions, which play an important role in the thymus as well. Importantly, Janus cells have broad chromatin accessibility and express genes encoding a variety of tissue-specific antigens, which suggests that AIRE functions in these cells in a manner much as it does in AIRE-expressing thymic medullary epithelial cells (mTECs).

Moreover, our view of the distinction between ILC3s and cDCs has recently been challenged by the simultaneous discovery of three different groups of a subset of cells that can induce the differentiation of peripheral iTregs in the intestine. Although the three groups agreed that the interaction between these cells and T cells occurs through the TGF-β-activating integrin αvβ8, they disagreed on the identity of the APC in charge of this phenomenon, described in detail below.

The group of Dr Dan Littman suggested that the iTreg-inducing cells are either ILC3s or the recently described Janus cells (59, 93). They employ *Helicobacter* infection because it can induce Tregs peripherally, but induces Th17 cells when iTregs are compromised. They found that this process is mediated by antigen presentation by cells that express RORyt and are distinct from classical DCs. Using *CD11c^{Cre} I-Ab$^{fl/fl}$* mice, they hypothesize that these cells are either ILC3s or Janus cells. They find that their candidate cells express CCR7, which is known to be used by both ILC3s and cDCs to migrate from the intestine to the draining mLNs, as well as the TGF-β activator integrin αv; both factors were necessary and sufficient for the induction of peripheral Tregs and suppression of Th17 cells. Though they showed that *Rorc^{cre} Aire$^{fl/fl}$* mice have no defect in iTreg differentiation, they concluded that they cannot rule out the contribution of Janus cells to this process, since AIRE itself may not be necessary for microbiota-dependent iTreg induction. Moreover, using a fate reporter mouse line, Itgb8-IRES-tdTomato, they showed that both Janus cells and a fraction of LTi-like ILC3s expressed tdTomato.

The group of Dr Gregory Sonnenberg postulated that iTreg-inducing cells are MHC-II^{+} ILC3s (12). Using single-cell transcriptomic analysis of MLNs of mice, they identified RORγt-expressing LTi-like ILC3s that co-localize with Rorγt-expressing iTregs at inter-follicular regions. These cells bear transcriptomic profiles distinct from those of extrathymic AIRE-expressing cells (which they name eTACs I and II), and express high levels of MHC-II. To delineate the mechanism, they used *Rorc^{cre} H2-Ab1$^{fl/fl}$* mice, in which MHC-II is ablated in all *Rorc*-expressing cells, particularly in MHC-II^{hi} LTi-like ILCs relative to other immune cells in the draining LN and the large intestine. In these mice, they found a marked reduction in iTregs, thus suggesting that MHC-II expression on LTi-like ILC3s is both necessary and sufficient to promote iTregs and prevent their differentiation into Th17 cells. They postulate that these MHC-II^{+} ILC3s present antigen via integrin αvβ3 (using *Rorc^{cre}Itgav$^{fl/fl}$* mice), which can process latent TGF-β, and that this process is partially dependent on competition for IL-2. They show that the interaction between these two cell types is altered in inflammatory bowel disease. Finally, using an infection model with *Helicobacter hepaticus,* they postulate that the interaction between these MHC-II^{+} ILC3s and RORγt^{+} Tregs is antigen-specific using detection on *H. hepaticus*-specific T cells and they serve to establish tolerance to microbiota.

Finally, Drs Brown and Rudensky *et al*. together claim that iTreg-inducing cells are a unique lineage of cells that do not arise from the classically described cDC and ILC lineages; they dub these Thetis cells (TCs), which seem to be critical during neonatal seeding of iTregs into tissues (60). All the mice used in the experiments in this study were young, and TCs peaked around post-natal day 15. The distinction between TCs and LTi-like cells becomes important. Although both cell types express RORγt, RORα, MHC-II, and are Lin^{-}, CD64^{-} and Ly6C^{-}, TCs are distinct from LTi-like ILC3s because they lack expression of CXCR6. TCs are sub-grouped into four distinct categories: TCI I cells, which are CD11c$^{-/lo}$, SiglecG^{+}, CCR6^{+}, AIRE^{+} and are reminiscent of Janus cells or RORγt^{+} eTACs; TC II cells, which are CD11c^{+} CCR6^{+}; TC III cells, which are CD11c^{+} Siglec G^{-}, CCR6^{-} and AIRE^{+}; and TC IV cells, which are CD11c^{+} and CD11b^{+} and presumed to be RORγt^{+} cDC2s. The term 'Thetis' refers to the shape-changing God from Greek mythology and has its root in the computational comparison of the signature of these cells to known cell types; a resemblance was found to thymic epithelial cells, as well cDCs and ILC3s, thus highlighting the hybrid nature of these cells. Whereas TC I and III shared expression of AIRE with mTECs, they found that TC IV was the TC subset that instructed extrathymic Treg development. These results suggest that there is an early wave of tolerogenic TCs that is necessary for life-long tolerance to commensal microbiota.

In all three cases, deletion of these RORγt cells led to a significant impairment in intestinal iTreg differentiation with ensuing expansion of Th17 cells. Other studies also suggest that cDCs and ILCs have many commonalities, and this further suggests probable interactions (Fig. 2). One of the most studied cDC–ILC interactions is the production of IL-23 by cDCs, which in turn activates ILC3s. Studies of *Citrobacter rodentium*, a mouse model for the enteropathogenic *Escherichia coli* bacterium, have shown that IL-23 and IL-22 are important for early clearance of the bacterium (97, 98).

It was later shown that Notch2-dependent CD11b^{+} cDCs were the non-redundant cell type responsible for producing the IL-23 in response to *Citrobacter rodentium* (65) and this lack of Notch2 was superior in displaying increased susceptibility to infection as compared with *Irf4$^{-/-}$* and *Ccr7$^{-/-}$* mice (65), suggesting that tissue-resident cDCs that reside in the intestine produce IL-23, which is sufficient to induce IL-22 production from ILC3s and control pathogen clearance.

IL-23 produced by cDCs also has important roles in the skin, specifically in inducing γδT cells when driven by nociceptive sensory fibers (99). This interaction between cDCs and ILC3s is not the only one of its kind. In the intestinal crypto patches (CPs) and GALT, there is a recently described type of intestinal cDC that the authors refer to as the CPs and isolated lymphoid follicles-associated DC (CIA-DC), which interact with GALT-resident CCR6^{+} ILC3s by responding to

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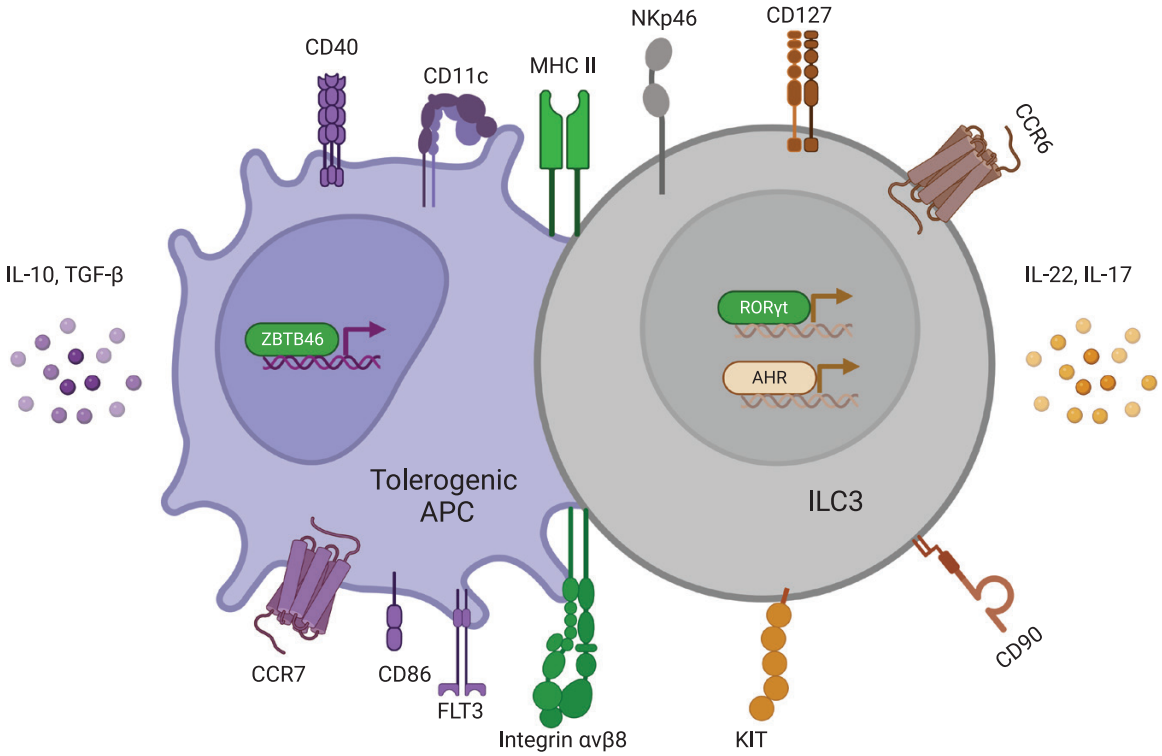

Fig. 2. The spectrum of features that distinguish tolerogenic APCs and ILC3s. On the left (purple) is a tolerogenic APC that characteristically produces immunosuppressive cytokines like IL-10 and TGF-β, expresses the co-stimulatory molecules CD40 and CD86, the chemokine receptor CCR7 and the integrin CD11c and develops by signaling through the FLT3 receptor. On the right (gray) is an ILC3, which can produce lymphotoxin, IL-22, IL-17 and GM-CSF. It expresses CD90 and can express NKp46 (NCR1) and the chemokine receptor CCR6. Its development is dependent on signaling through the receptors CD127 and KIT. Transcriptionally, tolerogenic APCs depend on ZBTB46 and ILC3s depend on RORγt and AHR. In green are represented the molecules that have been reported to be expressed in both cell types, like MHC-II, integrin αvβ8 and the transcription factors ZBTB46 and RORγt.

lymphotoxin β secreted by ILC3s (100). These cells can be generated *in vitro* only in the presence of ILC3s. Moreover, CIA-DCs was found to be a major source of IL-22 binding protein (IL-22BP) at a steady state, suggesting a possible regulatory mechanism for IL-22, which is constantly produced by ILC3s in these tissues (100).

The role of gut microbiota for cDC and ILC3 functions

It is estimated that in the large intestine alone, an adult human contains about 10^{14} bacteria of more than 1000 different species (101). Studies have shown that the normal intestinal microbiota is composed of bacteria from different phyla, including Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, Verrucomicrobia, Cyanobacteria and Spirocheates (101, 102). However, microbial quantity and diversity, as well as their derived metabolites, can vary between individuals because of several factors, primarily including host age, diet and health status (103). Interactions between the host and the commensal intestinal microbiota have begotten mutual benefits and promote resistance to pathogen colonization, thus constituting an exogenous biological defense mechanism (104–108).

In general, host-microbe interactions take place to and fro: epithelial cells and cDCs detect changes in the microbial environment and respond functionally to alert other immune cells; immune cells react in ways that impact the epithelial cells and commensal microbiota. In fact, intestinal immune cells contain and modulate the luminal bacterial allocation and composition (109–112). In this section, we summarize the crosstalk between commensal microbiota and intestinal innate immune cells—describing in more detail these effects on ILCs and cDCs (Fig. 3).

cDCs receive signals from the microbiota and in turn, interact with other intestinal cells in situations of intestinal insult. For instance, intestinal cDCs regulate the availability of hepcidin, which is required for adequate repair of the intestinal barrier, in response to microbial stimulation (113). Hepcidin then acts on ferroportin-expressing phagocytes to increase the local availability of iron, which in turn regulates the microbiota and promotes healing (113).

The microbiota also instructs tonic production of IFN-α (a type I IFN) by pDCs, which is continuously sensed by cDCs and keeps them prepared to respond when encountering an intestinal pathogen, though they are subdued by local tolerogenic mechanisms (114). For example, following infection by Chikungunya virus in antibiotic-treated or germ-free mice, there is greater Chikungunya burden and spread because of an alteration of the TLR7–MyD88 signaling in pDCs and abrogation of systemic IFN-α production, thus blunting monocyte IFN-mediated activation (115). This effect was found to be mediated by *Clostridium sciendens*

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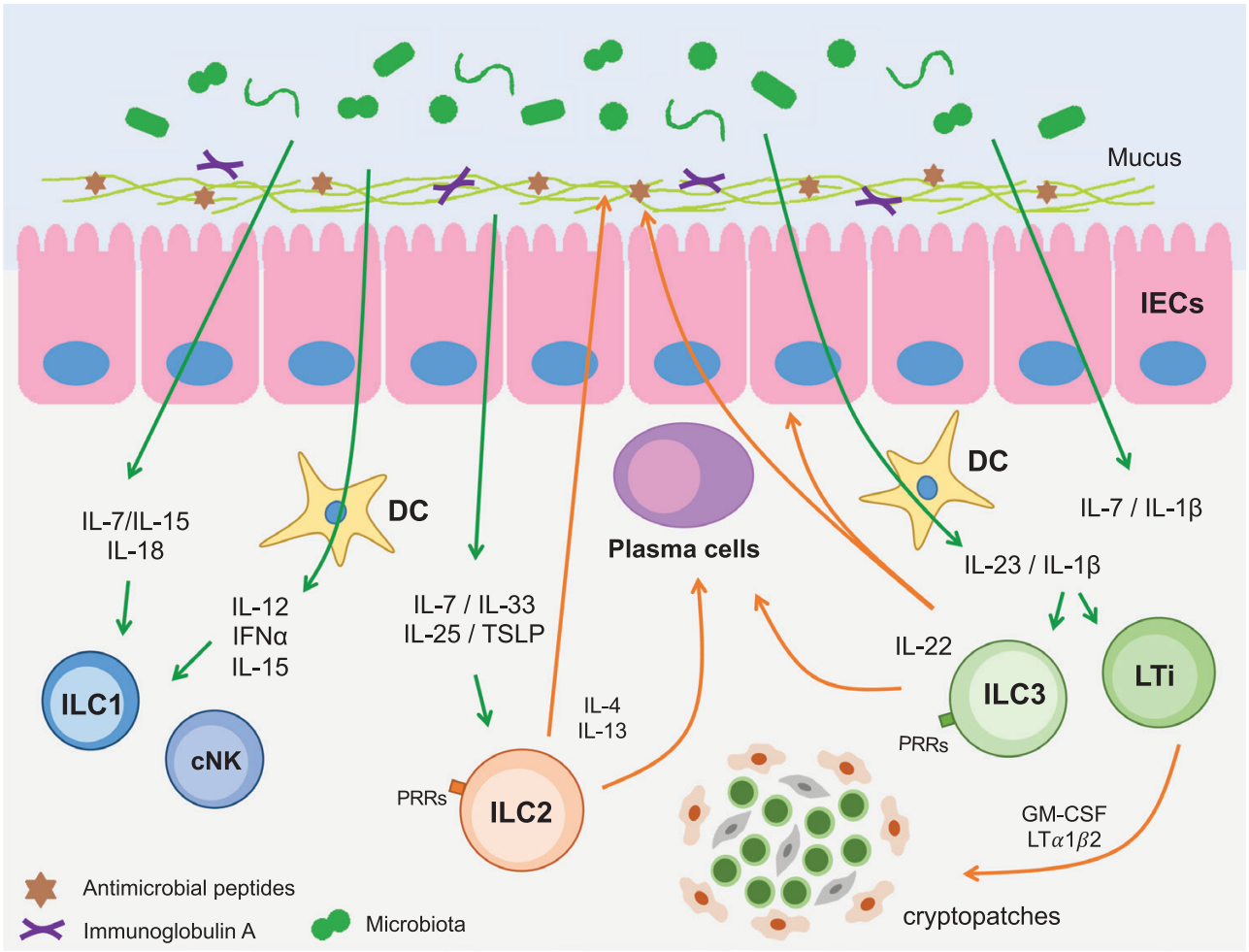

Fig. 3. Crosstalk between intestinal innate immune cells and the microbiota. Host-microbe interactions are two-way: intestinal epithelial cells especially can detect changes in the microbial environment and can respond functionally to adaptive immune cells, as well as cDCs and ILCs, which can react back to the commensal microbiota. In particular, ILCs are a heterogeneous population of cells that contribute to the maintenance of homeostasis and prevent the development of diseases. Together with cDCs, they are responsible for inducing tolerance to commensal microbiota and, during dysbiosis and/or infection, modulate the responses of other cells, such as phagocytes, and T and B cells.

and, specifically, its metabolite the secondary bile acid deoxycholic acid (115).

In addition, Th17 cells and ILC3s play crucial roles in the maintenance of the integrity of the epithelial cell barrier at homeostasis (116). For instance, it has been proposed that depletion of ILC3s can lead to systemic dissemination of commensal pathogens, which can be stopped by administration of IL-22 (117). Both the polarization of Th17 cells and the activation of ILC3s are achieved largely through cDCs. Specifically, intestinal Th17 cells are induced via antigen presentation (118) and production of IL-6 (119) and TGF-β (120) by intestinal IRF4-dependent $CD103^+CD11b^+$ cDC2s (119). ILC3s are activated by release of IL-23 by $CD103^+CD11b^+$ cDCs, which was shown in a model of TLR5 activation by flagellin (121).

Moreover, lysozyme-expressing cDCs and $CD11b^+$ PP cDCs sense mucosa-associated commensals through the Mincle and Syk pathways and secrete IL-6 and IL-23 p19, which in turn regulate production of IL-22 by both Th17 cells and ILCs (122). It has also been suggested by several groups that an increase in intestinal IgA, Th17 and Treg responses is associated with resistance to diet-induced obesity, which can change the composition of the microbiota (123).

Under homeostasis, ILC3s are the main source of IL-22, a cytokine that, among other actions, regulates the proliferation and the production of mucus and antimicrobial peptides by intestinal epithelial cells (85, 124–129). Thus, ILC3s indirectly influence the microbiota localization/composition by controlling the mucus layer and IL-22-regulated antimicrobial peptide expression, including that of the Reg3 family (130–132).

Given that IL-22 also impacts the microbiota composition, *Il22*$^{-/-}$ mice exhibited an altered intestinal microbiota and a major susceptibility to induced colitis (133). This gut microbiota phenotype was transmitted to co-housed wild-type (WT) mice and also increased their susceptibility to colitis, underscoring the importance of ILC3-driven IL-22 production in shaping the microbial communities (133). Segmented filamentous bacteria (SFB) expanded in the gut of mice lacking functional ILC3s (including *Rorc*^{Cre} *Id2*$^{fl/fl}$ and *Ahr*$^{-/-}$ mice), which was associated with resistance to colonization with

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Citrobacter rodentium (134–136). In the absence of LTi cells, ILC3s and Th17 cells (*Rorc*-deficient mice) the serum titers of specific IgG against commensal microbiota were increased, indicating potential bacterial translocation to the bloodstream and peripheral tissues through a disrupted gut epithelial barrier (116).

The commensal microbiota also promotes proficiency of the host immune responses and controls ILC3 activity (137, 138). APCs are able to produce IL-1β and IL-23 after microbiota stimulation, which enhances ILC3 production of IL-22 and GM-CSF (89, 121, 139–145).

In addition, the cooperation between intestinal epithelia and ILC3s is directly regulated by signals from the microbiota, which plays an important role in modulating ILC3 activity through recognition of bacterial components by innate receptors, such as TLRs and NCRs, and indirectly through the production of IL-7, IL-15, TSLP, IL-1β, IL-23 and IL-25 by immune and non-immune cells (121, 144, 146–148). In fact, germ-free and antibiotic-treated mice have decreased intestinal epithelial cell-derived IL-7, and consequently fewer NCR$^+$RORγt$^+$ ILC3s, as well as IL-22 production, in the small-intestinal LP (83, 125, 126, 149).

In addition, during embryonic development, fetal liver-derived LTi cells initiate the development of aggregated lymphoid nodules, also known as PPs. These lymphatic tissues have evolved to allow interactions between microorganisms and immune cells after birth and throughout life, monitoring intestinal bacterial populations and priming T-cell-dependent IgA production at barrier tissues (150–152). Similarly, LP CCR6$^+$ ILC3s express membrane-bound LTα1β2 and secrete soluble GM-CSF, a key for lymphoid organogenesis and CP formation; these are B-cell-containing isolated lymphoid follicles for preferentially T-cell-independent IgA production by plasma cells (153). CPs are controlled by microbiota-dependent IL-1β and mediate oral tolerance to dietary antigens (154). The contribution of ILCs, especially ILC3s, in the activation of B and T cells and lymphoid organogenesis further reinforces their indirect role in the composition of the microbial community.

cDCs and ILC3s control enteric infection

Intestinal innate immune cells are important players during viral, bacterial and fungal pathogen infection. As mentioned above, ILC3s resemble adaptive lymphocytes in responses against pathogens: ILC1s resemble Th1 cells, ILC2s resemble Th2 cells and ILC3s resemble Th17 cells, which act through type 1, 2 and 3 responses, respectively. Considering the similarities between cDCs and ILC3s, in this section, we will discuss the involvement of these cells in fighting enteric infections.

LTi cells and ILC3s cells play a critical role against enteric pathogens through IL-22 production and antimicrobial peptide-induced expression by intestinal epithelial cells. This cytokine-mediated ILC3 activity precociously controls proteobacteria-promoted intestinal infection, such as a murine enteropathogenic homolog of *E. coli*, *Citrobacter rodentium* (124–126, 155). Similarly, ILC3-derived IL-17 acts on neutrophil recruitment and is essential to promote a response against *E. coli* K1 and *Klebsiella pneumonia*, associated with less intestinal bacterial translocation and sepsis (156). Indeed, ILC3-derived IL-17 and IL-22 also regulate colonization by opportunist pathobionts, such as *Staphylococcus aureus* (157, 158), *Mycobacterium tuberculosis* (159) and fungal *Candida albicans* (160–162).

Additionally, IL-22-producing ILC3s also protect against infection by the intestinal pathogen *Salmonella enterica* serovar *Typhimurium* through IL-17 and IFN-γ (81, 140). However, another study showed a detrimental role of ILC3s during *Salmonella* infection because the activation of the ILC3–IL-22 axis enhances early infection by reducing competition with the microbiota; however, increased ILC3 pyroptosis during the late stages limits IL-22-dependent support of pathogen colonization (163). Similarly, another group showed that the production of IFN-γ by ILC3s potentiated *S. enterica* infection (81), as well as IL-17-induced intestinal fibrosis (164), showing a harmful role of intestinal ILC3s in some cases.

Likewise, ILC3s also extend their protective role to virus infection. IL-22- and IFN-γ-producing ILC3s potentiate the resistance of the intestinal epithelia to rotavirus (165). Moreover, NCR$^+$ ILC3s can be directly activated by the hemagglutinin of influenza virus and by antigens of mycoplasma-infected cells (166–168). Similarly, ILC3s also mediate immunological protection against the intracellular parasite *Toxoplasma gondii* (165, 169).

Knowledge of the role of intestinal cDCs in both fighting and disseminating viral pathogens has seen many advances in the recent years. Many of these studies have been shown using mice containing Cre targeting the integrin CD11c, which we now know is not specific for cDCs, as it can also be expressed on macrophages and many other cells, though the findings from these studies are important.

For instance, it was shown that murine norovirus (MNV) infects CD11c$^+$ cells (170) *in vitro* but, *in vivo*, depletion of CD11c$^+$ cells has two different consequences: on the one hand, at early post-infection timepoints, there is increased intestinal viral load, with subsequent defects in the generation of anti-MNV antibodies; on the other hand, depletion is protective as CD11c$^+$ cells enable MNV dissemination to secondary lymphoid tissues (171). With the description of CD300LF as the MNV receptor, it was shown that MNV primarily infects Tuft cells (172). Though cDCs also express CD300LF and can propagate the virus, they are not critical for MNV infection (172).

Specific molecules on cDCs have been shown to drive viral infection. One such molecule is CD40. In untreated HIV-infected patients, cDCs had increased CD40 and decreased CD83 (173). CD40$^+$ DC2s were positively correlated with HIV viral loads, as well as with frequencies of activated T cells both in the colon and the periphery, whereas frequencies of CD83$^+$CD1c$^+$ cDCs were negatively correlated with frequencies of Th1 cells and cytotoxic T lymphocytes (173). Interestingly, this study was also linked to pathogenic commensal bacteria, as CD40 on cDC2s was positively correlated with the abundance of species, which were shown to stimulate cDC2s (173). This suggested that HIV causes dysbiosis in which mucosal pathogenic bacteria appear, and this contributes to mucosal and systemic immune activation.

Finally, it is worth mentioning that cDC1-mediated cross-priming occurs both at a steady state and during viral infections. Specifically, in neonates infected with rotavirus, cDC1s

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are the only cells able to cross-prime, but compensatory cross-presenting mechanisms seem to exist in adults (174). cDC1s, by means of expressing the TGF-β-activating integrin αvβ8, are also critical for induction of rotavirus-specific IgA secretion in mLNs. However, the expression of this integrin by cDC1s was shown to be dispensable during homeostatic states, since homeostatic IgA secretion is a characteristic of cDC2s (175).

Intestinal cDCs are also important in controlling intestinal bacterial pathogens. *Salmonella,* which is a facultative intracellular pathogen, encodes a set of virulence factors collectively known as the type III secretion system (T3SS). Intestinal cDCs that express TLR5 recognizes the flagellum expressed by *Salmonella* (121, 176). Once taken up by cDCs, *Salmonella* induces CCR7 expression on the DC surface, inducing migration of cDCs into secondary lymphoid tissues following the chemokine gradient of CCL19 and CCL21 (ligands for CCR7). It was found that PP-resident CCR6$^+$ cDCs locally activate *Salmonella*-specific T cells (177). However, T3SS can interfere with cDC functions, as a specific component called the SPI2-T3SS forms a *Salmonella*-containing vacuole (SCV), which allows it to detach from the endolysosomal system of the cDC, rendering the cDC incapable of presenting *Salmonella*-specific antigens in the mLNs (178).

Other studies have shown that mice that are conditionally depleted of Notch2-dependent cells were unprotected against infection with *Citrobacter rodentium*, as the early stages of infection require IL-23 produced by Notch2-dependent cells (65). Interestingly, antigen presentation by cDCs seems to be dispensable for other infections, like *Clostridioides difficille*, as *Rag1*-knockout mice have identical infection kinetics to WT mice (87), despite evidence showing that cDCs are activated in response to infection (179, 180), suggesting that cDCs may interact with other cells to fight this pathogen.

Finally, intestinal cDCs are important to fight fungal infections. Interestingly, a subset of Langerin$^+$ DCs, found in the PPs, sample *Candida albicans* antigens via a process that is partially M-cell dependent (181). In fact, PP cDCs have the most pattern-recognition receptors that recognize *C. albicans*. Following antigen uptake, *Candida. albicans*-experienced cDCs can mount MyD88-dependent Th2 and Th17 responses, as well as Th1 and Treg responses by inducing TRIF signaling [reviewed in (182)].

Conclusion

Here, we have highlighted relevant concepts in the spectrum of knowledge about the functions played by intestinal cDCs and ILC3s, both in essence and in the context of infections and the gut microbiota. It is important to note that we have highlighted the most recent studies in the field that explored the functions performed by these cells to induce intestinal immunity. Certainly, the gut microenvironment does not exist in isolation and the only way to get a complete picture of gut immunity is to consider all these cells not only interacting with each other, but also co-inhabiting the gut with unicellular, multicellular and even acellular organisms.

In addition, the extent of the roles played individually by cDCs and ILC3s is still unclear, and we also note that the full spectrum of DC–ILC interactions has not been comprehensively explored. Current evidence suggests that a myriad of steps exist to ensure that antigen-independent ILCs, in their T-cell mirroring capacity, require cDC-derived signals to thrive, especially in the intestine. A compelling emerging topic is the blurring of cDC and ILC3 functions that have been suggested: can ILC3s interact and act as cDCs, and vice versa? Lastly, key interactions between these two cell types could pave the way for new therapies, especially for the treatment of human inflammatory bowel disease.

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