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Antigen-presenting cells as arbiters of mucosal tolerance and immunity

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Within the intestine, the immune system encounters a vast array of microbial antigens, as well as dietary components. Antigen-presenting cells (APCs) play a critical role in tailoring an appropriate immune response, ensuring both tolerance to innocuous antigens and protection from pathogens. An explosion of single-cell transcriptomic studies has revealed new subsets of APCs within mucosal tissues and lymph nodes, most notably within the gut. Harnessing their full potential to elicit protective immunity during oral vaccination or restoration of tolerance in inflammatory or allergic diseases requires an in-depth understanding of their unique functional roles and differentiation programs. Here we review the growing understanding of APC heterogeneity and discuss how balance and cooperation between distinct subsets shape mucosal immunity, inflammation and tolerance.

Maintaining homeostasis at mucosal surfaces represents a daunting task for the immune system. Diverse classes of pathogens can breach epithelial barriers, necessitating distinct types of inflammatory responses for successful clearance and host protection. At the same time, the immune system must establish and maintain tolerance to the trillions of symbiotic commensal microorganisms that colonize epithelial tissues. APCs express pathogen-sensing molecules and can initiate tolerogenic or inflammatory T cell responses, thereby serving as both sentinels and gatekeepers that regulate this delicate balance to achieve tissue homeostasis. Moreover, APCs provide critical signals, such as cytokines, that direct the differentiation of naive T cells into specific subsets, thus coordinating an immune response tailored to the nature of the antigen.

Mucosal barriers and associated lymphoid tissue harbor a spectrum of APCs, including classical dendritic cells (cDCs), monocytes, macrophages and epithelial cells. The role of these APCs in shaping T cell-mediated immunity and tolerance, and the mechanisms by which different APCs instruct diverse CD4$^+$ T cell programs remain incompletely understood. Recent single-cell transcriptomic studies have uncovered a wealth of new information regarding the diversity of these cell types, including identification of new DC subsets and previously uncharacterized retinoic acid receptor-related orphan receptor-γt (RORγt)-positive APCs. As the arsenal of identified APCs continues to grow, now encompassing as many subsets as effector T (T_{eff}) and regulatory T (T_{reg}) cell types, a 'one APC–one T cell fate' framework becomes plausible. Here, we review recent advances in our understanding of APC heterogeneity, the role of individual subsets in shaping mucosal immunity, inflammation and tolerance, and discuss emerging models for how APCs collectively regulate the full spectrum of T cell responses.

The expanding spectrum of APCs

APCs are defined by their expression of major histocompatibility complexes (MHCs) and their ability to stimulate T cells via their cognate T cell antigen receptor (TCR). Among APCs, cDCs are classically regarded as the principal initiators and modulators of both CD4$^+$ and CD8$^+$ T cell responses, owing to their superior ability to prime naive T cells relative to other known APC types. The division of cDCs into two subsets—cDC1 and cDC2—on the basis of mutually exclusive expression of cell-surface markers such as XCR1 and CD11b, demarcates a clear functional separation: cDC1s express key molecules required for cross-presentation of antigens[1], and have an essential role in activation of cytotoxic CD8$^+$ T cells during viral infection and tumor immunity[2]. Conversely, cDC2s are often suggested to preferentially instruct CD4$^+$ T cell priming. However, CD4$^+$ T cell responses are highly

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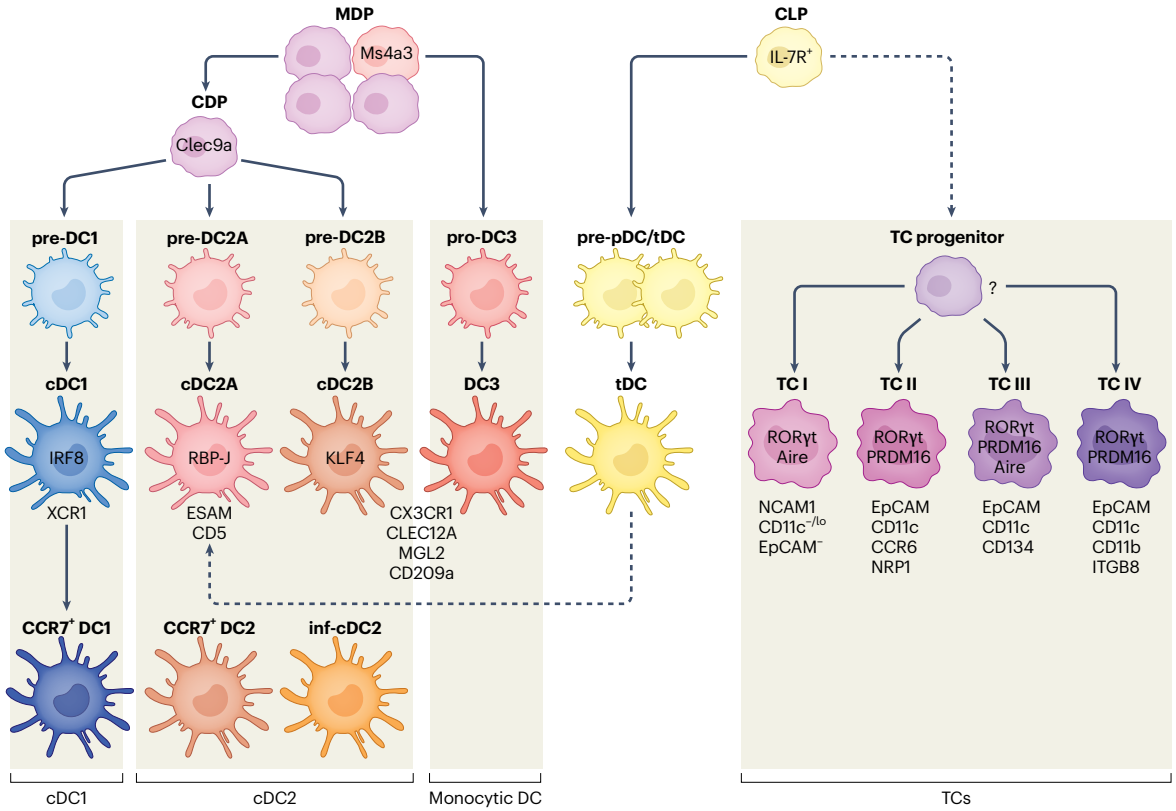

Fig. 1 | The expanding spectrum of APCs. cDCs are derived mainly from the CDP. cDCs are broadly divided into two major subgroups: cDC1 and cDC2; however, cDC2s are a heterogeneous group of cells, composed of two discrete subsets, cDC2A and cDC2B, that can be distinguished by characteristic cell-surface markers and transcription factors. In the spleen, cDC2As uniformly express ESAM; however, this marker is variably expressed by cDC2A in other lymphoid tissues. In mice, all cDC2As express the transcription factor T-bet. cDC2Bs are distinguished by expression of CLEC12A, CD209a and variable expression of MGL2/CD301b, depending on the tissue or LN. Although some of these genes are characteristic of monocytes, cDC2Bs are derived from the CDP. In contrast, a third 'DC' subset, DC3, that shares transcriptional features and cell-surface markers with cDC2B, is derived from monocytic progenitors. In addition to the classical pathway of cDC differentiation, tDCs that share similarities with pDCs, including a proposed lymphoid pathway for development, can give rise to both pDCs and cDC2s. Activating or inflammatory cues can promote the acquisition of additional dendritic cell states: cDC1 and cDC2s can differentiate into CCR7$^+$ DCs that migrate from tissue to LNs; inf-cDC2s arise during infection. A new family of APCs, TCs, that express RORγt have recently been identified within LNs and Peyer's patches. TCs are composed of four subsets (TC I–IV) that are distinguished by characteristic patterns of cell-surface markers and transcription factors. TCs can be distinguished from RORγt$^+$MHC class II$^+$ ILC3s by absence of CXCR6 and RORα expression, and expression of PRDM16 by TC II–IV. TC I and III express Aire. TCs are ontogenically distinct from cDCs. Lineage-tracing approaches suggested that TCs are derived from lymphoid progenitors. All cDC and TC subsets express Zbtb46. MDP, monocyte-dendritic cell progenitor; CLP, common lymphoid progenitor.

heterogeneous, spanning tolerogenic and inflammatory subsets, each with specialized functions. Different models have been proposed to account for the ability of cDCs to regulate the full gamut of CD4$^+$ T cell responses, including discrete subsets of cDCs, each endowed with particular CD4$^+$ T cell priming properties, versus cDC plasticity. In the latter scenario, environmental cues are thought to promote acquisition of specialized functions of APCs, such as loss of tolerogenic potential and expression of CD4-polarizing cytokines. The discovery of a previously uncharacterized RORγt$^+$ APC subset that instructs peripheral T_{reg} (pT_{reg}) cell differentiation and intestinal tolerance[3–5], as well as recently described cDC subsets[6–9] (Fig. 1), adds further weight to a division of labor among APCs, whereby each subset preferentially mediates a discrete branch of mucosal immunity or tolerance, akin to the functional division between cDC1 and cDC2 subsets.

cDC2 heterogeneity

Here we consider cDCs according to their ontogeny in which cDC subsets are predominantly derived from a CLEC9A$^+$ common dendritic cell progenitor (CDP) that undergoes progressive differentiation and commitment to a particular cDC type within the bone marrow[10,11]. These pre-DCs then seed lymphoid and nonlymphoid tissues, where they complete their differentiation, guided by environmental cues that shape their fate[10,11]. In contrast to cDC1s, which represent a relatively homogeneous group of cells, cDC2s are transcriptionally and functionally diverse. Initial studies identified subsets of cDC2s whose differentiation was dependent on Notch2 or KLF4 (refs. 12–14). Subsequent single-cell transcriptomic analysis of cDC2s demonstrated two major subsets, cDC2A and cDC2B, conserved across mice and humans[6]. cDC2As correspond to previously described ESAM$^+$ Notch2-dependent cDC2s[6,11], whereas cDC2Bs are dependent on KLF4 (ref. 15). Although no unifying pan-tissue cell-surface marker has been identified for cDC2As, their expression of T-bet affords the use of T-bet reporter mice for their characterization[6,11]. By contrast, cDC2Bs express signature cell-surface markers that include CLEC12A and CX3CR1. In addition, a variable proportion of cDC2Bs express MGL2 (also known as CD301b) and CLEC10A depending on tissue or lymph node (LN) of origin[6].

The original description of cDC2A/cDC2B subsets identified a small proportion of cells marked by RORγt genetic lineage tracing or gene expression within T-bet$^-$ cDC2s, which were presumed to be cDC2Bs[6]. However, subsequent studies demonstrated that these RORγt$^+$CD11c$^+$CD11b$^+$ APCs belonged to a lineage of RORγt$^+$ APCs that have a distinct ontogeny from cDCs[3–5,16]. An orthogonal study of human DCs in peripheral blood similarly identified a division in cDC2s, here termed DC2 and DC3 (ref. 17). Although it was initially

suggested that DC3 and cDC2B classifications were synonymous, owing to their transcriptional similarity in both mice and humans[8], DC3s were later shown to be descended from monocyte-dendritic progenitors[7,8,18,19], suggesting that these cells represent monocytes, whereas cDC2Bs are CDP-derived[11]. Given their transcriptional concordance, further studies are needed to determine whether cDC2Bs and DC3s are functionally distinct. The relative abundance of cDC2A and cDC2B subsets varies across LNs, with notable enrichment of cDC2As in gut-draining versus skin-draining LNs[6,11,20], suggesting that environmental cues shape cDC2A and cDC2B differentiation or recruitment of pre-committed bone marrow progenitors[11]. A quartet of signals encompassing Notch2, lymphotoxin-$\alpha_1\beta_2$ ($LT\alpha_1\beta_2$), retinoic acid (RA) and noncanonical NF-κB signaling have been identified as regulators of cDC2A differentiation[11–13,20–23], providing clues as to how the tissue or LN environment might drive particular cDC2 fates during steady state or inflammation.

Single-cell RNA-sequencing (scRNA-seq) analysis of intestinal DCs in wild-type mice identified a unique population of cryptopatch-associated and isolated lymphoid follicle-associated DCs (CIA-DCs), characterized by expression of *Il22ra2*, encoding IL22BP, a natural antagonist of interleukin (IL)-22, the mucosal barrier protective cytokine[24]. Similar cells have been identified in Peyer's patches[25], suggesting that environmental cues common to gut-associated lymphoid follicles promote CIA-DC differentiation. Independent strands of evidence have demonstrated dependency of IL-23$^+$ CIA-DCs on type 3 innate lymphoid cell (ILC3)-derived $LT\alpha_1\beta_2$, retinoic acid, Notch2 and noncanonical NF-κB signaling[24,26,27], the same signals that regulate cDC2A differentiation, suggesting a developmental link between these subsets.

During inflammation, a population of cDC2s termed inflammatory cDC2s (inf-cDC2s) have been identified by scRNA-seq analyses[28,29]. Inf-cDC2s arise in response to cell-intrinsic type I interferon signaling and share phenotypic and transcriptional features with both monocytes and cDC1s, including IRF8 expression[28]. Whether these inf-cDC2s correspond to cDC2As or cDC2Bs that have sensed type I interferons or represent a distinct subset of cells that directly arise from pre-DC2s has not yet been established. Although not dependent on IRF8 for their development, IRF8-deficient inf-cDC2s had reduced expression of genes associated with antigen presentation and activation[28]. The expression of the cDC1 lineage-determining transcription factor IRF8, by a subset of cDC2s, strikes a note of caution in attributing phenotypic changes following loss of a particular transcription factor solely to the missing cell type.

An additional DC subset or precursor has been described in humans and mice that exhibits overlapping transcriptional features and cell-surface markers with both cDC2s and plasmacytoid DCs (pDCs)[9,30–33]. These cells, variably termed transitional DCs (tDCs), pDC-like cells, pre-DC2s or, in humans, AXL or AS-DCs, share a lymphoid developmental pathway with pDCs[7,9,33], a lineage of DCs that serve as key producers of type I interferons during innate immune responses. In keeping with their moniker, tDCs can differentiate into pDCs and cDC2s[7,9], suggesting that tDCs represent a transient progenitor cell state. However, tDCs can prime T cells in vitro[32] and produce pro-inflammatory cytokines in response to viral sensing[9], and thus may still contribute to inflammatory responses during their differentiation into mature cDCs or pDCs.

CCR7$^+$ 'migratory' DCs—conduits between tissue and LNs

An essential feature of cDCs is their ability to migrate from tissues to LNs, providing a vital conduit for antigen to sites of T cell priming. cDC migration is intricately linked to their 'activation', which at the transcriptional level is reflected by expression of a signature gene program. This includes increased expression of MHC class II and co-stimulatory molecules, as well as molecules associated with cell migration, notably the chemokine receptor CCR7, which mediates homing to LNs in response to CCL19 and CCL21. Thus, activated CCR7$^+$ cDCs are predominantly found in LNs and are often referred to as migratory DCs, implying migration from tissues to LNs. However, spleen and LN-resident DCs can acquire expression of CCR7 and the signature gene profile associated with migratory DCs[6,34], allowing migration of CCR7$^+$ resident DCs to the LN T cell zone in response to the relevant chemokine gradients. Besides increased expression of co-stimulatory molecules, CCR7$^+$ cDCs express co-inhibitory molecules, including PD-L1, PD-L2 and CD200, and have been referred to as mreg DCs ('mature DCs enriched in immunoregulatory molecule' DCs)[35], although a predominant immunoregulatory role for these cells has not been established.

cDC activation and migration occur during both homeostasis and inflammation, driven by various signals, including uptake of apoptotic cells or cholesterol, nuclear mechanosensing and microorganism- and inflammation-associated signals[36–38]. The prevailing paradigm is that homeostatic or inflammation-induced CCR7$^+$ cDCs have tolerogenic or pro-inflammatory functions, respectively. However, further research is needed to delineate the type of T cell tolerance induced by CCR7$^+$ cDCs, given that recent studies have shown that cDCs are dispensable for intestinal pT_{reg} cell generation and tolerance to food and microbiota antigens[3–5,39–43]. In the steady state, cDC1s are the major cDC subtype that samples 'self' antigens within the periphery with subsequent activation and migration to LNs, as evidenced by uptake of model self-associated or tumor-associated antigens in mice[35,44], suggesting roles in self-tolerance. Although these CCR7$^+$ cDC1s can induce T_{reg} cell differentiation in vitro[35,44], the type of T cell tolerance induced after presentation of these antigens in vivo, for example, clonal deletion, anergy induction or T_{reg} cell differentiation, has not formally been established. Both imaging and intercellular contact labeling approaches have demonstrated that, among cDCs, T_{reg} cells predominantly interact with CCR7$^+$ cDCs[45–47]. Whether this reflects cDC regulation of T_{reg} cells or regulation of cDCs by T_{reg} cells—a canonical mechanism of T_{reg} cell-mediated immunosuppression—remains to be determined.

Thetis cells—a lineage of RORγt$^+$ APCs

A subset of RORγt$^+$ ILC3s, termed lymphoid tissue-inducer (LTi) or LTi-like cells, express MHC class II. At steady state, LTi cells lack expression of key co-stimulatory molecules that are required for T cell activation, although inflammatory MHC class II$^+$ ILC3s have been described in mouse models of central nervous system inflammation[48]. Early studies in mice examining the function of MHC class II expression on RORγt$^+$ cells attributed a number of roles to MHC class II$^+$ ILC3s, most notably tolerance to the gut microbiota[49,50]. However, recent single-cell transcriptomic studies identified a previously unappreciated group of RORγt$^+$ APCs that include bona fide professional APCs expressing gene programs associated with antigen processing and presentation, co-stimulation and cell migration[3–5,51].

The first clue to the existence of additional subsets of RORγt$^+$ APCs came from analyses of peripheral Aire-expressing cells in mice, which revealed a population of RORγt$^+$Aire$^+$ cells, variably termed ILC3-like Aire-expressing cells, RORγt$^+$ extra-thymic Aire-expressing cells (eTACs) or Janus cells[51,52]. Three parallel studies investigating the role of RORγt$^+$ APCs in microbiota tolerance provided further insights into these cells through scRNA-seq and the assay for transposase-accessible chromatin by sequencing analyses of RORγt$^+$ or Aire$^+$ cells[3–5]. Although two studies suggested that non-ILC3 RORγt$^+$ APCs spanned two subsets of RORγt$^+$Aire$^+$ cells (termed RORγt$^+$ eTAC I–II, or Janus cells 1–2)[4,5], we found that RORγt$^+$Aire$^+$ cells belong to a broader lineage of cells that we named Thetis cells (TCs). TCs comprise four subsets (TC I–IV) of which two subsets, TC I and III, represent eTACs[3]. This classification was subsequently validated by extensive single-cell transcriptomic analyses of RORγt$^+$ APCs in studies examining their role in oral tolerance[39,40]. Since the original description of the TC family, additional studies have not only provided new insights into

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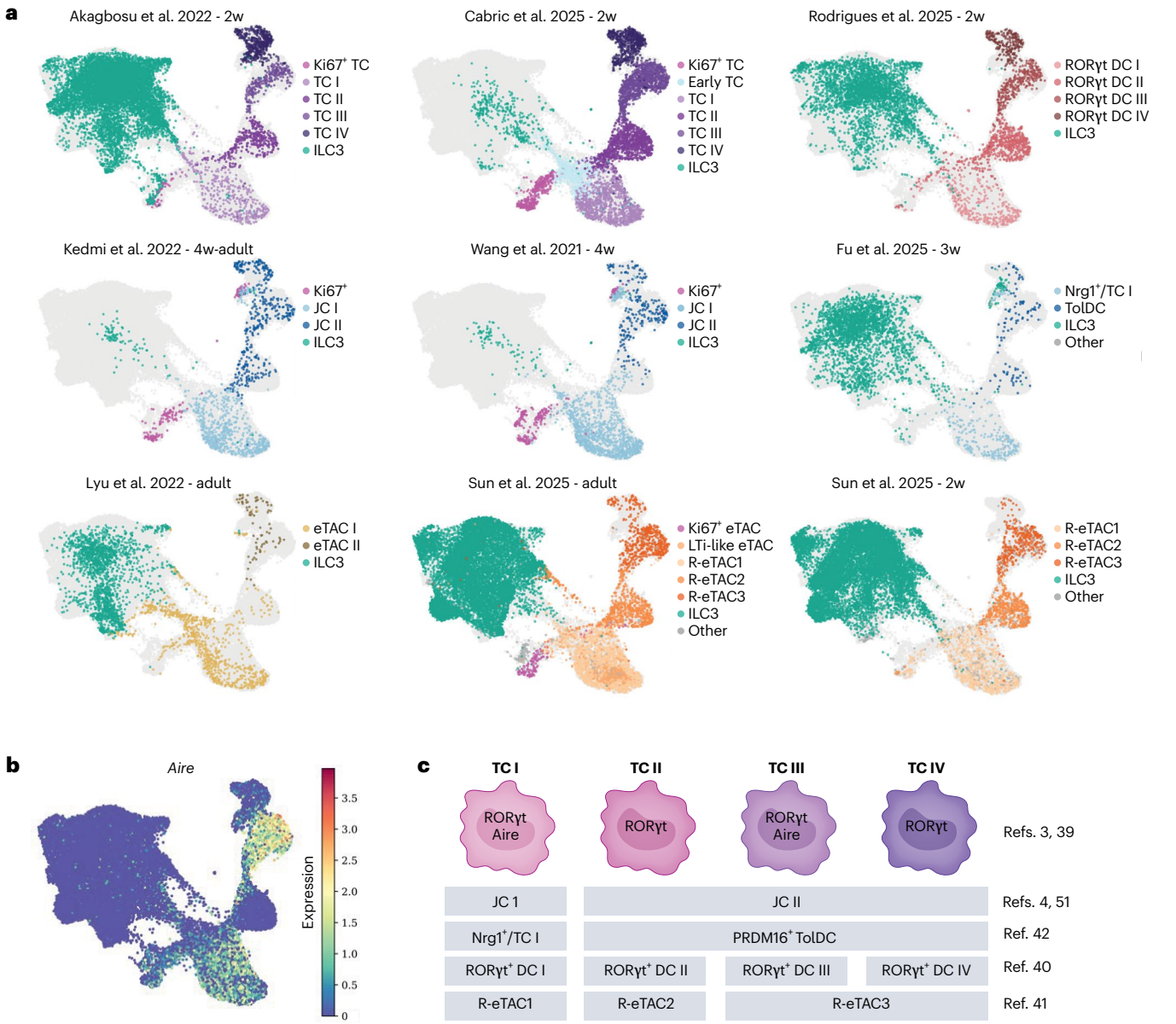

Fig. 2 | Reconciling the spectrum of RORγt$^+$ APCs. a, Uniform manifold approximation and projection visualization of integrated single-cell transcriptomes of RORγt$^+$ APCs from published studies[3,4,38–41,50]. Cells colored by their original cell-type annotation from available metadata[3,38–41] or expression of published signature genes in reanalysis of extracted RORγt$^+$MHC class II$^+$ single-cell transcriptomes[4,50]. **b**, Expression of *Aire* delineating TC I and TC III subsets. **c**, Reconciling nomenclature across individual studies. These integrated data indicate the presence of five RORγt$^+$ APCs: LTi ILC3 cells, and four subsets of TCs (TC I–IV). TC subset composition varies across LNs with almost complete absence of TC IV in non-gut LNs, suggesting that the presence of pT$_{reg}$-inducing TC IV may underlie the unique tolerogenic properties of mesenteric LNs that results in oral tolerance. TCs are enriched in the peri-weaning period but are present in low numbers in LNs of adult mice. JC, Janus cell; TolDC, tolerizing dendritic cell; R-eTAC, RORγt$^+$ extra-thymic Aire-expressing cell.

their biology, transcriptional regulation and function, but also given new names to the cells, including RORγt$^+$ DC I–IV[40], RORγt$^+$ eTAC 1–3 (ref. 41) or tolDCs (tolerizing DCs)[42]. To allow cross-comparison among studies, we have integrated published scRNA-seq analyses of RORγt$^+$ APCs, reconciling nomenclature for these subsets (Fig. 2). A unifying finding is the presence of TC I–IV subsets in all studies, although TC II, III and IV, or TC III and IV are often annotated as one subset, owing to cell sparsity. Although they are sometimes defined as a lineage of Aire-expressing cells, our integrated analysis confirmed that TC II and TC IV do not express Aire.

Each TC subset can be distinguished by unique cell-surface markers (Table 1), allowing phenotypic and spatial characterization. TCs possess an intriguing phenotype, exhibiting transcriptional similarity with both migratory CCR7$^+$ cDCs[3,16,42] and Aire$^+$ medullary thymic epithelial cells (mTECs)[3,51], the canonical tolerogenic APC that instructs T$_{reg}$ cell differentiation and tolerance to self-antigens within the thymus[3,16,42]. Lineage-tracing and genetic targeting approaches demonstrated that TCs are not derived from the Clec9a$^+$ CDP[3,5,43], or RORα-expressing ILC precursors[3,43]. While some studies have suggested myeloid ontogeny and DC identity, on the basis of shared expression of cDC-associated genes, such as *Zbtb46* and *Cd11c*, as well as chromatin accessibility at several genes typically expressed by DCs, none of these genes are uniquely expressed by DCs[41,42]. By contrast, other studies using ex vivo culture of progenitors or lineage-tracing approaches showed that TCs are derived from lymphoid progenitors[16,40], and suggested a developmental relationship with ILCs[40]. Adding to the lineage controversy, TCs were found to be absent in *Flt3l*$^{-/-}$ mice[16]. However, other studies have demonstrated modest or negligible effects of FLT3 or anti-FLT3L

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Table 1 | Distinguishing cell-surface markers and transcription factors for TC and cDC subsets in mice

Marker expression	TCs				ILC3	cDC2	
	TC I	TC II	TC III	TC IV	LTi	cDC2A	cDC2B
RORγt	Low	+	+	+	+	–	–
CXCR6	–	–	–	–	+	–	–
CD11c	–/lo	+	+	+	–	+	+
CD11b	–	–	–	+	–	+	+
NCAM1	+	–	–	–	–	–	–
EpCAM	–	+	+	+	–	–	–
CCR6	+	+	–	–	+	–/lo	–/+
NRP1	+	+	–	–	+	–	–/+
OX40	–	–	+	–	–	–	–
Aire	Low	–	High	–	–	–	–
AIRE	+	–	+	–	–	–	–
PRDM16	–/lo	+	+	+	–	–	–
CX3CR1	–	–	–	–	–	–	+
Mgl2	–	+	–	–	–	–	–/+
Clec10a	–	+	–	–	–	–	–/+

on TC differentiation[40,41]. Thus, at present, the lineage identity of TCs and their immediate precursor remains enigmatic.

Our understanding of the transcriptional regulation of TC differentiation is rapidly evolving. Two studies demonstrated that TC II–IV subsets were dependent on RORγt and identified a critical +7-kb *cis*-regulatory element in the *Rorc* locus (which encodes RORγt)[40,42]. The role of RORγt in TC I differentiation remains to be determined as genetic approaches to ablate RORγt relied on deletion of the enhancer, which is not active in TC I, or CD11c-cre driven RORγt ablation, which is likely to be inefficient in TC I, owing to low or absent CD11c expression[3]. TC II–IV were also shown to be dependent on PRDM16 (ref. 42) and IRF8 (ref.39), suggesting a bifurcation in TC I differentiation from that of TC II–IV. Future studies addressing the upstream cues that regulate this trio of transcription factors will provide important insights into TC development.

Thus far, functional roles have been identified for ITGB8$^+$ TC IV, which were found to instruct intestinal pT$_{reg}$ cell differentiation, discussed below. The functions of TC I, II and III are not known, although subset-specific gene expression programs suggest unique roles. Although expression of Aire by TC I and III subsets and overlapping transcriptomes with Aire$^+$ mTECs hint at roles in peripheral tolerance, Aire expression by RORγt$^+$ cells was required for T$_H$17 immunity to systemic *Candida albicans* infection[53]. Further studies are required to determine if Aire$^+$ TC I and III have distinct roles in tolerance versus immunity. Further clues about the roles of TCs are provided by their distribution. Although first characterized in gut- and skin-draining LNs, TCs are present in all LNs examined[16,39,40] but are extremely rare in nonlymphoid tissue[16]. Individual TC subsets exhibit LN-specific patterns of enrichment—TC I are notably absent from Peyer's patches, whereas TC IV are almost exclusively present in mesenteric lymph nodes (mLNs) and Peyer's patches[39]. The distribution of TC IV mirrors sites of pT$_{reg}$ cell induction[54], in keeping with their role in intestinal tolerance. A notable feature of TCs is their enrichment in LNs during early life, peaking in number in the peri-weaning period and declining rapidly thereafter[3,16,40,55]. Age and site of sampling are, therefore, major factors in capturing the full heterogeneity of these cells, as well as their functions. Technical issues related to isolation techniques may be an additional consideration, whereby the use of enzymatic digestion leads to underestimation of their true abundance in LNs, similar to previously reported findings for transcriptionally related mTECs[56].

Although first described in mice, human RORγt$^+$ APC populations with transcriptional homology to mouse TCs have been described by multiple groups across a spectrum of tissues, including nasal mucosa, intestinal lamina propria, tonsils and mLNs[16,42,57]. Human TCs express the transcriptional regulator PRDM16, a defining feature of TC II–IV, as well as the polymeric immunoglobulin Fc receptor, PIGR, a TC II signature marker. However, they do not express Aire. By contrast, a population of RORγt$^-$ Aire$^+$CCR7$^+$ APCs has been described in human tonsils[58], mLNs[3] and thymi[59]. Whether these cells represent Aire$^+$ TC I, which in mice express low levels of RORγt[3], or CCR7$^+$ cDCs, remains to be determined. Given that in mice, TCs are enriched during early life, and TC IV are rare outside gut LNs, further studies profiling larger numbers of cells across the spectrum of development are required to determine whether human TCs encompass distinct subsets, and exhibit a developmental wave in early life, akin to their mouse counterparts. One challenge to the investigation of human TCs is their scarcity within nonlymphoid tissues, as demonstrated by a recent analysis of TCs in human LNs and tissues using imaging approaches[60].

CX3CR1$^+$ mononuclear phagocytes

The intestinal lamina propria harbors abundant populations of CX3CR1$^+$ APCs, often referred to as CX3CR1$^+$ mononuclear phagocytes, a loosely defined heterogeneous group of myeloid cells that encompass monocytes, macrophages, cDC2Bs and DC3s[7]. Diphtheria toxoid (DT)-mediated ablation using CD11c-cre driven DT receptor (DTR) expression in CX3CR1$^+$ cells has revealed important roles for CD11c$^+$CX3CR1$^+$ cells in suppressing inflammatory responses to commensal fungi and bacteria, as well as enteric pathogens[61,62]. Further studies are required to delineate the exact CX3CR1$^+$ cell type responsible for these phenotypes.

Functional interrogation of APC subsets

Delineating the roles of specific APCs requires genetic tools for their selective ablation. For the vast majority of studies addressing DC function, CD11c has been used as a 'DC' Cre driver. However, despite its broad use and wide acceptance as a valid tool to target DCs, CD11c is expressed by a range of myeloid and lymphoid cell types. Among hematopoietic cells, Zbtb46 was shown to have restricted expression on cDCs[63]; however, recent studies revealed expression of Zbtb46 by TCs and ILC3s[3,64], highlighting a caveat to the use of this Cre or DTR driver for the study of cDC-specific functions. Moreover, Zbtb46 is expressed by endothelial cells, and bone marrow chimeras are needed to prevent lethality following ablation of Zbtb46$^+$ cells in Zbtb46-DTR mice[65]. Clec9a is expressed by the CDP and its expression is maintained on cDC1s[66]. Thus, *Clec9a^{cre}* mice can be used to target cDC1s, cDC2s and myeloid-derived pDCs, with increased efficiency in cDC2s when used at homozygosity[66,67]. The efficiency of *Clec9a^{cre}* for cDC2 targeting increases during the first weeks of life, owing to layered ontogeny of cDC2s[67]. By adulthood, >90% of cDC2s, encompassing cDC2A and cDC2B subsets, are effectively targeted by *Clec9a^{cre}* (refs. 20,43,67). cDC1s uniquely express XCR1 and multiple XCR1-cre alleles have been developed[68–70]. In addition, cDC1 differentiation is dependent on the activity of IRF8; thus, mice with conditional ablation of *Irf8* or specific enhancers can be used to study cDC1 functions[71]. Precise targeting of cDC2s has been more challenging, given their heterogeneity and overlapping markers with other myeloid cells, but new genetic tools are emerging. In an elegant study addressing the role of Zeb2 activity in DC differentiation, all cDC2 subsets were shown to be dependent on the combined activity of three enhancers in the *Zeb2* locus[72]. In addition to a block in cDC2 differentiation, *Zeb2$^{\Delta1+2+3}$* mice exhibit impaired differentiation of myeloid-derived pDCs, as well as of monocytes. Among cDC2 subsets, cDC2B, as well as a subset of CD11b$^-$CD24$^-$ DCs in skin-draining LNs, are dependent on KLF4 (ref. 15), whereas cDC2As are dependent on Notch signaling[11–13]. These divergent transcriptional dependencies allow their functional investigation, for example, with *Clec9a^{cre}* drivers

for specific ablation of cDC2A or cDC2B differentiation, with the caveat that loss of a particular transcription factor may perturb the function of the remaining DC subsets. Variable proportions of cDC2B express MGL2 (CD301b), and studies utilizing Mgl2-driven DTR ablation have been used to functionally interrogate these cells, albeit with the caveat that Mgl2 is also expressed by other myeloid cells, such as monocytes[73]. Currently, no specific tools exist for the DC3 subset although their descendancy from Ms4a3-expressing monocyte progenitors affords the use of intersectional approaches using *Ms4a3*^{cre} mice.

Delineating the role of newly identified TCs is complex, given their overlapping transcriptomes with DCs, as well as shared expression of RORγt by ILC3s and TCs. Comparative transcriptional analyses, as well as lineage tracing, identified *Clec9a* and *Rora* as driver genes that can be used to selectively target DCs and ILC3s, respectively[3]. In addition, a recent study identified Serinc2 as an ILC3-specific marker and demonstrated selective loss of ILC3s but not TCs in *Serinc2*^{cre}*Rorc*$^{fl/fl}$ mice[40]. Conversely, TC II–IV express PRDM16 and its loss in RORγt$^+$ cells led to impaired TC development, although specific TC subsets were not examined in this study[42]. Given that TCs encompass four transcriptionally, and likely functionally, distinct subsets, delineating their unique functions will require new genetic tools.

A division of labor among APCs

Effective immunity against intracellular microorganisms requires type 1 responses led by type 1 helper T (T_H1) cells, extracellular helminths activate type 2 immune responses, which are directed by type 2 helper T (T_H2) cells, and defense against extracellular bacteria and fungi is orchestrated by type 3 immunity and IL-17-producing helper T (T_H17) cells. In addition, follicular helper T (T_{FH}) cells contribute to all types of immune responses by promoting germinal center formation and humoral immunity. During T cell priming, APCs provide the critical cytokines that direct expression of lineage-defining transcription factors and downstream CD4$^+$ T cell programs, including IL-12 for T_H1 cells; IL-6 for T_H17 differentiation; and activation of latent transforming growth factor-β (TGFβ) for pT_{reg} cell induction. The expression of an array of pattern recognition receptors could, in theory, allow one APC type to decode the nature of the pathogen leading to pattern-specific rewiring of their transcriptional programs and acquisition of particular molecules, such as polarizing cytokines that induce corresponding T_H cell responses. Arguing against such a model, recently characterized cDC2 and RORγt$^+$ APC subsets express distinct patterns of Toll-like receptors, cytokines or related T cell fate-directing molecules, suggesting a less promiscuous relationship between APCs and their T cell partners. Emerging evidence, discussed below, lends credence to a 'division of labor' model whereby each APC subset is transcriptionally programmed to drive a particular T cell differentiation program both at steady state and during inflammation (Fig. 3). This APC specialization may be pre-instructed during their differentiation in the bone marrow or shaped by environmental cues within their tissue or LN niche.

In addition to the stereotypical CD4$^+$ T cell responses observed in response to particular classes of infectious microorganisms, certain commensals also have a propensity to elicit particular CD4$^+$ T cell fates. For example, segmented filamentous bacteria (SFB) induce mainly T_H17 cell differentiation[74,75], *Akkermansia muciniphila* promotes a dominant T_{FH} response in Peyer's patches with a mixed pT_{reg}, T_H1 and T_H17 cell response observed in intestinal lamina propria of specific-pathogen-free (SPF) mice[76], *Bacteroides thetaiotaomicron* induces pT_{reg} cells, and *Helicobacter hepaticus* induces both pT_{reg} cells and T_{FH} cells[77–80]. The factors that govern the nature of the T cell response to microbiota antigens are not well understood, but recent studies have shed light on the importance of particular APC subsets in regulating the balance between different pT_{reg} and T_{eff} cell subsets. When discussing the role of specific APCs in driving particular CD4$^+$ T cell fates, we consider immune responses to microbiota and pathogens together, as emerging evidence suggests that APC functions are conserved across different types of antigenic encounters.

cDC1 regulation of T_H1-mediated immunity and inflammation

In contrast to the CD8$^+$ priming abilities of cDC1s, cDC2s are often said to have a superior ability to prime CD4$^+$ T cell differentiation. However, accruing evidence strongly suggests that cDC1s are the chief DC subset responsible for T_H1 cell differentiation. Mice with a CD11c-driven deletion of *Irf8* and thus cDC1 deficiency, have reduced T_H1 cells both at steady state and in response to colonization with *Tritrichomonas musculis*[81]. Although these mice have additional defects in immune cells, a subsequent study established that small intestinal T_H1 cells arising in response to microbial colonization are dependent on both MHC class II antigen presentation and production of IL-27 by cDC1s[82]. Whereas cDC1-derived IL-27 is the key cytokine for microbiota-induced T_H1 differentiation, IL-12 production by cDC1s is essential for T_H1-mediated immunity during *Toxoplasma gondii* infection[83]. Although cDC1s were thought to induce tolerogenic T_{reg} cell responses to food antigens, two recent studies have shown that cDC1s are neither sufficient nor required for pT_{reg} cell priming, but instead promote both T_H1 immunity[39,43] and CD8$^+$ T cell immunity[43] to food antigens. The ability of cDC1s to promote both T_H1 and cytotoxic CD8$^+$ T cell differentiation highlights a role for cDC1s in coordinating type 1 inflammation against intracellular pathogens, as well as homeostatic 'type 1' responses to food and microbiota antigens.

cDC2B regulation of T_H2 immunity

Zeb2$^{\Delta1+2+3}$ mice, which lack all cDC2 subsets, exhibit a profound impairment in T_H2-induced immunity after infection with the helminth *Heligmosomoides polygyrus*[72]. Among cDC2s, cDC2Bs, or their migratory counterparts, seem to be the critical subset for T_H2 differentiation, as studies in mice that lack cDC2Bs, owing to loss of KLF4 in CD11c-expressing cells or DT-mediated ablation of Mgl2$^+$ cells have impaired T_H2 immunity in mouse models of type 2-mediated hypersensitivity or parasitic infection[14,73,84]. The exact mechanism underlying the ability of cDC2Bs to induce T_H2 cell differentiation remains to be determined, given that DCs do not produce the T_H2-polarizing cytokine IL-4, which is instead secreted by a variety of other innate immune cells associated with type 2 inflammation, including basophils, eosinophils, mast cells, tuft cells and ILC2s[85–87]. In the skin, ILC2s regulate DC2-mediated T_H2 differentiation in an IL-13-dependent manner[88,89]. In vitro studies indicated that IL-13 regulation of cDC2 differentiation required KLF4 (ref. 88), suggesting potential modulation of cDC2B differentiation. This axis of regulation may be specific to the skin, as IL-13 signaling on DCs is not required for T_H2 differentiation in house dust mite-induced lung hypersensitivity, a mouse model of allergic asthma[88]. Two recent studies have demonstrated an important role for IL-2 signaling in determining T_H2 cell fate[90,91]. In one study, Mgl2$^+$ cDC2Bs were shown to regulate IL-2 signaling via production of IL-2, as well as expression of CD25 (IL2RA)[91]. Besides cytokine regulation of T_H2 differentiation, targeted deletion of MARCH1 ubiquitin ligase on Zbtb46$^+$ or CD11c$^+$ cells abrogated T_H2 cell development following house dust mite-induced lung hypersensitivity[92]. This response was dependent on ubiquitylation of MHC class II and CD86, suggesting that reduced TCR stimulation promotes T_H2 differentiation at the expense of alternative CD4$^+$ T cell fates[92].

cDC2 regulation of T_H17 differentiation

T_H17 cells perform vital roles at mucosal barriers, protecting the host from bacterial and fungal infections. At steady state, T_H17 cells are most prevalent within the intestine, reflecting their induction in response to microbiota antigens. IL-6 and TGFβ promote initial T_H17 lineage commitment, but IL-23 and IL-6 are critically required for their maintenance[93,94]. A multitude of studies have implicated cDC2s in the regulation of T_H17 differentiation; however, a more recent study suggested a role for

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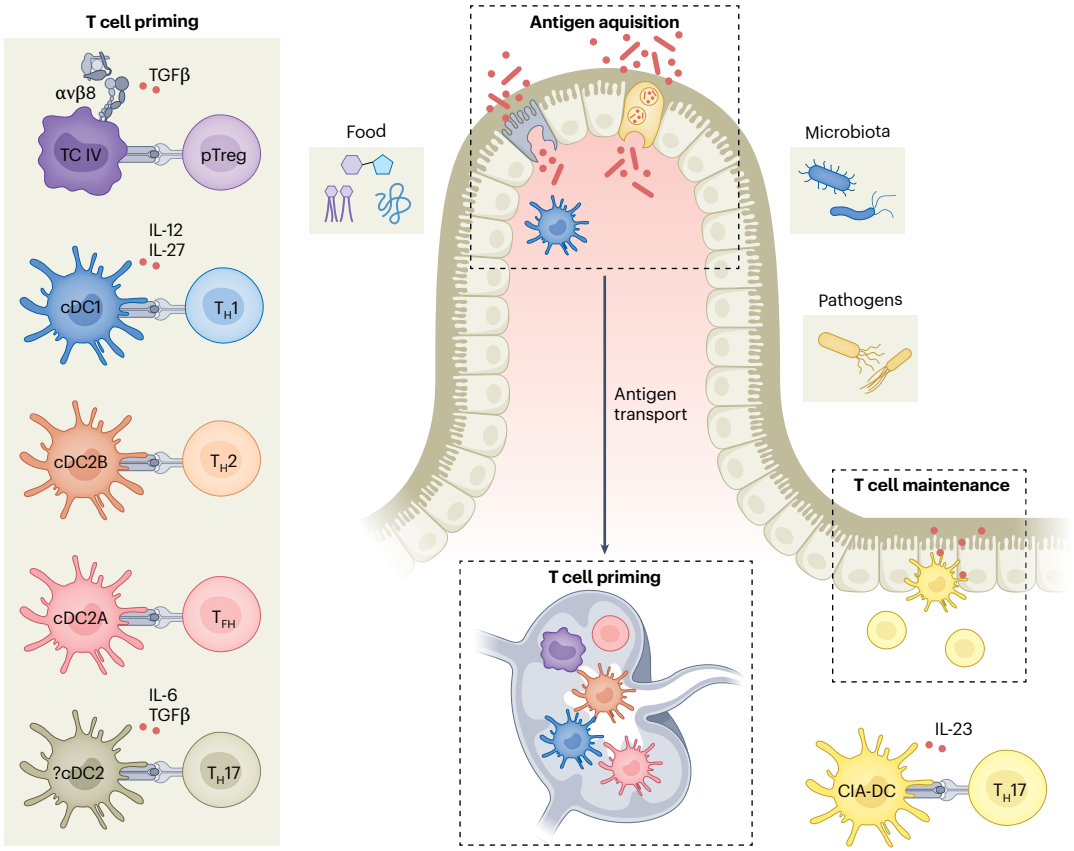

Fig. 3 | A division of labor among APCs. Commensal and dietary antigens are transported to LNs, either by lymphatic flow or by migration of cells residing beneath the intestinal epithelium. In addition to capture by phagocytic cells, antigens can be transported across the intestinal epithelium through goblet cell-associated passages or microfold cells. Naive T cells circulate and are primed within mLNs. The APC composition differs across anatomically distinct LNs. In early life, TCs are enriched within mLNs and the TC IV subset is almost exclusively found within mLNs and Peyer's patches. cDC2As are also enriched in gut LNs, pointing to a potential role for local environmental cues in shaping APC heterogeneity. Each DC or TC subset seems to have distinct roles in initiating particular $CD4^+$ T cell differentiation programs. MHC class II antigen presentation by cDC1s is required for T_H1 differentiation in response to food, commensal or pathogen-associated antigens. cDC2s, specifically KLF4-dependent $CD301b^+$ (Mgl2) cDC2Bs, promote T_H2 differentiation. In the spleen, cDC2A drive T_{FH} differentiation, although the specific role of cDC2As in intestinal T_{FH} responses has not been examined. The APC subset that primes T_H17 cells is debated and may depend on whether the antigen is associated with commensals, pathobionts or pathogens, which can induce different types of T_H17 responses. T_H17 differentiation is maintained by IL-23, for which DCs residing within cryptopatches and isolated lymphoid follicles (CIA-DCs) are likely the critical source.

alternative $CD11c^-$ APCs[4]. These conflicting results likely in part reflect the complex nature of T_H17 differentiation with spatiotemporally distinct phases of cytokine signaling during their induction in LNs versus maintenance within tissues[93]. Additionally, T_H17 cells are heterogeneous, comprising 'homeostatic' T_H17 cells that produce IL-10 and play a key role in maintaining epithelial barrier integrity and immunity to commensals and pathogens, and 'pathogenic' T_H17 cells that produce interferon-γ and play a role in autoimmune disease pathogenesis[95]. Thus, the APC that regulates T_H17 priming versus maintenance, as well as the differentiation of homeostatic versus pathogenic T_H17 cells, may differ. In mice, intestinal T_H17 cell differentiation has been most widely studied following colonization with SFB or *H. hepaticus*, which induce homeostatic or pathogenic T_H17 cells, respectively[74,78,96], or following infection with the attaching and effacing (A/E) pathogen *Citrobacter rodentium*.

Early studies examining the role of Notch2 signaling in DCs revealed a critical role for Notch2-dependent cDC2s in intestinal T_H17 cell differentiation and immunity to *Citrobacter*[12,13]. Orthogonal genetic approaches established that these cells expressed 'DC' markers Zbtb46 and CD11c and were the critical source of IL-23 (refs. 12,13). Recent phenotypic and spatial characterization of $IL\text{-}23^+$ DCs using a new IL-23 reporter mouse defined a unifying signature for $IL\text{-}23^+$ DCs that included Zbtb46, CD11c and CLEC4A4 (also known as DCIR2), and demonstrated their enrichment in cryptopatches and isolated lymphoid follicles in small and large intestine[27], indicating that these cells represent previously described CIA-DCs[24]. Interrogation of a spatially resolved gut cell atlas, available as a resource, confirmed that transcriptionally and spatially defined CIA-DCs are the sole source of IL-23 within the gut and express the $IL\text{-}23^+$ DC gene signature[97]. Of note, although previous studies have divided $IL\text{-}23^+$ DCs into subtypes on the basis of expression of CD103, in this dataset, CIA-DCs represent a transcriptionally homogeneous cluster of cells with variable expression of *Itgae* (which encodes CD103)[97].

Although these studies demonstrate a clear role for cDC2s in the IL-23-mediated maintenance phase of T_H17 differentiation, the exact identity of the APC that primes T_H17 cells has not been established. In one study, adoptively transferred SFB-specific 7B8Tg naive T cells failed to proliferate in the LNs of mice with acute ablation of $Zbtb46^+$ cells[98]. However, mice that lack MHC class II on $CD11c^+$ cells are still able to support the differentiation of *Helicobacter*-specific TCR-Tg T_H17 cells in mLNs[4]. Similarly, mice with $Clec9a^{cre}$-driven deletion of MHC class II on DCs have normal steady-state T_H17 cell numbers[3]. This finding suggests that an as-yet-unidentified $CD11c^-Zbtb46^+$ APC, distinct from cDCs, may contribute to intestinal T_H17 cell differentiation. Overall,

the T_H17 cell-inducing APC may depend on the type of commensal or pathogen, and the location of T_H17 cell priming.

cDC2 regulation of T_{FH} differentiation

Within the intestine, mLNs and Peyer's patches are the main sites of T_{FH} induction. Their presence during steady state, and absence in germ-free mice, indicates a homeostatic T_{FH} response to food or commensal microorganisms. While DCs initiate T_{FH} priming during immunization or infection, cognate interactions with B cells at the T cell–B cell border are required for further differentiation[99]. Among cDCs, several lines of evidence point toward a role for cDC2s in T_{FH} cell differentiation. Loss of cDC2 migration to the T cell–B cell border led to reduced T_{FH} cell priming during intranasal immunization[100]. Among cDC2 subsets, Notch2-dependent Esam$^+$ cDC2As are critical for T_{FH} differentiation in the spleen following immunization with sheep red blood cells[7,101]. Mechanistically, IL2RA expression by DCs has been shown to quench IL-2, creating a favorable environment for T_{FH} differentiation[102]. Bone marrow chimeric mice with deficiency of IL2RA on DCs have impaired T_{FH} responses following sheep red blood cell immunization, alum-OVA or listeria-OVA immunization[102]. Inflammation induces expression of IL2RA on DCs, although in LNs a subset of migratory DC2s constitutively express IL2RA[102].

Thus far, most studies examining DC regulation of T_{FH} priming have focused on T_{FH} differentiation elicited by systemic immunization. Whether similar APCs and mechanisms underlie intestinal T_{FH} cell responses to commensal microorganisms or enteric pathogens in gut LNs and Peyer's patches remains to be determined. In support of a role for cDC2 in intestinal T_{FH} differentiation, a recent study addressing the role of cDC2s in food tolerance using *Zeb2*$^{\Delta 1+2+3}$ mice revealed a global reduction in mLN T_{FH} cells, as well as specific impairment of food-induced T_{FH} differentiation[39]. However, huLangerin-DTA mice that lack Notch2-dependent CD103$^+$ DC2s exhibit only modest impairment in intestinal T_{FH} responses to OVA/cholera toxin immunization despite decreased IgA responses[103]. Further studies are required to delineate the role of specific cDC2 subsets in intestinal T_{FH} responses.

Regulation of food and microbiota-specific pT_{reg} differentiation by $\alpha_v\beta_8^+$ TCs

pT_{reg} cells play an essential role in maintaining intestinal homeostasis, suppressing harmful immune responses to innocuous commensal and dietary antigens. In the intestine, microbiota-specific pT_{reg} cells can be distinguished by expression of RORγt[104], whereas food-specific pT_{reg} cells exist in both RORγt$^+$ and RORγt$^-$ forms[39–43,105]. A longstanding paradigm was that cDCs determine the balance between T cell tolerance and immunity by using pathogen recognition 'switches' to convert from steady-state tolerogenic cells to pro-inflammatory cells. However, rather than all cDCs possessing T_{reg}-inducing abilities at steady state, parallel studies identified a superior ability of mLNs or small intestinal lamina propria CD103$^+$ DCs to promote in vitro T_{reg} (iT_{reg}) cell differentiation[106,107]. Of note, within mLNs and small intestinal lamina propria, CD103 is universally expressed by cDC1s as well as a proportion of cDC2s. Intestinal CD103$^+$ DCs express key molecules for iT_{reg} cell induction, including the retinoic acid-synthesizing enzyme RALDH2 and the TGFβ-activating integrin $\alpha_v\beta_8$ (refs. 106–110). A role for cDCs in intestinal pT_{reg} cell differentiation was supported by in vivo studies in which loss of integrin $\alpha_v\beta_8$ expression by CD11c$^+$ cells or deletion of MHC class II on Zbtb46-expressing cells, which resulted in reduced intestinal T_{reg} cell numbers and microbiota-dependent inflammation, seemed to support a role for cDCs in intestinal tolerance[63,111]. However, mice with MHC class II ablation on RORγt$^+$ cells, presumed at the time to be ILC3s, also exhibit loss of tolerance to the microbiota[49,50]. Reconciling these previous studies, RORγt$^+$ TCs, in particular TC IV, were found to express Zbtb46, CD11c, RORγt and $\alpha_v\beta_8$; notably, ILC3s do not express *Itgb8* (refs. 3,5). In three contemporaneous studies, loss of MHC class II, or integrin $\alpha_v\beta_8$ expression in RORγt$^+$ cells led to impaired differentiation of host RORγt$^+$ pT_{reg} cells or impaired *H. hepaticus*-specific pT_{reg} cell differentiation[3–5]. Moreover, deletion of MHC class II in CDP-derived cDCs or ILC3s, using Clec9a-cre or RORα-cre respectively, did not impact pT_{reg} cell generation. Together, these studies identified $\alpha_v\beta_8$ TCs as the pT_{reg}-inducing APCs and established a new paradigm for APC regulation of intestinal tolerance.

Building on this, five subsequent studies have shown that TCs, specifically $\alpha_v\beta_8^+$ TC IV, have a unique ability to promote food-induced pT_{reg} cell differentiation and oral tolerance[39–43]. Across these studies, several new genetic approaches were deployed to selectively target TCs or particular TC or cDC subsets. Deletion of a +7-kb *cis*-regulatory element in *Rorc* or ablation of *Prdm16* or *Irf8* in RORγt$^+$ cells led to loss of TC II–IV subsets and impaired food-specific or microbiota-specific pT_{reg} cell differentiation[39,40,42]. Among these TC subsets, food-specific pT_{reg} cell differentiation was dependent on the expression of $\alpha_v\beta_8$ by TCs[39,41,43]. While TC III also express low levels of $\alpha_v\beta_8$, a role for Aire$^+$ TCs in food-specific T_{reg} cell differentiation was excluded using a *Rorgt*^{cre}-driven *Aire*$^{flex\text{-}dtA}$ allele, which resulted in loss of TC I and III, but no impact on food-specific or microbiota-specific pT_{reg} cells[39], thus establishing TC IV as the pT_{reg}-inducing subset.

A number of complementary genetic approaches excluded a contribution of cDCs to food-specific T_{reg} cell priming, including cDC2-deficient *Zeb2*$^{\Delta 1+2+3}$ mice[39,112], cDC1-deficient *Clec9a*^{cre}*Irf8*$^{fl/fl}$ mice[39] or mice with restricted expression or deficiency of MHC class II in particular cDC subsets[39,42,43] Overall, these studies have led to a new paradigm for intestinal tolerance to microbiota and food antigens, highlighting the existence of TC IV as a dedicated tolerogenic subset of APCs. Given that cDCs also express $\alpha_v\beta_8$, it will be important to identify the other molecules or properties that endow TC IV with the unique ability to induce food-specific and microbiota-specific pT_{reg} cell differentiation. Interrogation of the TC IV-specific programs, as well as the cues that regulate their development in early life, may yield new insights into mechanisms of intestinal tolerance and provide new therapeutic strategies for intestinal inflammation and food allergy.

Cooperation between APCs

While induction of each CD4$^+$ T cell differentiation program seems to rely on one APC subset, accumulating evidence suggests cooperation between different APCs is required for terminal differentiation or maintenance of lineage-primed CD4$^+$ T cells. Collaboration between APCs, with two temporally distinct phases of antigen presentation, was originally described during subcutaneous immunization[113,114]. LN-resident DCs primed CD4$^+$ T cells, while subsequent MHC class II-dependent interactions with migratory DCs promoted their expansion and differentiation. An analogous model was suggested for food-induced T cell differentiation in which antigen presentation by $\alpha_v\beta_8^+$ TCs instructs initial pT_{reg} cell differentiation but a subsequent MHC class II-dependent interaction with a cDC is required for pT_{reg} cell maintenance[39,43,112]. In support of sequential interactions by distinct APC types, mice in which MHC class II antigen presentation is restricted to RORγt$^+$ APCs have reduced capacity for pT_{reg} cell differentiation compared to mice in which both cDCs and TCs can present antigen, despite an inability of cDCs to initiate pT_{reg} cell differentiation[39,43]. Biphasic interactions with distinct APCs have also been described for T_{FH}, T_H17 and CD8$^+$ T cell differentiation[93,115–119]. Besides TCR and co-stimulation, the second APC 'hit' can also provide additional cytokine signals that promote full effector functions[93,118,119].

Delineating these spatiotemporally distinct APC interactions is of therapeutic importance, given that clinical presentation of many inflammatory diseases occurs days to weeks after the initiation of an aberrant immune response. Therapeutic intervention may be best targeted to the APC interactions that sustain dysregulated T cell responses. In this regard, chronic antigen stimulation drives the differentiation of CD4$^+$ and CD8$^+$ stem-cell-like memory T cells that sustain T_{eff} cells. Stem-cell-like memory T cells have been associated with

several diseases, including chronic infection, cancer, food allergy and autoimmune disease[120–126]. Although these cells are critically dependent on TCR signaling[127], the exact nature of the APC that regulates their quiescence or differentiation has not been determined.

APCs as matchmakers between antigens and T cells

Although research into food- or microbiota-directed T cell differentiation has focused heavily on pT_{reg} responses, these antigens can elicit a spectrum of $CD4^+$ T cell responses, with parallel T_{eff} and T_{reg} responses to the same antigen[39–43,76–78,80,112,128]. In studies in which TCR-Tg T cells have been used to study pT_{reg} cell differentiation in response to *H. hepaticus* colonization or oral ovalbumin, only 10–20% of LN-primed cells are $Foxp3^+$ T_{reg} cells in adult mice, increasing to ~80% in peri-weaning mice, the remainder spanning different T_{eff} cell lineages[4,39–43,112,128,129]. Despite this difference, T_{reg} cells emerge as the dominant cells that are critically required for intestinal tolerance[39,112,129]. A multifaceted T cell response may serve to protect the host from pathobionts in the event that these microorganisms become pathogenic, or when food antigens are encountered in the context of food poisoning. Outgrowth of previously tolerated commensals may lead to sufficient activation of T_{eff} cells and their escape from T_{reg} cell-mediated suppression.

The occurrence of identical, microorganism-reactive TCRs across distinct T_{reg} and T_H cell lineages suggests T cell plasticity after priming, perhaps driven by cooperative sequential interactions with APCs, as discussed above. However, diverse responses of microbiota or 'food'-specific TCR-Tg T cells to their cognate antigen and the requirement for specific cDC or TC subsets for induction of these fates, suggest that antigen presentation by different APC types determines the heterogeneity of the T cell response. If such a division of labor exists, the relative abundance of different APC types within LNs, as well as their location and ability to access commensal, pathogen or food-derived antigens, would dictate the balance of T_{eff} and T_{reg} cell differentiation. The concept of APC subset abundance determining the diversity of T cell responses within particular LNs is perhaps best illustrated by the window of opportunity for enhanced commensal and food-specific pT_{reg} cell differentiation in early life that in mice coincides with the wave of TC differentiation in gut LNs[130]. It is tempting to speculate that enrichment of pT_{reg}-inducing TC IV during the peri-weaning period, alongside developmentally timed opening of goblet cell-associated antigen passages, small channels that facilitate transport of antigens across the epithelium[39,131], synergize to create an environment that favors uptake and presentation of antigens by tolerogenic TCs.

During homeostasis, the LN or tissue microenvironment may provide specific cues that promote the development of particular APC subsets to meet the demands of that tissue. For example, Notch2, RA, lymphotoxin and additional, as-yet-undefined signals could shape the APC landscape, with differential abundance of cDC2A, cDC2B and TC subsets, and altered $CCR7^+$ DC transcriptomes observed in mLNs and gut-associated lymphoid tissue[3,7,11,44,132]. If dedicated APCs are preprogrammed to prime particular $CD4^+$ T cell fates, as demonstrated for cDC1 regulation of T_H1 immunity or TC IV-induced pT_{reg} cell differentiation, how does the immune system ensure the appropriate balance of APC subsets during homeostasis and inflammation? The short lifespan of cDCs (estimated to be 3–6 days)[133], which requires constant replenishment from pre-DCs present within tissues and LNs, as well as the rapid increase in DC-poiesis observed during acute inflammation[27,29], in theory provides a responsive and adaptable APC landscape that can be quickly redrawn in response to microbial or inflammation-associated cues. Thus, it is conceivable that tailored $CD4^+$ T cell responses are achieved through changes in 'APC-poiesis' rather than APC plasticity at the cellular level, with subset-specific expression of particular pattern recognition receptors providing an additional mechanism to ensure activation of the relevant APC type. Although speculative, this notion is supported by the finding that homeostatic and inflammation-induced DC maturation exhibit concordant changes in their transcriptional programs, distinguished by a small subset of interferon-responsive genes that reflect sensing of the inflammatory milieu rather than a role for interferons in their differentiation[134]. Moreover, no studies have yet demonstrated transdifferentiation of an APC from, for example, a T_H1-inducing cell state to a T_H2- or T_H17-inducing cell state or vice versa; instead, the transcriptional changes observed in DCs after inflammation typically represent enhanced functions related to increased expression of molecules related to antigen presentation and co-stimulation. Better understanding of the ontogeny of individual DC and TC subsets, and the pathways that regulate the differentiation of immediate progenitors into functionally distinct subsets, is crucial to discerning how responses are coordinated between APCs, T cells and their cognate antigens.

Conclusions

Recent studies have revealed increased diversity of APCs, and investigations into their function have provided new insights into how the intestinal immune system balances tolerogenic and inflammatory immune responses. While a dedicated subset of tolerogenic APCs may be inherently less risky than APC plasticity, a monogamous relationship between preprogrammed APC subsets and particular T_H or T_{reg} cell lineages raises several interesting questions that require further investigation. What are the mechanisms that underlie the nonredundant functions of specific APC subsets? How are antigens delivered to the appropriate APC subset? To what extent are APC differentiation programs hard-wired during development or acquired in response to specific inflammatory or metabolic environments? New genetic tools that allow precise targeting of particular APC subsets are necessary to unravel their unique contributions to mucosal immunity and tolerance. Understanding the mechanisms by which diverse APC subsets regulate distinct immune responses to the wide range of antigens encountered at mucosal barriers has substantial therapeutic value for approaches aimed at promoting immunity to vaccine antigens or tolerance to allergens or self-antigens.

Code availability

The scripts used to annotate and integrate published scRNA-seq data can be accessed at https://github.com/pty0111/nat-imm-review-TC/.

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