



REVIEW ARTICLE

Antigen presentation by dendritic cells and their instruction of CD4⁺ T helper cell responses

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Dendritic cells are powerful antigen-presenting cells that are essential for the priming of T cell responses. In addition to providing T-cell-receptor ligands and co-stimulatory molecules for naive T cell activation and expansion, dendritic cells are thought to also provide signals for the differentiation of CD4⁺ T cells into effector T cell populations. The mechanisms by which dendritic cells are able to adapt and respond to the great variety of infectious stimuli they are confronted with, and prime an appropriate CD4⁺ T cell response, are only partly understood. It is known that in the steady-state dendritic cells are highly heterogeneous both in phenotype and transcriptional profile, and that this variability is dependent on developmental lineage, maturation stage, and the tissue environment in which dendritic cells are located. Exposure to infectious agents interfaces with this pre-existing heterogeneity by providing ligands for pattern-recognition and toll-like receptors that are variably expressed on different dendritic cell subsets, and elicit production of cytokines and chemokines to support innate cell activation and drive T cell differentiation. Here we review current information on dendritic cell biology, their heterogeneity, and the properties of different dendritic cell subsets. We then consider the signals required for the development of different types of Th immune responses, and the cellular and molecular evidence implicating different subsets of dendritic cells in providing such signals. We outline how dendritic cell subsets tailor their response according to the infectious agent, and how such transcriptional plasticity enables them to drive different types of immune responses.

Keywords: dendritic cells; CD4⁺ T cells; Th effector responses

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INTRODUCTION

The differentiation of CD4⁺ Th cells into functionally distinct and context-appropriate effector populations is an important aspect of adaptive immunity, ensuring host protection against a wide range of pathogens that differ greatly in size, structural makeup, and location within tissues and cells. Effector Th cells are typically characterized into Tbet⁺ IFN γ ⁺ Th1 cells, Gata3⁺ IL-4⁺/IL-13⁺ Th2 cells, ROR γ t⁺ IL-17⁺ Th17 cells, FoxP3⁺ regulatory T cells (Treg), or BCL6⁺ IL-21⁺ T follicular helper cells (Fig. 1). While it is clear that T cells may display intermediate phenotypes and cannot always be easily classified into clearly separated subsets, these Th subsets represent a useful paradigm for the study of Th cell heterogeneity and their development.¹ It is known that the differentiation of each Th subset requires a specific set of signals during antigen presentation, and as the primary antigen-presenting cell population dendritic cells (DC) are considered central players in orchestrating CD4⁺ Th cell responses. Through their ability to surveil their local environment, DC are able to sample self and foreign antigens, and integrate signals from pathogens and bystander cells to convey relevant differentiation signals to naive CD4⁺ T cells in local lymph nodes (Fig. 2).

Multiple factors contribute to determine DC expression of Th cell polarizing molecules in vivo, including lineage-specified factors and context-dependent adaptation. The existence of a

diverse range of DC subsets that express distinct repertoires of pattern-recognition receptors (PRR) suggests a division of labor and a specialized ability to recognize and respond to different stimuli.² However, DC are also a highly adaptable population and readily alter their transcriptional profile in a condition-specific manner to support the appropriate Th cell response. Studies mainly carried out in DC cultured in vitro from bone marrow precursors (BM-DC) suggest that DC metabolic state is also affected (recently reviewed in ref. 3). Single-cell (sc)RNASeq data from mice immunized with different inactivated pathogens show that immunization-induced transcriptional changes are highly enriched in a small population of cDC2 that have taken up fluorescent pathogen, suggesting that signals received directly from the pathogen or its immediate environment are important determinants of DC function.⁴ In this review, we outline the various factors that contribute to DC heterogeneity across tissues, and then discuss how individual DC subsets and their transcriptional plasticity can shape CD4⁺ T cell responses in vivo.

DENDRITIC CELL SUBSETS ACROSS TISSUES

Conventional DC

Conventional (c)DC are defined by the expression of the transcription factor zBTB46 that differentiates them from other

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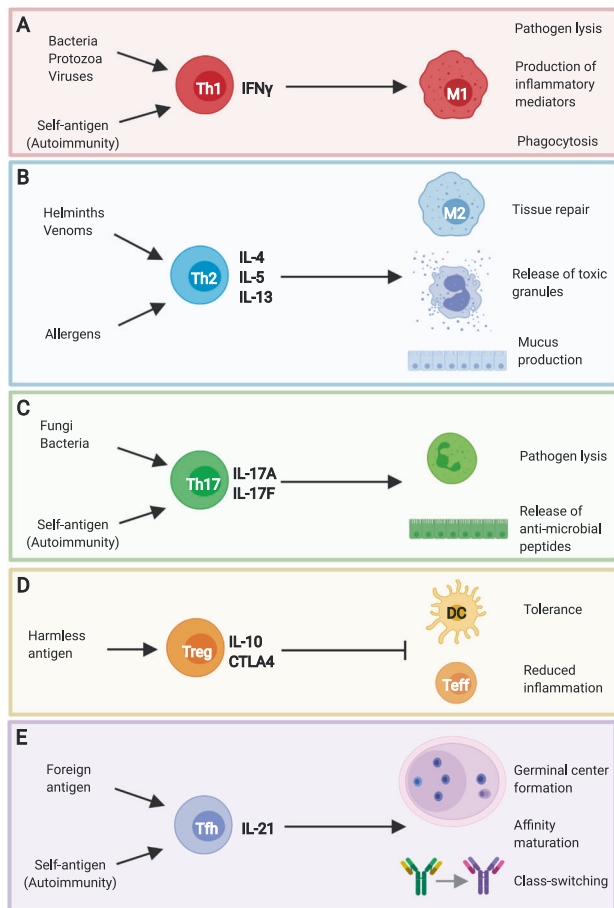


Fig. 1 Distinct effector T helper cell phenotypes are associated with different types of pathogens and immune-driven pathologies. **a** IFN γ ⁺ Th1 cells are associated with bacterial, protozoan, and viral infections, and promote microbicidal activity in macrophages. Similar pathways are invoked in some autoimmune diseases, where Th1 cells are directed against self-antigens. **b** Helminths and venoms drive IL-4/IL-5/IL-13⁺ Th2 cells that induce the release of toxic mediators from mast cells and eosinophils as well as promoting epithelial contractility and mucus production. Macrophages adopt a “M2” phenotype under these conditions to support tissue repair. Aberrant Th2 responses are strongly associated with allergic disease. **c** Extracellular bacterial and fungal infections drive IL-17⁺ Th17 cells that promote neutrophil activity and the release of anti-microbial peptides from the epithelium. Pathogenic Th17 cells are also associated with autoimmune diseases, such as multiple sclerosis and psoriasis. **d** Harmless antigens derived from self or foreign sources, such as the food proteins, are associated with the induction of IL-10⁺ Treg that act on dendritic cells and effector Th cells to regulate their function. **e** IL-21⁺ Tfh cells support antibody responses by promoting germinal center formation, affinity maturation, and antibody class switching. Tfh can be specific for foreign antigen, in the context of infection, or self-antigen, in the context of autoimmune disease. This figure was created using [BioRender.com](#)

DC populations such as lymphoid-derived plasmacytoid DC (pDC) and monocyte-derived DC (moDC).^{5,6} cDC populate almost all tissues and are the most abundant DC subtype under steady-state conditions. cDC are a heterogeneous population that have been organized into subsets using various criteria depending on the tissue or species under investigation. Aided by high-throughput and high-dimensional techniques, as well as by information on the transcription factors that drive cDC development in mice and humans,⁷ a unified classification strategy that can be applied across tissues and species has been identified.^{8,9} Using this strategy cDC can be stratified into one of four subsets based on

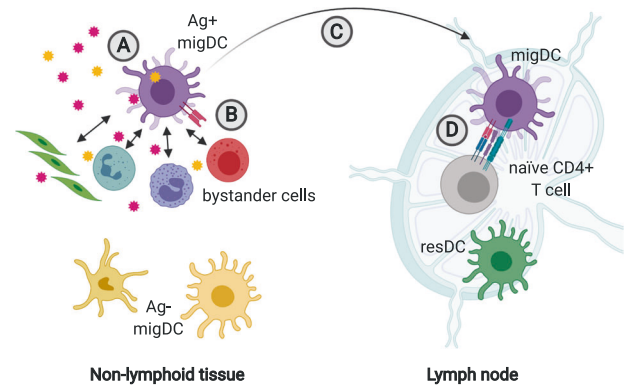


Fig. 2 Migratory dendritic cells integrate signals from antigen and bystander cells to inform CD4⁺ T cell differentiation in local lymph nodes. **a** Migratory DC (migDC) sample antigen in non-lymphoid tissues. The nature of the antigen (foreign vs. self, immunogenic vs. tolerogenic) determines migDC activation state and expression of CD4⁺ T cell polarizing factors. **b** DC communicate with epithelial, stromal, and immune cells in the vicinity. These interactions can inform migDC expression of CD4⁺ T cell differentiation factors. **c** Ag⁺ migDC alter expression of integrins and chemokine receptors to facilitate their migration from the tissue to local lymph nodes. **d** Ag⁺ migDC present peptide on MHCII to naive CD4⁺ T cells expressing a cognate TCR in local lymph nodes. During this interaction, DC provide co-stimulatory signals and instruct CD4⁺ T cell differentiation through expression of polarizing molecules. This figure was created using [BioRender.com](#)

whether they initially seed lymphoid or non-lymphoid tissues and their developmental requirements.

Migratory and lymphoid-tissue resident cDC populations cDC can be broadly divided into two populations based on their initial tissue of residence. Lymphoid-tissue-resident (res)DC directly seed lymphoid organs, such as the spleen, lymph nodes, or Peyer’s patches, where they remain as resident sentinels. In contrast, migratory (mig)DC initially seed non-lymphoid tissues, such as the lung, skin, or lamina propria, and migrate to local lymphoid organs either spontaneously under steady-state conditions or upon inflammation-induced activation in a CCR7-dependent manner.¹⁰ As such, lymphoid tissues can contain both resDC and tissue-derived migDC. Flow cytometry and histocytometry techniques are generally used to resolve LN resDC and migDC populations by their differential expression of MHCII, CCR7, and CD11c.^{8,11} MigDC highly express MHCII and CCR7 and have intermediate-to-low expression of CD11c, whereas resDC highly express CD11c and have intermediate expression of MHCII under steady-state conditions.¹² Analysis of LN tissue from organ donors suggests that migDC can also be identified by high HLA and CCR7 expression in human samples.^{13,14} However, it is important to note that the inducibility of MHCII and CCR7 expression on resDC populations during inflammation makes defining resDC and migDC by the sole use of these markers problematic. Unfortunately, stably expressed markers specific for resDC or migDC have yet to be identified. For this reason, the use of photoconvertible mouse models that enable tracking of cell migration are extremely valuable. Several studies have utilized the Kaede or KikGR mouse models to ascertain which LN cells originated from the skin. Cells present in the skin at the time of photoconversion fluoresce Kaede/KikGR-red, differentiating them from all other cells that are Kaede/KikGR-green.¹⁵ Following photoconversion migratory Kaede/KikGR-red cells can be detected in the draining LN and consist of migDC, neutrophils, monocytes, and CD4⁺ T cells, but not resDC.^{16,17} Similar studies have also highlighted the presence of MHCII^{hi} CCR7⁺ migDC expressing CD64, a marker previously thought to be restricted to monocyte and macrophage

Table 1. Transcriptional signatures and protein markers of described murine DC subsets as determined from published resources^{4,8,9}

Subset	Transcriptional signature (RNASeq, microarray)	Signature protein markers (flow/spectral cytometry, CyTOF, microscopy)
Total Lymphoid-resident cDC	<i>Tagap, Tagap1, Hhex, Tmem231, Snord13, Prdx6, Kynu</i>	CD11c ^{hi} , MHCII ^{int}
Total migratory cDC	<i>Fscn1, Ccr7, Cacnb3, Ltc4s, Clu, Epsti1, Ccl17, Il15, Il15ra</i>	MHCII ^{hi} , CCR7+
cDC1		
All	<i>Xcr1, Cadm1, Clec9a, Naaa, Irf8, Batf3, Id2, Cxcr3, Tlr3, Ifi205, Gcet2</i>	XCR1+, IRF8+
Migratory		CD103+, CD11b–
Lymphoid-resident		CD8α+, CD11b–
cDC2		
All	<i>Sirpa, Il2ra, Aldh1a2, Zeb2, Irf4, Sirpb1a, Sirpb1b, Dscam</i>	SIRPα+, IRF4+
Migratory		CD11b+, CD103– ^a
Lymphoid-resident		CD11b+, CD8α–
LC	<i>Cd207, Fam189a2, Tjp1, Pcdh7, Mtap2</i>	CD207+, CD326+, XCR1–
moDC	<i>Sirpb1c, Ly6c1, C3ar1, Ccr2, Plac8, Ms4a6b,</i>	CD64+, Ly6C ^{hi} , CD11c+, CCR2+
pDC	<i>Mctp2, Ly6d, Klk1, Siglech</i>	CD317+, Siglec H+

^aA subset of intestinal cDC2 co-express CD103 and CD11b.^{53,54} A subset of dermal cDC2 express low levels of CD11b.^{47,48}

populations^{8,18–20} that can also be expressed upon interferon (IFN) exposure (Immgen.com) or by activated cDC.^{21,22} Given these findings, the use of CD64 as an exclusion marker may not be appropriate for migDC analyses. The use of different markers such as CD88, Ly6C, or MerTK is then required to differentiate cDC from monocyte and macrophage populations in the LN and, more importantly, in non-lymphoid tissues such as the skin and mucosal surfaces. Together with CD26, a marker highly expressed by all cDC populations, the use of CD88 enables a clear distinction between cDC and monocyte/macrophages.^{8,23}

Despite the aforementioned limitations with current surface markers, resDC and migDC sorted from the same lymphoid organ using conventional gating strategies have distinct transcriptional profiles⁹, which are largely consistent with expression profiles generated using scRNASeq technology.⁴ Unsurprisingly, many of the differentially expressed genes are associated with cell migration, with transcripts such as *Ccr7* and *Fscn1* highly expressed by migDC but not resDC (Table 1). Transcripts for MHCII genes including *H2-Aa*, *H2-Ab1*, *Cd74*, and especially *H2-DMA* and *H2-DMab* are also expressed at lower levels in migDC compared with resDC, which is consistent with the reduced ability of mature migDC to process additional antigens in LN.^{4,9} Despite the profound transcriptional shutdown of MHCII genes upon maturation, MHCII protein expression remains high on the surface of migDC to facilitate efficient antigen presentation.⁴ This example highlights the power of techniques, such as index sorting and CITESeq, that enable the simultaneous assessment of mRNA and protein expression in the same assay.²⁴

Type-1 and type-2 cDC populations

cDC precursors develop from monocyte-dendritic cell precursors in the bone marrow where they commit to one of two distinct cDC lineages that differ in their developmental requirements.^{25–27} cDC1 preferentially develop from Ly6C– Siglec H– preDCs²⁵ in a BATF3- and IRF8-dependent manner^{27–29} and can be identified by their surface expression of XCR1³⁰ and, in non-lymphoid tissues, CD103.^{31,32} cDC2 preferentially develop from Ly6C+ Siglec H– CD115+ preDCs^{25,27} and are identified by their expression of SIRPα in combination with cDC-specific markers. The transcriptional regulation of cDC2 development has been shown to involve a number of transcription factors including *Zeb2*³³, *N* and *Irf4*,^{34–36} which positively regulate the development of all cDC2, and *Klf4*³⁷ and *Notch2*³⁸, which positively regulate the development of various cDC2 subsets. Similar populations of cDC1 and cDC2 precursors have been identified in bone marrow biopsies and peripheral blood samples from healthy volunteers

where they can be distinguished on the basis of SIRPα expression.³⁹

preDC seed both lymphoid and peripheral tissues where they undergo clonal expansion.^{26,40,41} Interestingly, cDC1 and cDC2 localize to different regions within lymphoid and non-lymphoid tissues, a factor that determines their access to antigen and interactions with other immune cell populations (recently reviewed in refs. ^{42,43}). Differential expression of chemokine receptors between cDC1 and cDC2 precursors may partially explain their distinct distribution patterns. Pre-cDC1 highly express CXCR3, whereas pre-cDC2 express CX3CR1.⁴⁴ Given that the CXCR3 ligands are the IFN-inducible chemokines CXCL9/10/11, preDC1 may be preferentially recruited to sites of IFN-mediated inflammation.⁴⁴ Further, the relative proportion of each cDC subset differs greatly across tissues.^{8,13} For example, in mice, cDC1 are rare in skin but are comparatively more abundant in the lung,⁸ whereas the cDC subset composition is compartmentalized along the intestinal tract.⁴⁵ These differences in abundance do not always extend to humans, as suggested by the low proportion of cDC1 compared with cDC2 in human lung.¹³ This variability may suggest a greater impact of environmental and infectious stimuli on human cDC development.

In addition to their divergent developmental pathways, cDC1 and cDC2 have distinct transcriptional profiles, with key transcriptional regulators such as IRF4 and IRF8 among the most differentially expressed genes. Markers used in cDC subset discrimination by flow cytometry, such as XCR1 and SIRPα, are also differentially expressed at the transcript level (Table 1). Interestingly, transcriptional segregation of cDC1 and cDC2 subsets in lymph nodes is much more evident within the resDC compartment.^{4,9} The relative similarity in transcriptional profiles among migDC subsets suggests that tissue-derived factors and/or cDC migration signals have a significant influence over the migDC transcriptome. Indeed, studies directly comparing the transcriptional and phenotypic profiles of cDC across human tissues have reported marked tissue-specific signatures.^{13,46}

cDC2 subsets

While cDC1 are accepted to be a largely homogenous population, cDC2 are highly heterogenous and have been divided into subpopulations according to various criteria that include the expression of CD11b,^{47,48} CD301b (MGL2),⁴⁹ CD206,⁵⁰ PDL2,⁵¹ Esam,³⁸ and Tbet,⁵² or the developmental reliance on *Notch2*,³⁸ or *Klf4*.³⁷ With the exception of CD301b and CD206 that are co-expressed on a subset of cDC2, there is no strong association between each of these classification strategies, highlighting the

extent of phenotypic heterogeneity within the cDC2 subset. Tissue-specific adaptation may account for some of this heterogeneity, with a population of Runx/Cbfb/TGFB-dependent CD103⁺ cDC2 unique to intestinal tissues^{53,54} and CD11b-low SIRPα⁺ cDC2 enriched in the skin.^{47,48} A recent single-cell transcriptional study of murine and human cDC2 has provided additional evidence for the existence of distinct cDC2 subsets across tissues.⁵² These data show that cDC2 can be segregated into Tbet⁺ and RORyt⁺ subsets that differ both transcriptionally and epigenetically. However, the study of these populations remains restricted by the need for appropriate reporter mouse strains for the phenotypic identification of the two subsets.

Other DC subsets

In addition to cDC, a number of other DC populations are found in mice and humans. These include moDC, Langerhans cells (LC), and pDC. Circulating Ly6C^{hi} CD11b⁺ monocytes are actively recruited to sites of inflammation or infection where they can adopt DC-like features, including expression of CD11c and enhanced antigen presentation capacity.^{55,56} In vitro, GM-CSF and IL-4 promote the differentiation of monocytes into moDC in an IRF4-dependent manner.^{57,58} In the absence of *Irf4*, GM-CSF and IL-4-treated monocytes differentiate into macrophages,⁵⁷ indicating that *Irf4* is an important regulator of moDC differentiation. Inflammation and infection are associated with the development of moDC in vivo,^{59–61} with IFNγ signaling implicated in driving monocyte-to-DC differentiation following *T. gondii* infection.⁶² However, the signals involved in the monocyte-to-moDC transition are likely to be context-dependent given that moDC are present in wide range of tissues and inflammatory settings.⁶³

LC are a radio-resistant population that share properties with both DC and macrophages⁶⁴ and are uniquely positioned in the epidermis where they reside in close contact with surrounding keratinocytes that provide factors required for LC survival and epidermal residence.^{65,66} Similar to tissue-resident macrophages, LC are continually replenished from a precursor pool⁶⁷ that is seeded into the epidermis during embryonic development,^{68,69} but can also be derived from monocytes following injury.⁷⁰ LC are readily identifiable by cell surface expression of langerin (CD207), E-cadherin, CD326, low CD11b, and, importantly, their lack of XCR1 and CD103 expression, distinguishing them from dermal cDC1.⁸

Finally, pDC are a distinct DC lineage that can be of lymphoid or myeloid origin^{71–73} and are identifiable by their expression of IRF7, Siglec H, and high levels of CD317.^{74–76} Unlike their lymphoid counterparts, myeloid-derived pDC are able to present antigen to T cells,⁷³ but their role in regulating CD4⁺ T cell responses remains to be determined.

ANTIGEN UPTAKE, TRANSPORT, AND PRESENTATION BY CDC SUBSETS

Antigen sampling and transport to lymphoid tissues

In their capacity as tissue sentinels DC continually sample antigen from their local environment and process it for presentation to CD4⁺ Th cells or CD8⁺ T cells on MHCII or MHCI molecules, respectively.^{77,78} Exogenous antigen is sampled by phagocytosis,^{18,79,80} receptor-mediated endocytosis^{81–83}, or micropinocytosis,^{84,85} depending on the subpopulation and activation status of the DC.⁸¹ At barrier sites, such as the epidermis and intestinal epithelium, LC and CX3CR1⁺ macrophages sample antigen by projecting dendrites through tight junctions into the cornified epithelial layer^{86,87} or intestinal lumen,^{88,89} respectively. Dermal cDC2 can also directly access epicutaneously applied antigen through hair follicles.⁹⁰ Following antigen uptake, human LC transit through the dermis in a CXCR4-dependent manner⁹¹ prior to CCR7-dependent migration to the skin-dLNs. LC can also transfer antigen-MHCII complexes directly to cDC within the dermis.⁹² This transfer likely increases the efficiency at which

epidermal-derived antigen reaches the LN as dermal cDC migrate to the LN at a faster rate, and distribute within the LN more widely, than LC.^{17,47,93} Similarly, sessile intestinal macrophages transfer antigens to migratory cDC via gap junctions for transport to the mediastinal LNs.⁹⁴

cDC2 are particularly adept at antigen uptake in the skin, making up the majority of antigen-positive (Ag⁺) cells in skin-dLNs following injection or application of fluorescently labeled particulate antigen.^{4,95–98} cDC2 also play an important role in the uptake of tumor antigens and their transport to LNs for presentation to T cells.⁵⁰ Soluble and particulate antigens < 70 kDa do not require active cellular transport to LN and are distributed throughout LN via a conduit network.^{99,100} It is therefore unsurprising that soluble antigens are taken up more ubiquitously than larger particles. Nevertheless, cDC2 seem to take up higher amount of soluble antigen per cell, and are overrepresented among Ag⁺ cells in a number of settings,^{47,101,102} suggesting that cDC2 have an intrinsic capacity for the uptake of exogenous antigens. Further, cDC2 may be optimally positioned for antigen capture within tissues as resDC2 are preferentially located near the subcapsular sinus within LNs, enabling easier access to lymph-borne antigens.^{98,101}

Conversely, cDC1 are located deep within the LN paracortex¹⁰¹ and are efficient at taking up cell-associated antigen and dead cells via receptors such as Clec9A, DEC205, Axl, and TIM3.¹⁰³ cDC1 preferentially process cell-associated antigen via the cross-presentation pathway for presentation on MHCI to CD8⁺ T cells, which is essential for antiviral and antitumor immunity.^{28,103} Indeed, CLEC9A and DEC205 have been successfully targeted with monoclonal antibodies to enhance antigen uptake and cross-presentation in the context of vaccination.^{104–106} moDC and cDC2 also cross-present antigen under certain conditions, such as in the presence of immune complexes,^{107–109} suggesting a level of redundancy in this pathway that is particularly apparent in humans.^{102,110,111}

Antigen presentation to naive CD4⁺ T cells

DC are the primary antigen-presenting cell population in vivo and are essential to elicit antigen-specific activation and expansion of naive CD4⁺ T cells though the peptide-MHCII interaction with the TCR and co-stimulatory signaling. In line with their superior capacity to take up exogenous antigens, cDC2 are particularly efficient at processing antigen for presentation on MHCII and induce superior CD4⁺ T cell proliferation compared with cDC1.^{104,112} *Irf4* expression by cDC2 is thought to account for this specialization, with IRF4 linked to enhanced peptide-MHCII formation in cDC.¹¹²

In addition to promoting the activation and proliferation of CD4⁺ T cells, communication between DC and CD4⁺ T cells during antigen presentation determines Th cell differentiation fate. A number of factors are implicated in regulating the Th cell differentiation program, including the strength and duration of co-stimulatory and peptide-MHCII-TCR interactions.¹¹³ For the purpose of this review, we will focus on DC-derived polarization signals, and how DC lineage and condition-specific adaptation regulate CD4⁺ T cell differentiation in vivo. This is discussed for the five major Th cell phenotypes below and is summarized in Fig. 3.

DC INSTRUCTION OF CD4⁺ T CELL DIFFERENTIATION

Instruction of Th1 differentiation

Th1 cells are defined by their production of IFNγ and are associated with protective immunity against intracellular pathogens and viruses.¹¹⁴ The Th1 transcriptional program is driven by IFNγ,^{115,116} IL-12,¹¹⁷ and IL-27,¹¹⁸ which promote the upregulation of Tbet and IL-12Rβ in CD4⁺ T cells.¹¹⁹ IL-12 signaling reinforces

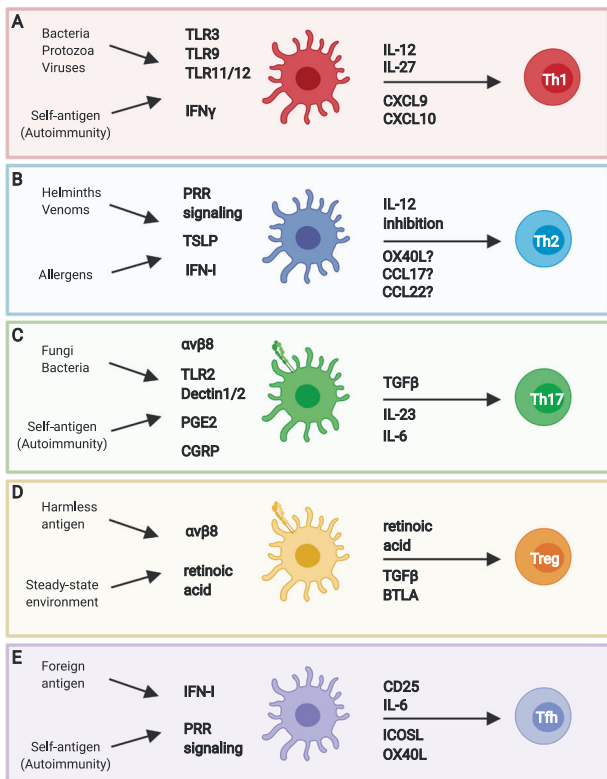


Fig. 3 Transcriptional plasticity of dendritic cells determines CD4⁺ T cell differentiation in vivo. Signals from pathogens, immunogens, and antigens, as well as signals from the local stromal, epithelial, and immune cells influence DC expression of CD4⁺ T cell polarizing factors in a context-specific manner. This ensures activation of the appropriate effector Th cell phenotype. **a** IFN γ from bystander cells and signaling through pattern-recognition receptors (PRR), such as TLR3, 9, 11, and 12, induces *Il12b* upregulation by DC. IFN γ also promotes transcription of the CXCR3 ligands *Cxcl9* and *Cxcl10*. Together with IL-27, DC production of these factors facilitates Th1 differentiation. **b** Helminth products condition DC to promote Th2 differentiation in vivo, potentially via PRR signaling, and DC integration of IFN-I and TSLP signals produced by bystander cells. The DC-derived factors that specifically promote Th2 differentiation are unclear but may include limiting IL-12 production in addition to a “positive” Th2 signal such as OX40L, CCL17, or CCL22. **c** Engagement of PRR, such as TLR2 and Dectin-1, promote DC production of IL-6 and IL-23. Fibroblast-derived prostaglandin E2 and neuron-derived calcitonin gene-related peptide also promote IL-23 production by DC. Together with $\alpha\text{v}\beta 8$ -mediated activation of TGF β , DC production of these factors promotes Th17 differentiation. **d** At steady state, DC are conditioned into a tolerogenic state by their local tissue environment. Retinoic acid may regulate this state in some tissues/settings. Tolerogenic DC express BTLA and the TGF β activating integrin $\alpha\text{v}\beta 8$, and produce retinoic acid. In the absence of immunostimulatory signals, the expression of these factors helps support the generation of Treg. **e** PRR and IFN-I signaling support IL-6 production by DC. Along with co-stimulatory signaling, IL-6 supports Tfh differentiation. DC also express CD25 to limit IL-2 availability to further support Tfh priming. This figure was created using BioRender.com

the Th1 program and is essential for optimal Th1 differentiation in both mice and humans.^{120–122}

cDC1 are a major source of IL-12 in vivo.^{123–125} They constitutively express high levels of *Il12b* transcripts, with evidence of constitutive IL-12p40 protein production in mice.^{126,127} As such, cDC1 are commonly associated with Th1 responses and are often portrayed as the principal Th1-inducing cDC subset. Indeed, cDC1 are more efficient than cDC2 at inducing Th1 differentiation in ex vivo coculture systems,¹²⁸ presumably

due to their baseline expression of IL-12. Th1 responses are also significantly compromised in some systemic,¹²⁹ cutaneous^{123,130} and intestinal infection models^{129,131–133} when cDC1 are absent, suggesting that cDC1 are required to support some protective Th1 responses in vivo. However, there is a notable body of literature implicating moDC in driving Th1 responses during infection with *Plasmodium*,¹³⁴ *T. gondii*,⁶² or *Salmonella*¹³⁵ as well as in CpG- or CFA-based immunization models.^{136,137} It is not clear whether moDC can serve as antigen-presenting cells in these settings given their low expression of MHCII and co-stimulatory molecules.^{18,138} Although inefficient providers of signals 1 and 2, moDC are a good source of signal 3 and can produce high levels of IL-12p40 in vitro^{137,138} and in vivo (Hilligan et al., submitted manuscript). A cooperation between moDC and cDC has been suggested by several studies that designated moDC as regulators of Th1 differentiation and cDC as inducers of Th1 proliferation.^{135,136,138,139} Finally, a recent study has demonstrated a role for TNFR2⁺ cDC2 in driving Th1 responses following intranasal immunization with a mucosal adjuvant.¹⁴⁰ Specific targeting of this cDC population reduced recall Th1 responses in the lung and abrogated immunization specific antibody production.¹⁴⁰ Together, these data suggest that a number of DC subsets have the capacity to support Th1 differentiation in vivo and the context and location of infection or immunization determines the DC subset requirements.

Given the critical requirement for IL-12 in Th1 immune responses, signals that regulate IL-12 expression in DC likely contribute to potentiating Th1 differentiation. Interestingly, constitutive IL-12p40 production by cDC1 is maintained under germ-free and antigen-free conditions (Kawabe et al., submitted manuscript) suggesting that IL-12 production is intrinsically coupled to the cDC1 lineage and is not the result of exogenous microbial signals. Homeostatic IL-12 is thought to function as a regulator of Th2 responses^{126,127} and support the generation of innate-like Tbet^{hi} memory-phenotype (MP) CD4⁺ T cells (Kawabe et al., submitted manuscript) that can produce IFN γ in the absence of TCR signaling and provide early host protection against infection.¹⁴¹

During infection or inflammation, a number of signals induce or enhance IL-12 production by DC. Firstly, signaling through PRR such as TLR3 and TLR9 enhances DC expression of IL-12p40,^{142–144} and the engagement of multiple PRR synergizes to boost IL-12 production.¹⁴⁵ Secondly, CD40:CD40L interactions between DC and T cells promotes transcription of the IL-12p35 subunit in DC,^{146,147} supporting the production of bioactive IL-12p70. Finally, IFN γ derived from NK or T cells feedbacks on DC to further promote IL-12 production.^{148–150} IFN γ also promotes cDC and moDC expression of CXCR3 ligands which recruit CXCR3⁺ pre-Th1 cells to a niche that favors Th1 differentiation.^{4,151,152} DC–NK cell crosstalk likely facilitates the generation of Th1 cells through this pathway.¹⁵³ It is possible that the main role of cDC1 in Th1 immunity is their involvement in eliciting early IFN γ from NK¹⁵⁴ and CD4⁺ MP cells¹⁴¹ that then conditions cDC2 and moDC towards a Th1-inducing phenotype.

Instruction of Th2 differentiation

Th2 cells produce IL-4, IL-5, and IL-13¹⁵⁵ and are associated with helminth infection, exposure to venoms, and allergic disease.¹⁵⁶ IRF4⁺ cDC2 are necessary for promoting Th2 differentiation in the skin,⁵¹ lungs,¹⁵⁷ and intestinal tract,^{132,158} with KLF4-dependent³⁷ and CD301b⁺ PDL2⁺ cDC2 subsets^{49,159} specifically implicated in Th2 models. LC may also support Th2 responses in the skin as LC depletion impairs IL-4 production following epicutaneous application of protein antigen¹⁶⁰ or a vitamin D analog that drives an atopic dermatitis-like phenotype in mice.¹⁶¹ In contrast, cDC1 negatively regulate Th2 responses through constitutive IL-12p40 production and their absence can increase the levels of Th2 cytokines in mouse models of

helminth infection^{126,132} and house dust mite allergy.¹²⁷ The inhibitory effect of cDC1 appears to vary depending on the Th2 model being used, and in some cases it can also be observed for LC,¹⁶² which also constitutively express some level of *Il12b*.⁹ In this respect it is also important to note that, while lower IL-12 production may result in increased Th2 responses in some models, IL-12-KO mice exposed to Th1 stimuli develop lower Th1 responses without defaulting to Th2.¹⁶³ Thus, lack of IL-12 may contribute but is not sufficient for Th2 differentiation.

Exposure to helminth products endows cDC2 with the capacity to induce Th2 differentiation, suggesting that DC instruction of Th2 responses is an active process rather than a default one.^{96,158,164–166} Indeed, there is evidence that the *S. mansoni* egg antigen (SEA)-derived protein Omega1 inhibits IL-12 production by DC and alters DC morphology to limit contact time with CD4⁺ T cells,^{167,168} two factors thought to contribute to Th2 differentiation.¹⁶⁹ In an in vitro model, SEA can also trigger Dectin-1 and Dectin-2 receptors on DC, leading to prostaglandin-dependent expression of OX40L and development of Th2 responses.¹⁶⁶ In the same study, in vivo experiments in mice also supported a pro-Th2 role of Dectin-2 in the SEA response.

Transcriptomic analysis of helminth- or allergen-conditioned cDC has identified thymic stromal lymphopoietin (TSLP) and IFN-I as upstream regulators of helminth or allergen-induced transcriptional networks in cDC.^{162,170} These signatures are restricted to Ag + cDC2, with comparatively little change observed in the transcriptional profile of Ag- cDC2 and cDC1 compared with baseline.^{4,162} TSLP has long been associated with allergic responses in mice and humans, and is known to be a potent inducer of DC activation accompanied by the upregulation of *Tnfrsf4* (encoding OX40L), and the expression of the chemokines *Ccl17* and *Ccl22* to attract CCR4-expressing Th2 cells (reviewed in ref. ¹⁷¹). However, it is important to note that the DC-specific effects of TSLP signaling are complicated by the finding that TSLP also acts directly on T cells to promote the secretion of type-2 cytokines.^{172,173} A recent OVA allergy model employing *Tslpr*^{fl/fl} animals crossed to cDC-specific (*Zbtb46*) or CD4⁺ T cell-specific (*Cd4*) Cre lines demonstrates that TSLPR signaling in both cell types is important for driving Th2 inflammation.¹⁷⁴ IFN-I signaling has been more recently associated with Th2 responses, and interestingly, is only associated with Th2 responses in some settings, such as intradermal injection of *N. brasiliensis* larvae or house dust mites. In these cases, IFNAR signaling is required to promote robust IL-4 production by Th2 cells in vivo.^{162,175} The IFN-I requirement for Th2 responses is likely due to a direct effect on DC rather than T cells, as suggested by experiments using immunization with IFNAR-deficient BM-DC.¹⁷⁵ IFNAR blockade does not impact cDC2 activation or migration,¹⁶² suggesting that other IFN-I driven signals within DC support instruction of Th2 responses. Further investigation of IFN-I driven transcriptional programs in DC may uncover previously unidentified signals involved in Th2 differentiation.

In conclusion, the DC-derived signals required to initiate and reinforce the Th2 differentiation program remain currently unclear. A source of IL-4 appears to be required to support IL-4 production by Th2 cells in vivo,¹⁷⁶ a finding that may reflect the recognized role of IL-4 in Th2 differentiation in vitro.¹⁷⁷ However, the origin of this early IL-4 production remains undefined, with no evidence suggesting that DC might be the source of such IL-4. While TSLP and IFN-I can condition DC and promote Th2 development, neither is essential except in rare cases. OX40L–OX40 interactions between DC and CD4⁺ T cells, as well as the DC-derived chemokines CCL17, CCL22, and possibly CCL8, may support Th2 differentiation;^{178–182} however, it remains to be determined whether these signals are definitive Th2 polarizers or are merely involved in co-stimulation¹⁸³ and Th2 cell trafficking,^{184,185} respectively. Future studies may provide new information on this important question.

Instruction of Th17 differentiation

Th17 cells are characterized by production of IL-17 and are required for resolving extracellular bacterial and fungal infections. Th17 cells are also strongly implicated in pathogenesis of many autoimmune diseases, including multiple sclerosis and psoriasis.¹⁸⁶ IL-6 and TGFβ are critical signals for Th17 differentiation in vitro,¹⁸⁶ whereas IL-1β and IL-23 can support Th17 differentiation in vivo in the presence of IL-6 or TGFβ.^{187,188}

LC have a documented association with cutaneous Th17 responses and accumulate in psoriatic lesions.¹⁸⁹ Studies in mice have shown that LC are a significant source of IL-6 and depletion of LC in the context of an epicutaneous *C. albicans* infection significantly reduces the number of antigen-specific Th17 cells in the dLN.^{130,190} However, other studies have found no role for LC in driving cutaneous Th17 inflammation.¹⁹¹ Instead, dermal cDC2 have been implicated,¹⁹² which is in line with a large body of data showing that IRF4-dependent cDC2 are required to support Th17 differentiation across a number of tissues including the lung^{36,193} and intestine.^{38,35,54,194–196} Unlike the cDC2 involved in promoting Th2 immune responses, Th17-promoting cDC2 require Notch2, but not KLF4, for their development.¹⁹⁵ Finally, CCR2-dependent moDC have also been associated with promoting Th17 responses in an oropharyngeal candidiasis model. Similar to Th1 models, moDC are thought to cooperate with cDC to facilitate optimal Th17 differentiation.¹⁹⁷

DC provide many of the required Th17 differentiation signals in vivo^{192,193,195,198} and facilitate the activation of latent TGFβ via αvβ8 integrin. Indeed, DC-specific deletion of αvβ8 significantly blunts Th17 responses in the lamina propria¹⁹⁹ and limits IL-17-mediated pathology in an experimental autoimmune encephalitis model.^{200,201} A number of signaling pathways are involved in inducing IL-6 and IL-23 production by cDC, including signaling via TLR2 and Dectin-1, PRR that recognize glucans enriched in fungal cell walls.^{148,190} TLR2 engagement preferentially induces IL-23 over the related cytokine IL-12,²⁰² suggesting that the distribution of PRR activation directly influences DC expression of Th cell polarization factors. Signaling via other PRR can also promote IL-23 and IL-6 production by DC, including imiquimod engagement of TLR7 in dermal cDC¹⁹¹ and flagellin activation of TLR5 in lamina propria cDC2.¹⁹⁶ More recently, interactions between stromal cells, neural networks, and DC have been implicated in eliciting and potentiating Th17 immunity, particularly in the skin. Fibroblast-derived prostaglandin E2 (PGE2) can promote IL-23 production by preactivated human cDC, and skin fibroblasts from psoriasis patients were found to express considerable levels of COX-2, an enzyme involved in prostaglandin synthesis. In turn, cDC-derived TNFα and IL-1β promote fibroblast secretion of PGE2, implicating fibroblast-DC cross-talk in potentiating Th17 inflammation.²⁰³ Similarly, calcitonin gene-related peptide (CGRP), a neuropeptide derived from sensory TRPV1⁺ neurons, promotes IL-23 production by dermal cDC2 following *C. albicans* infection, or induction of psoriasiform inflammation in mice.^{204,205} TRPV1⁺ neurons can also trigger CGRP release at sites adjacent to infection, pre-empting cDC responses to help contain infection.²⁰⁶

Instruction of iTreg differentiation

In the periphery, CD4⁺ T cells can differentiate into inducible regulatory Th cells (iTreg) that limit inflammation and establish immunological tolerance.²⁰⁷ iTreg can be distinguished from the thymus-derived natural Treg, which are not considered in this review, through their expression of the transcription factor Helios, which is expressed by natural Treg but not iTreg.²⁰⁸ TGFβ and IL-2 signaling are important determinants of iTreg differentiation,^{209–211} with cDC-dependent activation of TGFβ important for optimal iTreg induction.²¹² In addition, cDC expression of BTLA²¹³ and cDC-derived retinoic acid^{214,215} support iTreg differentiation in vivo by negatively regulating molecules associated with the differentiation of other effector CD4⁺ T cell subsets.^{216,217} Treg-DC

cross-talk may also play a role in limiting inflammatory responses as Treg actively deplete CD80, CD86,^{218,219} and peptide-MHCII complexes from cDC.²²⁰

The involvement of DC in instructing iTreg differentiation has been largely studied in the context of the intestine where DC-derived signals are known to play an important role in inducing immunological tolerance to food proteins.²²¹ IRF8- and BATF3-dependent cDC1 are necessary for optimal iTreg induction,^{129,221} which is in line with their selectively high expression of BTLA,²¹³ *Aldh1a2* (encoding retinaldehyde dehydrogenase 2 or RALDH2), an enzyme involved in retinoic acid production, *Tgfb2*, and *Itgb8* (encoding a subunit of $\alpha v \beta 8$), an integrin required for TGF β activation.^{131,221,222} However, oral tolerance is maintained in animals lacking IRF8-dependent cDC1, suggesting a level of redundancy in intestinal iTreg induction.^{31,221,223} Experiments in which ovalbumin-specific CD4⁺ T cells were co-cultured with various mesenteric LN cDC subsets pulsed with ovalbumin peptide showed that CD103⁺ CD11b⁺ migDC2 and CD8 α ⁺ resDC were also capable of inducing an iTreg differentiation program, albeit to a lesser extent than CD103⁺ CD11b⁺ migDC1.²²¹

Redundancy in DC subset instruction of iTreg differentiation is further exemplified in the skin where LC and cDC2 also have demonstrable roles in Treg induction. The proximity of epidermal LC to commensal microbes colonizing the surface of the skin has led to the proposal that the main function of LC is to induce tolerant responses. Indeed, in animals where MHCII expression was restricted to LC, epicutaneous immunization failed to induce effector or memory CD4⁺ T cell responses.²²⁴ Further, other studies have shown that depletion of LC reduces Treg numbers and improves effector immune responses in a *Leishmania major* model.²²⁵ LC were also found to mediate the induction of Treg following skin exposure to ionizing radiation.²²⁶ In the skin, RALDH2 expression is greatest within the dermal cDC2 population, which were also found to promote iTreg induction ex vivo.²¹⁴

The signals involved in promoting tolerogenic functions of DC are currently unclear. NF κ B signaling may be involved as DC-specific deletion of IKK β impairs Treg induction, resulting in spontaneous autoimmunity.²²⁷ The observation that IKK β deficiency also reduces the accumulation of tissue-derived cDC in LNs suggests that NF κ B signaling may regulate cDC migration rather than a tolerogenic program specifically.²²⁷ Instead, adaptation to the tissue microenvironment may be important for initiating a tolerogenic cDC phenotype. TGF β R, retinoic acid, and MyD88 signaling are required for optimal *Itgb8* expression by intestinal cDC1 and are sufficient to promote *Itgb8* expression by splenic cDC1 ex vivo.²²² Further, *Aldh1a2* expression is specifically enriched in cDC1 situated in the proximal intestine where iTreg induction is most prominent, indicating strong regionalization of tolerogenic programs within cDC1.⁴⁵ These data suggest that tolerogenic programming likely occurs in the tissue prior to migration, which may explain the largely concordant nature of transcriptional changes upon homeostatic or immunogenic maturation of cDC1 populations.²²⁸

Instruction of Tfh differentiation

Tfh cells are a specialized Th cell subset that localize to B cell areas within secondary lymphoid organs where they support germinal center formation, affinity maturation, and antibody class switching (recently review in ref. ²²⁹). The differentiation pathway of Tfh cells is a multistep process that generally requires signals from both DC and B cells.²³⁰ IL-6 and inducible co-stimulator (ICOS) signaling initiate the Tfh differentiation program by promoting the expression of Tfh-defining proteins, including BCL6, CXCR5, and IL-21.^{231–235} An IL-21 autocrine signaling loop further supports their differentiation.²³⁵ IFN- γ ,²³⁶ IL-27,²³⁷ IL-1 β ,²³⁸ OX40,²³⁹ and Notch signaling²⁴⁰ can also support Tfh differentiation in vivo. In contrast, IL-2 is a potent inhibitor of Tfh differentiation^{241,242} and

Tfh-inducing cDC express the IL-2 receptor (CD25) in order to limit the availability of local IL-2.²⁴³

DC are thought to play an important role in directing the initial phases of Tfh differentiation, with B cells providing subsequent signals to reinforce the Tfh program. However, a recent study has challenged this notion in the context of *Plasmodium* infection where cDC were reported to be dispensable and B cells necessary for the generation of antigen-specific Tfh responses.²⁴⁴ Nevertheless, most cDC subsets have the capacity to induce Tfh differentiation in the appropriate conditions (recently reviewed in ref. ²⁴⁵) and cDC2 populations are necessary for regulating Tfh differentiation and humoral responses in a number of settings.^{98,140,243,246–249} Several mouse models in which cDC2 were specifically depleted report a reduction in Tfh, germinal center B cells, and antibody titers following an immunization or infection protocol.^{140,246,247,249} In humans, cDC2 isolated from tonsils were identified as the most effective DC subset at inducing Tfh polarization.²⁵⁰ These data suggest that cDC2 support optimal Tfh differentiation in both mice and humans. Interestingly, cDC2 can also have a role in limiting Tfh and antibody responses in the absence of sufficient adjuvanticity. This function is performed by CD301b⁺ cDC2 and likely serves to prevent the generation of autoantibodies and restrict the generation of antibodies against allergens.²⁵¹

LC and cDC1 also have the capacity to induce Tfh differentiation, but this only appears to be efficient if antigen is directly targeted to these cell types with Clec9A or Langerin antibodies.^{252–255} In experiments employing untargeted immunization protocols, LC only partially contributed to Tfh responses,²⁵⁶ and there was no documented defect in Tfh differentiation or antibody responses in animals lacking cDC1.^{140,246,247,249,256} The role of monocyte-derived cells, including moDC, in Tfh responses is less well defined, with examples of monocytes supporting, inhibiting or having no effect on Tfh numbers or antibody responses depending on the model being assessed. Depletion of monocyte-derived cells had no impact on antibody production in *Salmonella* infected mice¹³⁵ or the number of germinal center B cells in papain immunized mice,²⁵¹ suggesting that cells other than moDC are supporting Tfh responses in these animals. In other models, moDC are shown to negatively regulate Tfh differentiation. Monocyte depletion was associated with increased Tfh differentiation at the expense of Th1 differentiation following *Plasmodium* infection,¹³⁴ suggesting that monocyte-derived signals may regulate Th1/Tfh bifurcation.¹³⁴ There is also evidence that monocytes are required to provide critical Tfh-inducing factors in some settings. MoDC-derived IL-6 boosted Tfh responses in a CpG-B immunization model²⁵⁷ and IL-1 β derived from CX3CR1⁺ patrolling monocytes was required for Tfh differentiation following administration of heat-killed *E. coli*.²³⁸ Together, these studies underscore the context-specific nature of the cell types involved in Tfh differentiation and the extent of cellular redundancy in this pathway.

As mentioned above, a number of redundant signals from cDC, monocytes, and B cells are involved in Tfh differentiation, which makes defining a “Tfh-inducing” profile difficult. One of the most critical factors for Tfh differentiation are early co-stimulatory interactions between DC and naive CD4⁺ T cells. DC-dependent ICOS signaling is required to induce BCL6 and CXCR5 in CD4⁺ T cells within the first few days of infection.²³¹ Similarly, cDC expression of CD40,²⁵⁸ OX40L,^{239,248,259} and Notch ligands²⁴⁰ support Tfh differentiation to varying degrees. The non-canonical NF κ B pathway is required for cDC2 expression of ICOSL and OX40L following polyIC stimulation,²⁴⁸ and B-cell expression of ICOSL,²⁶⁰ suggesting that this pathway may facilitate Tfh priming.

In addition to co-stimulation, DC promote Tfh responses through IL-6, with IL-6-KO DC inefficient at promoting IL-21⁺ Tfh cells.^{261,262} Early IFN- γ is an important inducer of DC-derived IL-6²⁶³ and IFNAR deficiency in the DC compartment leads to blunted Tfh responses after VSV infection.²⁶² TRIF-dependent

signaling in monocytes has been implicated in endowing these cells with the capacity to induce Tfh differentiation through production of IFN- γ and inflammasome activation.²³⁸ T cell intrinsic expression of IFNAR and IL-1R β was required for optimal Tfh differentiation in this model,²³⁸ suggesting that IFN- γ supports Tfh responses through actions on DC and T cells. This may explain the IFN- γ -dependent enhancement of Tfh cell and germinal center responses in animals co-administered dsRNA with an influenza vaccine.²⁶⁴

Finally, given that co-localization of pre-Tfh with B cells is a requirement for full Tfh differentiation,²⁶⁵ chemokine signaling is an important factor in determining Tfh fate. CXCR5 expression mediated by ICOS signaling occurs within two cell divisions and is necessary for positioning pre-Tfh near B-cell areas.²³¹ In their study, Lonnberg et al. used pseudo-time modeling of scRNASeq data to show *Plasmodium*-specific Th cells co-express *Cxcr3* and *Cxcr5* prior to Th1/Tfh bifurcation. This observation coupled with their finding that depletion of monocyte-derived cells favors a Tfh fate over a Th1 fate led them to propose that IFN-dependent mDC production of CXCR3 ligands may act to limit Tfh differentiation by preferentially recruiting antigen-specific CXCR3 + CXCR5⁺ Th cells to a niche that supports Th1 differentiation.¹³⁴ This proposal is supported by data demonstrating a requirement for Th cells to locate to areas rich in CXCR3 ligands for optimal Th1 differentiation.^{151,152} Therefore, chemokine gradients generated by DC and monocytes at the time of CD4⁺ T cell priming may play a key role in Tfh vs. Th differentiation.

CONCLUDING REMARKS

Advances in transcriptional profiling and high-dimensional flow cytometry have highlighted the lineage-derived and tissue-imprinted heterogeneity present within the DC compartment,^{8,13,46,52} as well as the remarkable level of plasticity DC exhibit when responding to different types of stimuli.⁴ Together, these factors ensure that the DC compartment as a whole has the capacity to sense and respond to a wide variety of pathogens, immunogens, and antigens, and elicit the appropriate CD4⁺ T cell response.

The tissue environment in which DC capture antigen has a marked impact on their ability to induce particular Th cell differentiation programs. At steady state, tissue-derived signals likely play an important role in programming a tolerogenic vs. immunogenic state in DC,²²² as was recently demonstrated in the intestinal tract.⁴⁵ During infection or inflammatory responses, DC integrate signals derived from the tissue environment as well as the pathogen itself to inform their expression of Th cell polarization factors. Direct interaction with antigen appears to be required for this transcriptional plasticity, as condition-specific transcriptional profiles are only evident among the small proportion of DC that have taken up fluorescent-labeled pathogens.⁴ This may explain why cDC2, which are superior at taking up exogenous antigen,^{4,95–98} are pivotal in directing Th cell differentiation in many settings, whereas cDC1, which specialize in the uptake of cell-associated antigen,¹⁰³ excel at cross-presentation.²⁸

In addition to pathogen-derived signals, DC also sense signals, such as cytokines, derived from surrounding cells responding to the same pathogen. Again, responses to these signals are enriched in Ag⁺ DC,⁴ likely due to the relative abundance of the cytokine in the vicinity of the pathogen. However, cytokines derived from bystander cells or DC themselves can have far reaching effects and prime DC at distal sites to anticipate their response, as has been shown with the neuropeptide CGRP promoting IL-23 production by cDC2 at sites adjacent to infection.²⁰⁶ Further, observations of context-specific adaptation of distal DC are now being extended to the epigenetic level in bone marrow precursors.²⁶⁶ These new findings open up the possibility for conditioned responses in DC

exceeding the lifespan of tissue-resident cells or the longevity of infection, potentially modulating DC responses to subsequent infections.

Overall, context-dependent adaptation of DC plays a central role in determining Th cell differentiation in vivo. Further study focused on the rare population of DC that take up antigen will likely aid in understanding the specific signals involved in the early regulation of Th cell responses.

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AUTHOR CONTRIBUTIONS

K.L.H. wrote the manuscript and drafted the figures; F.R. provided edits and comments; and both authors agreed on the final manuscript and figures.

ADDITIONAL INFORMATION

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