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Functions of Murine Dendritic Cells

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Abstract

Dendritic cells (DCs) play critical roles in activating innate immune cells and initiating adaptive immune responses. The functions of DCs were originally obscured by their overlap with other mononuclear phagocytes, but new mouse models have allowed for the selective ablation of subsets of DCs and have helped to identify their non-redundant roles in the immune system. These tools have elucidated the functions of DCs in host defense against pathogens, autoimmunity, and cancer. This review will describe the mouse models generated to interrogate the role of DCs, and will discuss how their use has progressively clarified our understanding of the unique functions of DC subsets.

eTOC

Durai et al review the progress made in developing new mouse models for the analysis of the functions of dendritic cell subsets and what these models have revealed about the roles of these cells in immune responses

Introduction

The vertebrate immune system has evolved the remarkable capacity to robustly and precisely eliminate the wide variety of potential threats it encounters, from single cell bacteria to multicellular parasites to even transformed oncogenic versions of its own cellular components. To achieve this goal, many diverse lineages of effector cells must act together in different capacities throughout the course of the immune response. As with any system possessing such complexity, the careful control and coordination of the numerous components of the immune system is critical for its proper functioning. As our understanding of each cell type acting within this system has grown, it has become increasingly apparent that dendritic cells (DCs) act as the central regulators of the entire

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immune response, responsible both for sensing the nature of the threats faced and for activating the precise combination of effectors required to eradicate them.

First isolated by Ralph Steinman and Zanvil Cohn, DCs were identified by their stellate morphology and capacity to stimulate naïve T cells (Steinman and Cohn, 1973; Steinman and Witmer, 1978; Nussenzweig et al., 1980). DCs comprise two major branches, the classical DCs (cDCs) identified by Steinman and the lymphocyte-like plasmacytoid DCs (pDCs) that produce Type 1 interferon in response to pathogens (Perussia et al., 1985; Heath and Carbone, 2009; Cella et al., 1999; Siegal et al., 1999). cDCs can be further divided into two major subsets recently renamed cDC1s and cDC2s (Guilliams et al., 2014). All DCs originate from bone marrow (BM) progenitors arising from hematopoietic stem cells, starting with the macrophage/dendritic cell progenitor (MDP) (Fogg et al., 2006; Auffray et al., 2009), which gives rise to the common dendritic cell progenitor (CDP) (Naik et al., 2007; Onai et al., 2007), which finally gives rise to committed progenitors for each branch of DC such as the pre-cDC1 and the pre-cDC2 (Grajales-Reyes et al., 2015; Schlitzer et al., 2015).

cDCs express the integrin CD11c and MHC class II (Steinman et al., 1979; Metlay et al., 1990), and each subset can be distinguished by additional markers. Resident cDC1s in the spleen and lymph nodes (LNs) express CD8α, CD24, and XCR1, while cDC2s express CD4 and Sirpα (Mildner and Jung, 2014; Murphy et al., 2016). In nonlymphoid tissues, all cDCs express CD24, which distinguishes them from macrophages that instead express CD64 (Schlitzer et al., 2013; Plantinga et al., 2013; Langlet et al., 2012). Nonlymphoid tissue cDC1s also express XCR1 and CD103, while cDC2s express CD11b and Sirpα. Migratory cDCs that traffic from nonlymphoid tissues to LNs express these same markers they expressed in the periphery. There are several exceptions to these rules, however, such as $CD11b^+$ cDC2s in the small intestine that comprise both $CD103^+$ and $CD103^-$ fractions (Bogunovic et al., 2009; Satpathy et al., 2013). While these varied markers have historically been used to identify cDC subsets, a recent analysis suggests that a more simple and consistent identification of these cells across most tissues is possible by gating cDCs as $CD11c^+MHCII^+CD26^+CD64^-F4/80^-$, and within this population cDC1s as $XCR1^+$ and cDC2s as $Sirp\alpha^+$ (Guilliams et al., 2016). pDCs also express CD11c and MHCII, but can be segregated by their additional expression of B220, Siglec-H, and Bst2 (Blasius et al., 2006; Zhang et al., 2006).

While cDCs were discovered for their ability to serve as potent antigen-presenting cells (APCs), it is now clear that they also have non-redundant roles in innate immune responses (Mashayekhi et al., 2011; Satpathy et al., 2013). Their early recognition of pathogens and rapid cytokine production activates innate immune cells such as innate lymphoid cells (ILCs) and natural killer (NK) cells to limit pathogen spread until adaptive immunity can be initiated. Indeed, the heterogeneity of cDCs can itself be viewed as an evolutionary adaptation for the coordinated activation of the specific innate and adaptive effector responses best suited to control various forms of pathogens (Briseno et al., 2014). cDC1s, for example, recognize intracellular pathogens and initiate type 1 immune responses that require the early activation of ILC1s and NK cells as well as eventual Th1 polarization (Mashayekhi et al., 2011). Some cDC2s, on the other hand, govern type 2 immune responses

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against parasites in which they activate ILC2s and Th2 cells (Tussiwand et al., 2015). Other cDC2s sense extracellular bacteria and initiate type 3 immune responses by activating ILC3s and Th17 cells (Lewis et al., 2011; Satpathy et al., 2013). In this way, each cDC subset acts as the gatekeeper for a specific module of the immune response, recognizing a particular type of threat and activating both the innate and adaptive defenses best suited for overcoming it.

Since the time of their discovery, there has been considerable debate over whether DCs possess unique functions or whether they are redundant with macrophages and other mononuclear cells (Hume, 2008). Fortunately, increasingly sophisticated mouse models for the specific ablation of DCs or for the conditional deletion of genes in these cells have helped resolve some of these controversies, revealing how immune responses are compromised in the absence of DCs and thus the roles these cells play in the normal response. These mouse models have been critical for elucidating the unique functions of DCs and resolving previously ambiguous conclusions. This review will provide an overview of the mouse models that have been developed to interrogate DC function and how these models have progressively clarified the role of DCs in various types of immune responses. As recent reviews have discussed DC development (Murphy et al., 2016; Mildner and Jung, 2014), this review will focus primarily on how DCs function in immune responses to pathogens, autoimmunity, and cancer.

Mouse models for studying dendritic cells

Numerous models of constitutive and inducible DC depletion have been generated and used to identify the specific functions of DC subsets. In this section, we will first describe the different strains developed, their specificity, and their limitations. We will organize this by categories of DTR/DTA based models, Cre strains, and transcription factor knockout mice. In the next section we will discuss the discoveries made with these models regarding the functions of DCs in various immune responses.

Depletion of dendritic cells by DTR/DTA systems

The first models of genetic ablation of cell lineages were transgenic mice in which cell type-specific promoters drove expression of the diphtheria toxin (DT) A-chain (Palmiter et al., 1987; Breitman et al., 1987). This toxin disrupts protein translation by catalyzing the ADP-ribosylation of polypeptide chain elongation factor 2 and eventually leads to cell death (Honjo et al., 1968; Robinson et al., 1974; Van Ness et al., 1980). While DT efficiently ablates the cells in which it is expressed, it can be problematic as even low levels of off-target expression can lead to death of unintended cells or even to effects on embryogenesis or morphogenesis (Breitman et al., 1990). Later mouse models overcame this problem by expressing the human or simian DT receptor (DTR) under the control of a cell type-specific promoter, with subsequent administration of DT to these mice (Saito et al., 2001). As the mouse ortholog of the DTR is orders of magnitude less sensitive to DT (Mitamura et al., 1995; Pappenheimer, Jr. et al., 1982), this allows for the efficient depletion of only DTR-expressing cells and has the added benefit of allowing for inducible depletion of these target cells rather than constitutive ablation.

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The first DTR based model used for DCs was the *Itgax*-DTR strain, a transgenic line expressing a DTR-GFP fusion protein under the control of the murine promoter for the gene *Itgax*, which codes for the integrin CD11c (Jung et al., 2002). DT administration completely depleted $CD11c^+$ DCs within 24 hours, with no observable depletion of splenic B cells or $F4/80^+$ macrophages. DCs began to reappear 3 days after DT treatment. While this strain was vital for early work confirming the functions of DCs in T cell priming and pathogen responses, several limitations have emerged. First, repeated administration of DT led to death in these mice, restricting the duration through which depletion could be studied (Jung et al., 2002). This lethality was likely due to off-target expression of the *Itgax*-DTR transgene in radioresistant or non-hematopoietic tissues, since BM chimeras of WT BM into *Itgax*-DTR recipients also died after repeated DT treatment. This limitation can be overcome by generating chimeras of *Itgax*-DTR BM into WT recipients, which tolerate repeated DT treatment (Zaft et al., 2005). Another caveat derives from CD11c expression by non-DCs and the depletion by DT of such cells, which include splenic metallophilic and marginal zone macrophages, LN sinusoidal macrophages (Probst et al., 2005), alveolar macrophages (van Rijt et al., 2005), activated CD8 T cells (Jung et al., 2002), and plasma cells (Hebel et al., 2006). Ablation of these cells complicates analysis with this strain, since phenotypes observed may result from their depletion rather than that of DCs. Some $CD11c^+$ cells, notably pDCs, are not depleted in this model, indicating that the level of CD11c expressed by cells influences whether they are depleted (Sapoznikov et al., 2007).

A similar DTR strain, *Itgax*-DOG (Hochweller et al., 2008), is a BAC transgenic line in which the *Itgax* promoter drives expression of a fusion protein composed of DTR, a portion of ovalbumin, and GFP (DOG). Continuous DT treatment in these mice does not cause lethality, perhaps due to more faithful expression of the BAC compared with the promoter-based transgene. Depletion of splenic macrophages was also observed after DT treatment in these mice, but whether CD8 T cells and plasma cells were similarly affected has not been evaluated.

The recently developed *Zbtb46*-DTR mouse allows for more specific depletion of cDCs (Meredith et al., 2012). This strain has sequences for an internal ribosome entry site (IRES) and a DTR-mCherry fusion protein inserted into the 3′ untranslated region (UTR) of the endogenous *Zbtb46* gene, which codes for the zinc finger transcription factor Zbtb46 (also known as zDC) whose expression in hematopoietic lineages is restricted to cDCs. However, a single administration of DT into *Zbtb46*-DTR mice or into chimeras of WT BM into *Zbtb46*-DTR recipients proved lethal, indicating zDC expression in vital radioresistant or non-hematopoietic cells. Further work indicated these vital cells may be endothelial cells (Satpathy et al., 2012). DT treatment of *Zbtb46*-DTR BM into WT recipient chimeras depleted cDCs alone without ablation of B cells, T cells, neutrophils, natural killer cells, pDCs, or monocytes. This strain therefore allows for the specific and complete deletion of cDCs, with the caveat that BM chimeras must be used. To circumvent this need, the *Zbtb46*-LSL-DTR mouse was generated (Loschko et al., 2016a), in which the IRES-*mCherry*-DTR is preceded by a loxP flanked transcriptional Stop cassette (LSL) that terminates transcription of the locus before the DTR. Transcription of the DTR proceeds only after Cre-mediated excision of the Stop cassette. After crossing the *Zbtb46*-LSL-DTR strain to a *Csf1r-cre* strain (Loschko et al., 2016a), DT administration specifically depleted cDCs and

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did not lead to lethality. This model allows for the continuous depletion of cDCs for at least 4 weeks without the need for BM chimeras.

Several DTR strains allow for depletion of specific DC subsets. The first were two strains that expressed DTR from the endogenous *Cd207* gene that codes for langerin, a C-type lectin expressed on epidermal Langerhans cells and dermal cDC1s. One strain was generated by inserting an IRES DTR-*egfp* into the 3′ UTR of the *Cd207* gene (Kissenpfennig et al., 2005), while in the second strain the first *Cd207* coding exon was replaced with a DTR-*egfp* cassette (Bennett et al., 2005). In both strains, DT treatment depletes Langerhans cells as well as cDC1s in skin-draining LNs. Additionally, a BAC transgenic *CD207*-DTA mouse strain was generated using a BAC in which an IRES DTA was inserted into the 3′ UTR of the human *CD207* gene that codes for langerin (Kaplan et al., 2005). This strain has constitutive ablation of Langerhans cells but not of cDC1s in skin-draining LNs, suggesting that the human *CD207* promoter is controlled differently from its murine equivalent. It was later demonstrated that these mice also lack $CD103^{+}CD11b^{+}$ cDC2s in the intestinal lamina propria (Welty et al., 2013).

The *Ly75*-DTR strain was generated by inserting an IRES DTR-eGFP into the 3′ UTR of the *Ly75* gene that codes for CD205, an endocytic type I C-type lectin-like receptor also known as DEC-205 (Fukaya et al., 2012). DT administration to this mouse also resulted in lethality, necessitating the use of *Ly75*-DTR into WT recipient BM chimeras. DT treatment of such chimeras depleted $CD205^{+}$ cDCs, the majority of which were $CD8\alpha^{+}$ cDC1s. CD205 is also expressed on B cells at 10–50 fold lower levels than on BM derived DCs (Inaba et al., 1995) and accordingly a minor decrease in splenic B cells was observed upon DT treatment to *Ly75*-DTR mice. However, CD205 is also expressed at much higher levels in germinal center (GC) B cells (Victora et al., 2010) as well as all migratory cDCs and Langerhans cells (Idoyaga et al., 2013), but it was not determined whether DT treatment also depleted these cells.

The *Clec9a*-DTR BAC transgenic mouse, in which the first *Clec9a* coding exon in the BAC was replaced with DTR, also allows for depletion of cDC1s (Piva et al., 2012). DT treatment completely ablates cDC1s, but also causes a ~50% decrease in pDCs, which may be due to the lower expression of Clec9a in mature pDCs (Sancho et al., 2008; Caminschi et al., 2008). Clec9a is expressed in the common dendritic cell progenitor (CDP) (Schraml et al., 2013), so continuous DT treatment may deplete this progenitor and therefore all DCs over prolonged periods. Though this issue was not examined in the original study, at least one report indicated that cDC2s were unaffected after 15 days of DT treatment (Muzaki et al., 2016).

Two more recent DTR strains specifically deplete cDC1s. In the *Xcr1*-DTRvenus strain, the first *Xcr1* coding exon was replaced by a cassette encoding a DTR-venus fusion protein (Yamazaki et al., 2013). XCR1 is a chemokine receptor uniquely expressed by cDC1s in humans, mice, and sheep (Crozat et al., 2010; Crozat et al., 2011). DT treatment to this strain completely depleted cDC1s with no decrease in T cells, B cells, NK cells, granulocytes, monocytes, cDC2s, or pDCs. Expression of the Venus fluorescent protein was likewise restricted to cDC1s. This subset was depleted by 24 hours after DT treatment and

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began to recover on day 4 after treatment. A similar mouse strain, referred to as *Karma*, has an IRES *tdTomato*-2A-DTR inserted into the 3′ UTR of the endogenous *a530099j19rik* gene (Alexandre et al., 2016), which like XCR1 is highly specific for cDC1s. Both tdTomato expression and cell ablation after DT treatment were specific to cDC1s.

The *Clec4a4*-DTR strain, a BAC transgenic line in which an IRES DTR was inserted into the 3′ UTR of the *Clec4a4* gene on the BAC, allows for ablation of cDC2s (Muzaki et al., 2016). Clec4a4 (also known as DCIR2) is a C-type lectin expressed by cDC2s and recognized by the 33D1 antibody first used to define this subset (Nussenzweig et al., 1982; Dudziak et al., 2007). DT treatment in this strain severely reduces $CD103^+CD11b^+$ cDC2s in the colonic lamina propria and $CD11b^+$ cDC2s in the mesenteric LNs, and partially depletes $CD103^-CD11b^+$ cDC2s and $CD64^+CX3CR1^+$ macrophages in the colonic lamina propria. Whether depletion of other cells occurs and whether cDC2s in other organs are similarly depleted remains to be determined. A second strain thought to deplete a subset of cDC2s is the *Mgl2*-DTR strain, in which the first coding exon of the endogenous *Mgl2* gene was replaced by a DTR-*gfp* cassette (Kumamoto et al., 2013). DT treatment in this strain depletes the $Mgl2^+$ population of dermal cDC2s, but whether DCs in other tissues are also depleted remains to be analyzed.

Several DTR strains have been generated for pDC depletion. The first was the *CLEC4C*-DTR BAC transgenic strain, which replaced the exons of the human gene *CLEC4C* in the BAC with the sequence for DTR (Swiecki et al., 2010). *CLEC4C* codes for BDCA-2, a C-type lectin uniquely expressed by human pDCs with no equivalent in mouse pDCs, but its human promoter remains active in mouse pDCs as DT treatment in *CLEC4C*-DTR mice specifically and completely depleted pDCs. No reduction was observed in B cells, cDCs, T cells, NK cells, macrophages, monocytes, or neutrophils. Second, a *Siglech*-DTR strain was generated by knocking an IRES DTR-*egfp* sequence into the 3′ UTR of the endogenous murine *Siglech* gene (Takagi et al., 2011). Homozygous *Siglech*-DTR mice lacked expression of Siglec-H on pDCs, implying that the IRES DTR cassette somehow altered native Siglec-H expression. In this strain, DT administration completely depletes pDCs in the spleen, mesenteric LNs, and BM, with no apparent effect on cDCs. However, Siglec-H deficiency has been associated with altered cytokine responses (Swiecki et al., 2014) and perhaps abnormal kidney and testes function (Orr et al., 2013), which may influence the phenotypes of this strain. Third, a *Siglech*-DTR BAC transgenic strain was generated on the Balb/c background by replacing the coding exons of *Siglech* in a BAC with the sequence for DTR (Piva et al., 2012). DT treatment in these mice completely depleted pDCs with no effect on cDCs. A separate BAC transgenic *Siglech*-DTR line on the C57BL/6 background was later generated, and analysis of both *Siglech*-DTR lines found that Siglec-H was also expressed by marginal zone macrophages and DT treatment ablated these cells as well as pDCs (Swiecki et al., 2014). This indicates that cell ablation in *Siglech*-DTR strains may not be limited to pDCs, complicating analysis with these lines.

Finally, several DTR based systems for the depletion of monocytes and macrophages (reviewed in (Lauvau et al., 2015)), have been generated that are driven by genes such as CCR2 and CX3CR1. These have helped segregate the independent functions of monocytes and cDCs. One useful model for this purpose is the *Cx3cr1*-LSL-DTR strain (Diehl et al.,

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2013), in which an LSL-DTR was inserted into the endogenous *Cx3cr1* locus. This strain was crossed to *Itgax-cre* so that DT treatment specifically depletes $CD11c^{+}CX3CR1^{+}$ cells, which include $CD103^{-}CD11b^{+}$ cDC2s and monocyte-derived $CD11b^{+}$ macrophages in the intestinal lamina propria (Bogunovic et al., 2009). Another useful model is the *MM*-DTR strain, which was a cross between the *Csf1r*-LSL-DTR and *LysM-cre* strains, and in which DTR expression is limited to monocytes and monocyte-derived macrophages (Schreiber et al., 2013). Table 1 provides a summary of DTR strains useful for the study of DC function.

An important caveat for some DTR strains is the reduced LN cellularity seen even without DT treatment (van Blijswijk et al., 2015). In a cross between the *Clec9a-cre* strain and a *Rosa26*-LSL-DTR strain (Buch et al., 2005), reduced cellularity was observed in skin-draining and mesenteric LNs even without DT treatment. There was also reduced cellularity in skin-draining LNs of *Itgax*-DTR mice, *Cd207*-DTR mice, and a cross between *Itgax-cre* and *Rosa26*-LSL-DTR mice. There was no decrease observed in *Itgax*-DOG mice, so this phenomenon must be evaluated in each individual strain. The basis for this effect is unclear.

Cre strains for conditional deletion of genes in dendritic cells

The Cre-loxP system allows for conditional deletion of genes in specific cell lineages (Sharma and Zhu, 2014). In this system, two 34-bp *loxP* sites are inserted on either side of a gene or exon, which is then said to be “floxed”. Cell type-specific promoters are then used to express the bacteriophage P1 *cre* gene, which encodes an integrase that mediates recombination between two adjacent *loxP* sites, leading to deletion of the intervening DNA and inactivation of the floxed gene in Cre^{+} cells.

The earliest Cre strains developed for cDCs were based on CD11c expression. First was an *Itgax-cre* BAC transgenic line in which the first coding exon of *Itgax* was replaced with *cre* (Caton et al., 2007). When crossed to the *Rosa26*-LSL-*yfp* strain (Srinivas et al., 2001), in which Cre-mediated excision of a STOP cassette allows YFP expression, splenic DCs were >95% labeled and pDCs were ~86% labeled. However, background deletion of 5–12% was seen in splenic T, B, and NK cells. A later study also found deletion in 20–40% of blood monocytes, nearly ~100% of alveolar macrophages, 70% of splenic red pulp macrophages, 35% of marginal zone macrophages, and 20% of peritoneal macrophages (Abram et al., 2014). Therefore, as with *Itgax*-DTR strains, *Itgax-cre* is active in non-DC populations. The second *Itgax-cre* strain is a transgenic line expressing *cre*-IRES-*gfp* under the control of the *Itgax* promoter (Stranges et al., 2007). GFP expression in this line was found to be uniformly high in cDC1s and cDC2s as well as in pDCs, but no expression was seen in T cells or B cells. Whether deletion of genes occurred in cell types other than DCs, especially other $CD11c^{+}$ populations such as macrophages, was not evaluated and remains to be resolved.

The *Clec9a-cre* strain was generated by replacing the first two exons of the endogenous *Clec9a* gene with *cre* (Schraml et al., 2013). Clec9a is first expressed at the CDP stage and is maintained in cDC1s and pDCs, but not in cDC2s (Schraml et al., 2013; Sancho et al., 2008; Caminschi et al., 2008). The *Clec9a-cre* strain achieved deletion in ~100% of cDC1s, ~50% of cDC2s, and ~20% of pDCs, suggesting that the transient expression of Cre at the CDP stage is insufficient for full deletion in cDC2 precursors and that pDCs do not express high

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enough levels of Cre for complete deletion. Deletion was not observed in any other cell types.

Recently a *Zbtb46-cre* line was produced by inserting an IRES-*cre* cassette into the 3′ UTR of the endogenous *Zbtb46* gene, allowing for more specific deletion of genes in cDCs (Loschko et al., 2016b). When crossed to *Rosa26*-LSL-*yfp* mice, the *Zbtb46-cre* achieved deletion in ~65% of cDCs, which was lower than the ~100% deletion with *Itgax-cre*, but also demonstrated <10% deletion in T cells, B cells, and monocytes, which was also consistently lower than the deletion seen with *Itgax-cre*. Also <10% deletion was seen in red pulp macrophages, pDCs, and small intestinal macrophages, in contrast to the *Itgax-cre* strain where ~70% of red pulp macrophages and pDCs and ~100% of small intestinal lamina propria macrophages showed deletion. *Zbtb46-cre* also induced ~95% deletion of a floxed MHCII allele in cDCs, indicating that deletion of some alleles may be more complete than others. It was not evaluated whether *Zbtb46-cre* is as active in endothelial cells as the *Zbtb46*-DTR strain is.

The first Cre strain for targeting specific DC subsets was the *Cd207-cre*, in which a *cre* cassette was inserted into the second exon of the endogenous *Cd207* gene (Zahner et al., 2011). This strain achieved nearly complete deletion in Langerhans cells and langerin^{+} cDC1s in the dermis, skin-draining LNs, and lung. A second Cre line useful for targeting particular DC subsets is the *Xcr1-cre* strain, in which the first exon of the endogenous *Xcr1* gene was replaced by c*re* (Ohta et al., 2016). This was crossed to *Rosa26*-lacZbpA^{flox}DTA mice (Brockschnieder et al., 2006), in which Cre excises a loxP flanked lacZ-polyadenylation (bpA) sequence and allows for DTA expression and cell ablation. This cross resulted in nearly complete ablation of cDC1s in the spleen, MLN, and intestinal lamina propria, but no reduction in cDC2s in these organs. There was no reduction in CD4 or CD8 T cells in the spleen or MLN, but T cells and intraepithelial lymphocytes in the intestinal lamina propria were reduced, which was attributed to the absence of cDC1s. Finally, in the BAC transgenic *Siglech-cre* strain, the first exon of *Siglech* was replaced with a *cre* IRES *mCherry* cassette (Puttur et al., 2013). However, this strain only achieved deletion in ~30% of pDCs and ~2% of all lymphoid cells, and so is better suited for lineage tracing than functional analysis.

Transcription factor based depletion of dendritic cells

Several transcription factors have been identified whose deletion selectively depletes specific subsets of DCs, and these knockout mice provide useful models for studying DC function. One of the earliest identified was the *Irf8*$^{-/-}$ strain, which lacks cDC1s (Schiavoni et al., 2002; Aliberti et al., 2003), but also has impairments in B cells (Wang et al., 2008), monocytes (Kurotaki et al., 2013), eosinophils (Milanovic et al., 2008), and basophils (Sasaki et al., 2015). *Irf8*$^{-/-}$ mice were also thought to lack pDCs (Schiavoni et al., 2002), but more recently it was determined that pDCs do not require IRF8 for their development but instead that loss of this factor affects their expression of cell-surface markers and their ability to produce interferon (Sichien et al., 2016). *Irf8*$^{-/-}$ mice therefore do not serve as a model of specific cDC1 depletion, but this can be overcome by crossing a floxed *Irf8* allele to the *Itgax-cre* strain (Luda et al., 2016), which depletes cDC1s and CD64^{+}CD11b^{+}

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macrophages in the intestinal lamina propria, or to the *Zbtb46-cre* strain (Esterhazy et al., 2016), which specifically depletes cDC1s.

Id2$^{-/-}$ mice lack cDC1s (Hacker et al., 2003), but also have defects in NK cells (Yokota et al., 1999) and ILCs (Serafini et al., 2015). *Nfil3*$^{-/-}$ mice similarly lack cDC1s (Kashiwada et al., 2011), NK cells (Gascoyne et al., 2009), and ILCs (Geiger et al., 2014; Seillet et al., 2014). The similarity of *Id2*$^{-/-}$ and *Nfil3*$^{-/-}$ mice may result from an epistatic action of Nfil3 on the expression of Id2 during DC development, as has been suggested for NK cell development (Male et al., 2014).

Batf3$^{-/-}$ mice are selectively deficient in cDC1s (Hildner et al., 2008). Batf3 and the AP-1 transcription factor Jun form a heterodimer that interacts with IRF8 to bind AP-1/IRF consensus elements (AICEs) (Tussiwand et al., 2012; Glasmacher et al., 2012). Batf3 acts to maintain the expression of IRF8 by autoactivation during development of cDC1s, without which cDC1s divert to cDC2 fate (Grajales-Reyes et al., 2015). *Batf3*$^{-/-}$ mice on the 129SvEv and Balb/C backgrounds have an almost complete lack of cDC1s in lymphoid and nonlymphoid tissues (Edelson et al., 2010), but interestingly in the C57BL/6 background they retain cells resembling cDC1s in skin-draining LNs (Tussiwand et al., 2012).

Few known transcription factors selectively control cDC2 development. *Relb*$^{-/-}$ mice have DCs with reduced immunogenicity (Burkly et al., 1995), and reportedly a partial cell-intrinsic defect in cDC2 development (Wu et al., 1998). RelB acts in the noncanonical NFκB signaling pathway activated by lymphotoxin-β receptor (LTβR) signaling, and *Ltbr*$^{-/-}$ mice also have a cell-intrinsic but partial reduction in CD4^{+} cDC2s (Kabashima et al., 2005). It has not been tested whether the impact of RelB deficiency on DCs is secondary to deficient LTβR signaling. As *Relb*$^{-/-}$ mice develop severe multiorgan inflammation and hematopoietic abnormalities (Weih et al., 1995), and *Ltbr*$^{-/-}$ mice lack lymph nodes (Futterer et al., 1998), neither strain is a convenient model for testing DC function. Conditional deletion of *Ltbr* with *Itgax-cre* has been used to overcome this limitation (Tumanov et al., 2011).

Other factors that influence cDC2 development include Notch2 and Klf4. Conditional deletion of the Notch cofactor *Rbpj* (Caton et al., 2007) or *Notch2* (Lewis et al., 2011; Satpathy et al., 2013) using *Itgax-cre* demonstrated a specific depletion of CD11b^{+}ESAM^{+} cDC2s in the spleen and CD103^{+}CD11b^{+} cDC2s in the intestinal lamina propria and MLNs, without apparent loss of other cell types. *Notch2*$^{f/f}$ *Itgax-cre* mice did exhibit gene expression changes in remaining cDC1s and cDC2s, however, indicating possible effects of Notch2 in these lineages (Satpathy et al., 2013). Conditional deletion of the transcription factor *Klf4* with *Itgax-cre* also led to the depletion of subsets of cDC2s (Tussiwand et al., 2015) similar to migratory CD24− CD11b− cDC2s in skin-draining LNs (Ochiai et al., 2014).

Irf4$^{-/-}$ mice do not lack cDC2s, but instead have partial impairments in the number or functioning of these cells. CD11b^{+} cDC2s are present in the skin of *Irf4*$^{-/-}$ mice, but fail to migrate to LNs (Bajana et al., 2012; Bajana et al., 2016). Similarly, conditional deletion of *Irf4* with *Itgax-cre* resulted in a partial reduction in CD11b^{+} cDC2s in the lung but an almost

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complete absence of $CD11b^+$ cDC2s in the lung draining LNs (Schlitzer et al., 2013), as well as a partial reduction in $CD103^+CD11b^+$ cDC2s in the intestinal lamina propria and a more complete reduction in mesenteric LNs (Schlitzer et al., 2013; Persson et al., 2013). Finally, conditional deletion of *Irf4* with *Itgax-cre* depletes a $PDL2^+Mgl2^+$ population of cDC2s in skin-draining LNs but not the dermis (Gao et al., 2013). But although $Irf4^{f/f}$ *Itgax-cre* mice can serve as a useful model for cDC2 deficiency, IRF4 also plays a role in other $CD11c^+$ cells such as M2 macrophages (Satoh et al., 2010) and GM-CSF activated monocytes (Briseno et al., 2016), and these may also be affected in this strain.

Several transcription factors have been identified that regulate pDC development. The first model of specific pDC deficiency was conditional deletion of *Tcf4*, which encodes the factor E2-2, with *Itgax-cre* (Cisse et al., 2008). Recently, conditional deletion of the transcription factor *Zeb2* using *Itgax-cre* also demonstrated selective pDC deficiency (Scott et al., 2016). Table 3 provides a summary of the transcription factor knockout mice useful for the study of DCs.

Functions of Dendritic Cells

Since their discovery, cDCs were recognized as possessing a remarkable capacity to stimulate naïve T cells and to initiate adaptive immune responses (Steinman and Witmer, 1978; Nussenzweig et al., 1980). Subsequent work, based in large part on the models described above, has shown that DCs are also critical for - not just participants in - early innate immune responses, activating innate lymphoid cells (ILCs) and other cells involved in the immediate response to pathogens. Additionally, subsets of cDCs play varied roles in autoimmunity and responses to tumors. Below we discuss the studies that identified these functional aspects of DCs, what tools were used to arrive at these conclusions, and confounding factors that may require further resolution.

General Adaptive Immune Responses

The first study to use *Itgax*-DTR mice showed that the absence of $CD11c^+$ cells led to the loss of CD8 T cell priming to cell-associated antigens and to the intracellular pathogens *Listeria monocytogenes* and malaria (Jung et al., 2002). This provided the first direct *in vivo* evidence for the role of $CD11c^+$ cells in T cell priming. Subsequent use of the *Itgax*-DTR strain showed a requirement for $CD11c^+$ cells in priming CD8 T cells against lymphocytic choriomeningitis virus (LCMV) (Probst and Van Den, 2005), herpes simplex virus (HSV) (Kassim et al., 2006), vesicular stomatitis virus (VSV) (Ciavarra et al., 2006), and influenza (GeurtsvanKessel et al., 2008; Kim et al., 2010), as well as in CD4 T cell priming against HSV (Kassim et al., 2006), *Mycobacterium tuberculosis* (Mtb) (Tian et al., 2005), and immune complexes (de Jong et al., 2006). Depletion of $CD11c^+$ cells in *Itgax*-DTR mice also impaired the expansion of antigen-specific memory CD8 T cells during rechallenge with Listeria, VSV, or influenza (Zammit et al., 2005) or with LCMV (Lauterbach et al., 2006).

Later studies used additional models to validate the specific role of cDCs and not other $CD11c^+$ cells in T cell priming. Specific ablation of cDCs with the *Zbtb46*-DTR strain resulted in a complete inability to prime CD8 or CD4 T cells against soluble antigen

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(Meredith et al., 2012), and also a failure to prime CD4 T cells against Mtb (Samstein et al., 2013). Finally, cDC specific deletion of MHCII using *Zbtb46-cre* led to a complete reduction in CD4 T cell priming to soluble antigen (Loschko et al., 2016b).

Targeting antigen to specific DC subsets has indicated that there may be a specialization of roles between subsets, with cDC1s preferentially priming CD8 T cells and cDC2s priming CD4 T cells (Dudziak et al., 2007). cDC1s have also been recognized as the cells most efficient in cross-presentation of exogenous antigens on MHC class I (den Haan et al., 2000). Studies using mouse models with selective depletion of one subset have provided evidence to support this dichotomy. *Batf3*$^{-/-}$ mice failed to prime CD8 T cells against cell-associated antigen or West Nile virus (WNV), but still had intact priming of CD4 T cells (Hildner et al., 2008). *Batf3*$^{-/-}$ mice also mounted reduced CD8 T cell responses to cytomegalovirus (CMV) (Torti et al., 2011), influenza (Helft et al., 2012; Waithman et al., 2013), cowpox virus (Gainey et al., 2012), HSV (Nopora et al., 2012; Zelenay et al., 2012), and malaria (Lau et al., 2014). Depleting cDC1s using the *Xcr1*-DTRvenus strain also abrogated CD8 T cell priming against soluble and cell-associated antigen as well as against *Listeria* infection (Yamazaki et al., 2013). CD4 T cells could still be primed against soluble antigen in these mice. A study using *Karma* mice similarly showed that depletion of cDC1s led to deficient CD8 T cell priming to soluble protein as well as failure to reactivate memory CD8 T cells in response to *Listeria*, vaccinia virus, and VSV (Alexandre et al., 2016). Taken together these studies suggest that cDCs alone are responsible for priming T cells, with a specific role for cDC1s in priming naïve CD8 T cells and activating memory CD8 T cells during recall responses. Future work with specific depletion of cDC2s is needed to confirm their unique role in priming CD4 T cells.

Recent work has also indicated that CD4 T cell help in CD8 T cell responses might be mediated through “licensing” of cDC1s. Analysis of immune responses suggests that CD4 T cells are primed by DCs earlier than CD8 T cells, and that later in the response clusters of these three cell types form during which CD4 T cells may “license” cDC1s in order to enhance priming of CD8 T cells (Hor et al., 2015; Eickhoff et al., 2015). In agreement with this model, one study found that mixed bone marrow chimeric mice made of *Xcr1*-DTR*:H2-Ab1*$^{-/-}$ BM, in which following DT treatment all remaining cDC1s lack MHCII expression, generated fewer antigen-specific CD8 T cells upon vaccinia virus infection (Eickhoff et al., 2015). MHCII expression in cDC1s may therefore serve as a mechanism by which they receive CD4 T cell help rather than for their direct priming of naïve CD4 T cells.

Several studies have implicated DCs in the induction of Tfh cells and the initiation of the germinal center (GC) response. Depletion of all CD11c^{+} cells by *Itgax*-DTR was found to diminish the number of Tfh cells induced in response to *Toxoplasma* infection (Goenka et al., 2011). Specific cDC depletion with *Zbtb46*-DTR mice also abolished GC responses and antibody production against allogeneic red blood cells (RBCs) (Calabro et al., 2016). This was attributed to the function of cDC2s, as *Batf3*$^{-/-}$ mice had intact antibody responses but *Irf4*$^{f/f}$ *Itgax-cre* mice did not (Calabro et al., 2016). Another study found that CD25 expression by cDCs was crucial for Tfh induction (Li et al., 2016). In this study, mixed BM chimera mice reconstituted with *Zbtb46*-DTR*:Il2ra*$^{-/-}$ BM, in which after DT treatment all remaining cDCs lack the IL-2 receptor alpha chain (CD25), failed to generate antibodies or

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GC B cells upon sheep RBC immunization. cDC2s in the spleen can induce expression of CD25, suggesting these may be the specific cells involved in this process (Li et al., 2016). Finally, IgA class switching in Peyer's patches was also found to be regulated by DCs (Reboldi et al., 2016). This study utilized mixed BM chimeras made from *Itgax*-DTR:*Ltbr*$^{-/-}$ BM, in which following DT treatment all remaining DCs lack the LTβ receptor and thus certain cDC2s fail to develop. After DT treatment, these chimeras had fewer IgA class-switched GC B cells in Peyer's patches. This appears to involve the activation of latent TGF-β by the integrin chains Itgβ8 and Itgαv on DCs and presentation of the active cytokine by DCs to B cells, as *Itgax-cre* mediated deletion of *Itgb8* also resulted in reduced IgA^{+} GC B cells (Reboldi et al., 2016).

Type 1 Immune Responses

Type 1 immune responses are mounted against intracellular pathogens that require IFN-γ activated macrophages and cytotoxic CD8 T cells for their clearance. Early nonspecific sources of IFN-γ are NK cells and ILC1s, while antigen-specific Th1 and CD8 T cells are responsible for producing this cytokine later in the immune response. IL-12 is critical for the activation of type 1 responses, as it induces NK cells and ILC1s to secrete IFN-γ (Sonnenberg and Artis, 2015), and is also responsible for the polarization of naïve T cells to Th1 cells (Hsieh et al., 1993). Several studies have established that cDC1s are vital for mounting type 1 responses because of their non-redundant production of IL-12 and their ability to prime CD8 T cells.

The first indication that cDC1s are important in these responses was a study demonstrating that *Irf8*$^{-/-}$ mice had increased susceptibility to *Toxoplamsa gondii* infection and reduced serum IL-12 levels (Scharton-Kersten et al., 1997), though given the myriad defects in this mouse this could not be attributed specifically to cDC1s. Later, *Itgax*-DTR mice were used to show that CD11c^{+} cells were required for IL-12 and IFN-γ production and protection against *Toxoplasma* infection (Liu et al., 2006). Finally, *Batf3*$^{-/-}$ mice that specifically lack cDC1s were found to be susceptible to *Toxoplasma*, demonstrating that these cells were indeed critical for resistance (Mashayekhi et al., 2011). Mixed chimeras made from *Batf3*$^{-/-}$:*Il12a*$^{-/-}$ BM, in which all cDC1s that develop are deficient in IL-12 production, were susceptible to *Toxoplasma* infection, indicating that cDC1s are the non-redundant source of IL-12 necessary for resistance to infection (Mashayekhi et al., 2011). Additionally, depletion of cDC1s using the *Karma* strain abolished IL-12 production in response to soluble *Toxoplasma* antigen (Alexandre et al., 2016). *Batf3*$^{-/-}$ mice also have reduced IFN-γ production from NK cells during *Toxoplasma* infection, suggesting that IL-12 from cDC1s is critical for NK cell activation in this response (Askenase et al., 2015).

The role of cDCs in type 1 responses to several other intracellular pathogens has also been documented. During infection by *Listeria*, *Itgax*-DTR (Neuenhahn et al., 2006), *Batf3*$^{-/-}$ (Edelson et al., 2011), *Ly75*-DTR (Fukaya et al., 2012), and *Xcr1*-DTRvenus (Yamazaki et al., 2013) mice all had reduced CD8 T cell responses. However, many of these strains also exhibited a reduced splenic *Listeria* burden and *Batf3*$^{-/-}$ mice actually demonstrated increased resistance to infection. This is because *Listeria* must infect cDC1s in the splenic marginal zone in order to spread to and proliferate in the lymphoid areas of the spleen

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(Neuenhahn et al., 2006), so *Batf3*$^{-/-}$ mice lacking these cells show reduced susceptibility to infection. Finally, constitutive deletion of CD11c^{+} cells, achieved by crossing *Itgax-cre* mice to *Rosa26*-LSL-DTA mice (Voehringer et al., 2008), diminished IFN-γ production from NK cells, NKT cells, and T cells during Listeria infection (Kang et al., 2008).

Leishmania major, another intracellular pathogen, similarly requires cDC1s for its clearance, as *Batf3*$^{-/-}$ mice exhibit greater disease burden from infection (Ashok et al., 2014) and generate fewer Th1 cells (Martinez-Lopez et al., 2015). Another study used *Batf3*$^{-/-}$ and *Cd207*-DTR mice to demonstrate that cDC1s are required for the priming of CD8 T cells and Th1 cells against skin infection by the fungus *Candida albicans* (Igyarto et al., 2011).

Several studies have implicated DCs in NK cell homeostasis. Depletion of CD11c^{+} cells in *Itgax*-DTR mice diminished steady-state numbers of NK cells (Guimond et al., 2010) and reduced their cytotoxicity and IFN-γ production in several infection models (Lucas et al., 2007; Schleicher et al., 2007; Kassim et al., 2006). NK cell survival and activation requires trans-presentation of IL-15, whereby a non-NK cell that expresses the non-signaling receptor chain IL-15Rα binds IL-15 and presents to the full IL-15αβγ receptor on NK cells (Burkett et al., 2004). One study implicated a CD11c^{+} cell as the trans-presenting cell by using mixed BM chimera mice made from *Itgax*-DTR:*Il15*$^{-/-}$ BM or from *Itgax*-DTR:*Il15rα*$^{-/-}$ BM (Mortier et al., 2008). In these chimeras, DT treatment causes all remaining CD11c^{+} cells to lack IL-15 or IL-15Rα, respectively, and after DT treatment these chimeras show severely reduced NK cell activation *in vivo*. Future studies are needed to clarify whether cDCs are involved in this activity, and if so which subsets.

Type 2 Immune Responses

Type 2 responses are carried out against multicellular parasites at barrier surfaces in order to aid in their expulsion. Many cytokines play key roles in this response. These include IL-25 and IL-33, which activate ILC2s to produce effector cytokines such as IL-4, IL-5, and IL-13 (Sonnenberg and Artis, 2015), and IL-4, which polarizes naïve T cells to Th2 cells (Le Gros et al., 1990). Much remains unknown about the initiation of type 2 immune responses, and it has been controversial whether DCs are even involved directly.

An early study found that depletion of CD11c^{+} cells in *Itgax*-DTR mice did not impair Th2 cell polarization in response to immunization with papain (Sokol et al., 2009), although another study using the same strain found that it did (Tang et al., 2010). A later study also found that depletion of CD11c^{+} cells in *Itgax*-DTR mice abrogated type 2 responses, this time to inhaled house dust mite (HDM) allergen (Hammad et al., 2010). This study implicated an FcεRI expressing CD11c^{+}MHCII^{+} cell population as being the principal APC responsible. Subsequent studies also found that CD11c^{+} cell depletion by *Itgax*-DTR reduced numbers of Th2 cells after infections with the helminthes *Schistosoma mansoni* (Phythian-Adams et al., 2010), *Heligmosomoides polygyrus* (Smith et al., 2011), and *Nippostrongylus brasiliensis* (Smith et al., 2012).

Other models have suggested putative Th2 inducing DC subsets. Two groups demonstrated that depletion of an Mgl2^{+} subset of cDC2s, either with the *Mgl2*-DTR strain (Kumamoto et al., 2013) or by conditional deletion of *Irf4* with *Itgax-cre* (Gao et al., 2013), diminished Th2

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priming in response to papain and *Nippostrongylus* infection. *Irf4*$^{f/f}$ *Itgax-cre* mice also had reduced Th2 responses after HDM allergen challenge (Williams et al., 2013). Another study found that depletion of a subset of cDC2s in the *Klf4*$^{f/f}$ *Itgax-cre* strain increased susceptibility to *Schistosoma mansoni* infection and diminished allergic inflammation after intransal HDM challenge (Tussiwand et al., 2015). Also, a subset of CXCR5^{+} DCs, which was depleted by using *Itgax*-DTR:*Cxcr5*$^{-/-}$ BM chimeras, appeared to be required for Th2 responses to *Heligmosomoides* infection (Leon et al., 2012). Finally, *Batf3*$^{-/-}$ mice develop somewhat stronger Th2 responses to helminth infection, suggesting that the IL-12 produced by cDC1s regulates Th2 polarization carried out by other DCs (Everts et al., 2016).

Cytokine production by DCs has not been established in any of these models, so whether they control type 2 responses by this mechanism or some other remains to be determined. Recent studies have suggested that ILC2s may be the critical source of cytokines acting to induce type 2 responses (Halim et al., 2014; Halim et al., 2016). Conceivably, ILC2s and DCs may cooperate in Th2 priming, but this area awaits further studies.

Type 3 Immune Responses

Type 3 immune responses at barrier surfaces such as the lungs and intestines control infections by extracellular bacteria and fungi and require several cytokines, including IL-23 and IL-6. IL-6 and TGF-β initiate Th17 cell polarization (Bettelli et al., 2006), and IL-23 increases the survival and expansion of committed Th17 cells (Veldhoen et al., 2006). IL-23 is also crucial in innate responses for activating ILC3s to produce IL-22, which in turn promotes production of bactericidal lectins such as RegIIIγ from small intestinal epithelial cells (Sonnenberg and Artis, 2015; Kinnebrew et al., 2012).

Citrobacter rodentium, a mouse pathogen used to study type 3 immune responses, requires IL-23 and IL-22 for its clearance (Zheng et al., 2008; Basu et al., 2012; Mundy et al., 2005). An early study that implicated cDCs in response to oral *Citrobacter* infection found that conditional deletion of *Ltbr* by *Itgax-cre*, which depleted a subset of cDC2s, reduced colonic IL-22 production and increased pathogen burden (Tumanov et al., 2011). Later studies demonstrated that depletion of all cDCs in *Zbtb46*-DTR mice (Satpathy et al., 2013; Schreiber et al., 2013) led to severe susceptibility to *Citrobacter* infection, as did depletion of cDC2s with *Notch2*$^{f/f}$ *Itgax-cre* mice (Satpathy et al., 2013). Mixed BM chimeras reconstituted with *Notch2*$^{f/f}$ *Itgax-cre:Il23a*$^{-/-}$ BM were also severely susceptible to *Citrobacter* infection, indicating that Notch2-dependent cDC2s are the critical source of IL-23 in defense against this pathogen (Satpathy et al., 2013). NFκB signaling in CD11c^{+} cells is also critical, as mice with conditional deletion of *Myd88* with *Itgax-cre* also showed susceptibility to *Citrobacter* (Longman et al., 2014). Importantly, mice depleted of all cDCs or *Notch2*-dependent cDC2s die at ~day 10 after *Citrobacter* infection, while *Rag2*$^{-/-}$ mice lacking B and T cells die at ~day 30 after infection (Zheng et al., 2008). This suggests that cDC2s are critical for activating innate defenses during *Citrobacter* infection and not just for initiating adaptive immunity.

Several recent studies have suggested contributions from multiple cDC2 subsets in defense against *Citrobacter*. First, *CD207*-DTA mice, which lack intestinal CD103^{+}CD11b^{+} cDC2s, were not found to be susceptible to *Citrobacter* infection (Welty et al., 2013). Thus, the

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lethality seen with *Notch2* deficiency may be due to an impact on the function of CD103-CD11b$^+$ cDC2s, rather than the depletion of CD103$^+$CD11b$^+$ cDC2s. In accordance with this possibility, *Cx3cr1*-LSL-DTR mice crossed to *Itgax-cre*, in which DT treatment depletes CD103$^-$CD11b$^+$CX3CR1$^+$ cDC2s and CD11b$^+$ macrophages, also showed susceptibility to *Citrobacter* and reduced IL-22 production (Longman et al., 2014). Finally, MM-DTR mice, which lack monocytes and monocyte-derived macrophages, did not display susceptibility to Citrobacter, suggesting that CD103$^-$CD11b$^+$ cDC2s and not CD11b$^+$ macrophages are the critical cells for defense against this pathogen (Schreiber et al., 2013). However, direct comparison of these different models may be required to eliminate confounding effects such as variable microbiota in the strains used in each study.

Studies have also implicated cDC2s in various other type 3 responses, but the reported mechanisms by which they act have varied. Several groups found that depletion of intestinal CD103$^+$CD11b$^+$ cDC2s, using *Notch2$^{f/f}$ Itgax-cre*, *Irf4$^{f/f}$ Itgax-cre*, or *CD207*-DTA mice, resulted in fewer small intestinal Th17 cells at steady state (Lewis et al., 2011; Schlitzer et al., 2013; Welty et al., 2013). Another study using the *Irf4$^{f/f}$ Itgax-cre* strain similarly found reduced numbers of Th17 cells at steady state in the small intestine lamina propria and mesenteric lymph nodes, and also reduced Th17 polarization after immunization with antigen plus αCD40 and LPS (Persson et al., 2013). This study observed reduced levels of IL-6 in *Irf4$^{-/-}$* DCs, which might explain the reduced Th17 priming. A second group found deficient Th17 priming in *Irf4$^{f/f}$ Itgax-cre* mice after lung infection by the fungal pathogen *Aspergillus fumigatus*, which was attributed to the loss of CD11b$^+$ cDC2s that normally express high levels of IL-23, IL-6, and TGF-β (Schlitzer et al., 2013).

In agreement, another study used BM chimeras to show that CD11c$^+$ cells must produce IL-6 and TGF-β to induce Th17 responses to *Streptococcus pyogenes* (Linehan et al., 2015). They found that BM chimeras of *Itgax*-DTR:*Il6$^{-/-}$* or *Itgax*-DTR:*Tgfb1$^{f/f}$* BM, in which DT treatment causes all remaining DCs to be deficient in either IL-6 or TGF-β respectively, had reduced Th17 responses to this pathogen. This was confirmed in *Tgfb1$^{f/f}$ Itgax-cre* mice and specifically found to involve Mgl2$^+$ cDC2s, as DT treated *Mgl2*-DTR:*Il6$^{-/-}$* mixed BM chimeric mice also had reduced Th17 induction upon *Streptococcus pyogenes* infection (Linehan et al., 2015). A separate study used *Mgl2*-DTR:*Il23a$^{-/-}$* mixed BM chimeras to determine that Mgl2$^+$ cDC2s must produce IL-23 in order to activate IL-17 secretion from dermal γδ T cells during cutaneous *Candida albicans* infection (Kashem et al., 2015). Finally, a study using the *Il23a$^{f/f}$ Itgax-cre* strain concluded that IL-23 from CD11c$^+$ cells was important for Th17 and Th1 induction in response to *Helicobacter hepaticus* infection (Arnold et al., 2016), although the relative contributions of cDCs and macrophages in this infection remains to be defined with more specific methods. While depletion of cDC2s has revealed defects in type 3 responses to several pathogens, responses to many of these infections, specifically *Citrobacter* (Satpathy et al., 2013), *Streptococcus* (Linehan et al., 2015), and *Candida* (Kashem et al., 2015; Trautwein-Weidner et al., 2015), are intact in mice lacking cDC1s. This again underscores the functional specialization of different cDC subsets and the non-redundant roles they play in host defense.

Control of type 3 responses against segmented filamentous bacteria (SFB) may differ from those described above. SFB are a commensal organism present in some mice microbiota that

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induce an antigen-specific Th17 response in colonized mice (Ivanov et al., 2009). Conditional deletion of MHCII with *Itgax-cre* reduced Th17 induction after inoculation with SFB, which was interpreted to mean cDCs were responsible for priming against this organism. But *Batf3*$^{-/-}$*CD207*-DTA mice, which lack cDC1s and CD103^{+}CD11b^{+} cDC2s, and *Flt3l*$^{-/-}$ mice, which have substantially reduced numbers of all cDCs (McKenna et al., 2000), had no impairment in Th17 priming (Goto et al., 2014; Geem et al., 2014). Instead, depletion of monocytes with the *Ccr2*-DTR strain abrogated Th17 induction against SFB, and this could be restored by transfer of wildtype monocytes, suggesting that monocyte-derived intestinal macrophages prime this response (Panea et al., 2015). This example illustrates how some conclusions regarding cDC function drawn from CD11c-based systems may need re-interpretation with follow-up studies using more precise genetic models.

Dendritic cells in autoimmunity

DCs are important for the induction of tolerance to self-antigens and for the pathogenicity of several autoimmune syndromes. Early studies found a relationship between DC and Treg numbers, and that depletion of Tregs with a *Foxp3*-DTR strain greatly expanded cDCs (Kim et al., 2007). This expansion was due to increased numbers of cDC progenitors and was dependent on Flt3L, a crucial growth factor for cDCs (Liu et al., 2009). Conversely, depletion of DCs in *Itgax*-DTR mice or in *Flt3l*$^{-/-}$ mice reduced splenic Treg numbers, while expansion of DCs by exogenous Flt3L increased Treg numbers (Darrasse-Jeze et al., 2009). This phenomenon held for certain antigen-specific Treg clones as well (Leventhal et al., 2016).

DCs primarily control Treg numbers by regulating their proliferation rather than their induction, as Tregs transferred into *Itgax*-DTR mice treated with DT divided less than when transferred into WT mice (Darrasse-Jeze et al., 2009). This control of proliferation required MHCII expression by DCs, as both *Itgax*-DTR*:H2-Ab1*$^{-/-}$ mixed BM chimeric mice treated with DT and *H2-Ab1*$^{f/-}$ *Itgax-cre* mice also showed a decrease in Treg numbers and proliferation (Darrasse-Jeze et al., 2009). Later work also concluded that DCs do not play a primary role in Treg induction in the thymus, as neither constitutive depletion of DCs in mice with *Itgax-cre* crossed to *Rosa26*-LSL-DTA nor *H2-Ab1* $^{f/f}$ *Itgax-cre* mice had differences in the absolute number of Foxp3^{+} cells that develop in the thymus (Birnberg et al., 2008; Liston et al., 2008).

However, several recent studies have demonstrated that at least some Treg clones are induced in the thymus by DCs, especially clones that recognize self-antigens expressed by the transcription factor AIRE. One group identified certain T cell clones that convert to Tregs in the thymus in response to AIRE-dependent antigens in wildtype mice, but not in mice that were depleted of CD11c^{+} cells by crossing the *Itgax-cre* to the *Rosa26*-LSL-DTA strain (Perry et al., 2014). These same T cells also did not convert in *Batf3*$^{-/-}$ mice, suggesting that cDC1s controlled their induction. A subsequent study reported that both polyclonal Tregs and a specific AIRE-dependent Treg were reduced in number in mice with conditional deletion of MHCII by *Itgax-cre*, but in contrast to the previous study found that this AIRE-dependent Treg still developed in *Batf3*$^{-/-}$ mice (Leventhal et al., 2016). This

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suggests that the specific Treg clone or the nature of the antigen it recognizes may determine which cDC subset is responsible for its induction.

DCs also play a role in several models of autoimmunity. Studies using *Itgax*-DTR mice show that CD11c$^+$ cells are required for pathogenesis in ovalbumin-driven asthma (van Rijt et al., 2005) and in Th2-mediated peanut food allergy (Chu et al., 2014). Conditional deletion of *Myd88* with *Itgax-cre* also diminished colitis in *Il10*$^{-/-}$ mice (Hoshi et al., 2012) and decreased lupus-like symptoms in *Lyn*$^{-/-}$ mice (Hua et al., 2014), suggesting that NFκB signaling in CD11c$^+$ cells contributes to autoimmunity. Constitutively depleting CD11c$^+$ cells in a model of lupus, achieved by crossing the *Rosa26*-LSL-DTA and *Itgax-cre* alleles onto the lupus-prone MRL.Fas^{lpr} background, reduced disease severity, in part by decreasing T and B cell activation (Teichmann et al., 2010). Disease incidence partly involved MyD88 signaling in CD11c$^+$ cells, since *Myd88*$^{f/f}$ *Itgax-cre* mice on this background also had reduced dermatitis and T cell activation, but not reduced nephritis (Teichmann et al., 2013). In the nonobese diabetic (NOD) model of murine diabetes, cDC1s are required for disease initiation, as *Batf3*$^{-/-}$ NOD mice are completely free of lymphocyte infiltration into and destruction of pancreatic islets (Ferris et al., 2014). Conversely, increased activity of DCs can lead to autoimmunity, often from aberrant cytokine production or inappropriate activation of autoreactive T cells. This was seen when *Itgax-cre* was used to delete inhibitory signaling molecules such as Fas (Stranges et al., 2007) β-catenin (Manicassamy et al., 2010), FADD (Young et al., 2013), A20 (Hammer et al., 2011), TRAF6 (Han et al., 2013), Lyn (Lamagna et al., 2013), and Caspase-8 (Cuda et al., 2014).

Recent work has also implicated cDCs in establishing oral tolerance to ingested antigens. Using *Zbtb46*-DTR mice, one study demonstrated a loss of oral tolerance in mice lacking all cDCs (Esterhazy et al., 2016). These mice failed to induce Tregs against orally fed antigen and generated an immune response to subsequent peripheral antigen challenge. Oral tolerance was intact in *Irf8*$^{f/f}$ *Zbtb46-cre* mice that lack cDC1s, although antigen-specific Treg conversion was partially impaired. This suggests cDC1s are important in establishing oral tolerance but that other cDCs can compensate for their loss. A separate study found that for orally fed antigen to be tolerized it is phagocytosed by CX3CR1$^+$ macrophages in the intestinal lamina propria and subsequently transferred to CD103$^+$CD11b$^+$ cDC2s through gap junctions made up of connexin-43 (Mazzini et al., 2014). Conditional deletion of *Gja1*, which encodes connexin-43, with *Itgax-cre* reduced the number of Tregs induced to orally fed antigen and also diminished oral tolerance established to this same antigen. Also, conditional deletion of *Itgb8*, which encodes the integrin Itgβ8 that activates latent TGF-β, with *Itgax-cre* resulted in autoimmune inflammatory bowel disease and reduced numbers of colonic Tregs, suggesting that CD11c$^+$ cells are critical sources of active TGF-β for the induction of Tregs in response to gut antigens (Travis et al., 2007).

Dendritic cells in tumor immunity

Recent work has expanded our understanding of the roles of DCs in antitumor immune responses. Studies using *Itgax*-DTR mice demonstrated that depletion of CD11c$^+$ cells reduced CD8 T cell responses against transferred tumor cells (Casares et al., 2005; Shimizu et al., 2007) and reduced survival in oncogene-driven tumor models (Scarlett et al., 2012).

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cDC1s in particular were critical for antitumor responses, as *Batf3*$^{-/-}$ mice failed to reject transplanted immunogenic fibrosarcomas (Hildner et al., 2008) and failed to induce T cell infiltration into autochthonous oncogene-driven melanoma (Spranger et al., 2015). cDC1s are also required for the antitumor effects of many cancer therapies, as *Batf3*$^{-/-}$ mice have reduced responses to therapeutic PD-L1 blockade, Flt3L injection, or polyI:C injection (Salmon et al., 2016) as well as to anti-CD137 or anti-PD1 therapy (Sanchez-Paulete et al., 2016). cDC1s must migrate to LNs to activate tumor-specific CD8 T cells, as mixed BM chimeras made with *Xcr1*-DTR*:Ccr7*$^{-/-}$ BM, in which DT treatment causes all remaining cDC1s to lack CCR7, are not able to reject tumors (Roberts et al., 2016). Finally, cDCs play a role in opposing the establishment of metastatic foci, as *Zbtb46*-DTR mice depleted of cDCs mice had increased numbers of melanoma metastases to the lung (Headley et al., 2016).

Two studies have demonstrated that interferon signaling in DCs is necessary for the initiation of antitumor immune responses. In the first, conditional deletion of the type 1 interferon receptor *Ifnar1* with *Itgax-cre* prevented rejection of transplanted fibrosarcomas (Diamond et al., 2011). IFNAR deficient cDC1s also displayed deficient cross-presentation of antigens to CD8 T cells, suggesting that an inability to cross-present tumor antigens and activate CD8 T cells underlies the inability to reject tumors in this strain (Diamond et al., 2011). In the second, *Ifnar1*$^{-/-}$*:Batf3*$^{-/-}$ mixed BM chimeras failed to prime CD8 T cells to tumor antigens, suggesting that interferon signaling is specifically necessary in cDC1s (Fuertes et al., 2011).

Comparison of *Zbtb46*-DTR and *Itgax*-DTR mice reveals a partial redundancy between cDCs and other CD11c^{+} cells in some aspects of the antitumor response. When immunized with tumor antigens and challenged with melanoma, DT treatment in either strain led to a failure to reject tumors (Meredith et al., 2012). But mice depleted of CD11c^{+} cells succumbed to tumors faster than mice depleted of cDCs, suggesting that CD11c^{+} non-cDCs can compensate in driving antitumor responses. Others studies have distinguished various macrophage and DC populations present within the tumor microenvironment (Franklin et al., 2014; Broz et al., 2014), and future studies may provide a better understanding of the roles of each in tumor immunity. Figure 1 summarizes the many known roles of cDCs in immune responses.

Concluding Remarks

The increasing precision by which cDC subsets can be ablated in mouse models has greatly expanded our understanding of their functions. It is now clear that cDCs are a unique lineage comprised of distinct functional subsets critical for both innate and adaptive immunity. Further work is needed to resolve the apparent heterogeneity in cDC2s (Jaitin et al., 2014), and to test for their potential activities. In particular, how cDC2s control type 2 immune responses is unclear, including whether they provide instructive signals themselves, whether they are the primary APCs, and whether they, or other cells such as epithelial tuft cells, directly sense the pathogen. While these and other questions remain, it is now clear that DCs are the critical regulators of the entire immune response and that to understand the immune system we must fully analyze these remarkable cells.

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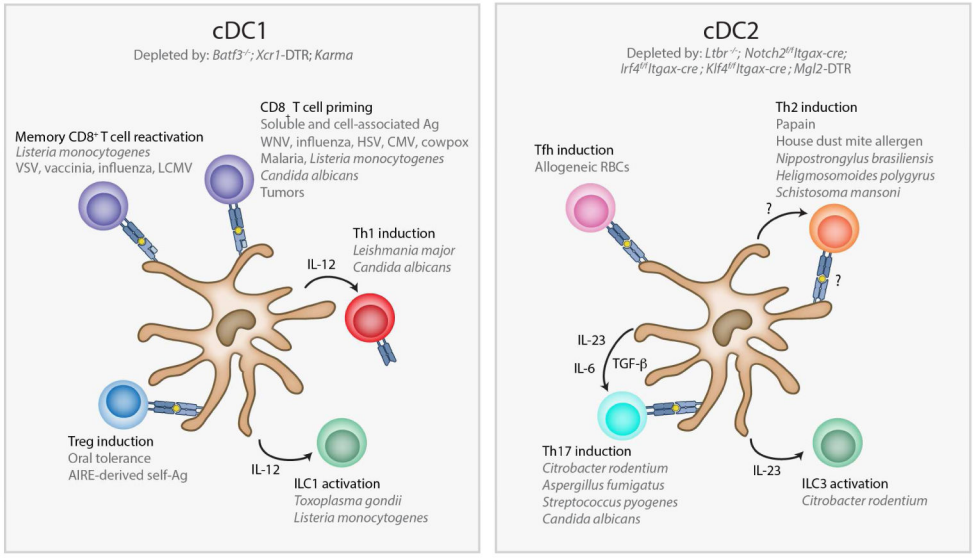

Fig. 1. Functions of dendritic cell subsets in the immune response

Classical dendritic cells (cDCs) play critical roles in both innate and adaptive immunity. Furthermore, each subset of cDC appears to possess unique functions and to control the immune response against specific forms of pathogens. cDC1s control type 1 immune responses against viruses and intracellular pathogens (left panel). In these responses they prime naïve CD8 T cells, reactivate memory CD8 T cells, activate ILC1s, and induce Th1 cells. Their production of the cytokine IL-12 is vital for many of these functions. cDC1s also play a role in inducing Tregs against orally fed antigens and AIRE-dependent self-antigens expressed in the thymus, though it appears that in some cases cDC2s can also mediate Treg conversion to these antigens. Some cDC2s, on the other hand, regulate type 2 immune responses against parasites in which they induce Th2 cells (right panel). The exact mechanism by which they do this is unclear. Other cDC2s control type 3 immune responses against extracellular bacteria and fungi (right panel). In these responses, cDC2s produce IL-23 in order to activate ILC3s and to induce Th17 cells. Their production of IL-6 and TGF-β also contributes to the polarization of Th17 cells. Finally, cDC2s are also responsible for the induction of T follicular helper (Tfh) cells that regulate the germinal center response. DTR, diphtheria toxin receptor; VSV, vesicular stomatitis virus; LCMV, lymphocytic choriomeningitis virus; WNV, west nile virus; HSV, herpes simplex virus; CMV, cytomegalovirus; AIRE, autoimmune regulator; Ag, antigen.

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Table 1

Comparison of DTA/DTR based mouse models for dendritic cell depletion

Strain	Cells Depleted	Caveats
***Itgax*-DTR** (Jung et al., 2002)	cDCs, metallophilic, marginal zone, sinusoidal, and alveolar macrophages, activated CD8 T cells, plasma cells	Repeated DT treatment leads to death, requires CD11c-DTR into WT BM chimeras No depletion of pDCs
***Itgax*-DOG** (Hochweller et al., 2008)	cDCs, splenic macrophages	Depletion of other cell types not evaluated
***Zbtb46*-DTR** (Meredith et al., 2012)	cDCs	DT treatment leads to death, requires zDC-DTR into WT BM chimeras
***Zbtb46*-LSL-DTR** (Loschko et al., 2016a)	cDCs	Requires crossing to Cre strain active in cDC lineage
***Cd207*-DTR (Knockin)** (Kissenpfennig et al., 2005)	Langerhans cells, cDC1s	
***Cd207*-DTR (BAC)** (Bennett et al., 2005)	Langerhans cells, cDC1s	
***CD207*-DTA** (Kaplan et al., 2005)	Langerhans cells, $CD103^+CD11b^+$ cDC2s in small intestine	
***Ly75*-DTR** (Fukaya et al., 2012)	cDC1s, ~15% splenic B cells	DT treatment leads to death, requires CD205-DTR into WT BM chimeras Depletion of germinal center B cells, migratory cDCs, and Langerhans cells untested but possible
***Clec9a*-DTR** (Piva et al., 2012)	cDC1s, ~50% pDCs	
***Xcr1*-DTRvenus** (Yamazaki et al., 2013)	cDC1s	
***Karma* (*a530099j19rik*-DTR)** (Alexandre et al., 2016)	cDC1s	
***Clec4a4*-DTR** (Muzaki et al., 2016)	cDC2s and $CD64^+CX3CR1^+$ macrophages in intestinal lamina propria and MLNs	DC depletion in other organs not analyzed
***Mgl2*-DTR** (Kumamoto et al., 2013)	$Mgl2^+$ dermal cDC2s	
***CLEC4C*-DTR** (Swiecki et al., 2010)	pDCs	
***Siglech*-DTR (Knockin)** (Takagi et al., 2011)	pDCs	Loss of SiglecH expression in homozygous knockin mice Depletion of marginal zone macrophages untested but possible
***Siglech*-DTR (BAC)** (Piva et al., 2012)	pDCs, marginal zone macrophages	
Cx3cr1*-LSL-DTR *Itgax-cre (Diehl et al., 2013)	$CD103^-CD11b^+CX3CR1^+$ cDC2s, $CD11b^+CX3CR1^+$ macrophages	Requires crossing to Cre strain active in cDCs
MM-DTR (Schreiber et al., 2013)	Monocytes and monocyte-derived macrophages	

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Table 2

Comparison of Cre strains for conditional deletion of genes in dendritic cells

Strain	Cells Affected	Caveats
***Itgax-cre* (BAC)** (Caton et al., 2007)	~100% of cDCs, pDCs, alveolar macrophages, ~70% red pulp macrophages, 20–40% monocytes, marginal zone macrophages, peritoneal macrophages, ~5–12% T, B, NK cells	Germline deletion possible
***Itgax-cre* (Transgene)** (Stranges et al., 2007)	cDCs, pDCs	Deletion in other cell types was not analyzed, most likely similar to other *Itgax- cre* strain
Clec9a-cre (Schraml et al., 2013)	~100% of cDC1s, ~50% of cDC2s, ~20% of pDCs	Begins deletion at CDP stage, low or transient expression may affect deletion efficiency in cDC2s and pDCs
Zbtb46-cre (Loschko et al., 2016b)	65–95% cDCs, <10% in all other cell types	Deletion efficiency variable depending on floxed allele
Cd207-cre (Zahner et al., 2011)	Langerhans cells, Langerin+ cDC1s in dermis, skin-draining LNs, and lung	
Xcr1-cre (Ohta et al., 2016)	cDC1s	
Siglech-cre (Puttur et al., 2013)	~30% of pDCs, ~2% of other lymphoid cells	Incomplete deletion in pDCs Deletion in marginal zone macrophages untested but possible

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Table 3

Comparison of transcription factor knockout mice that affect dendritic cell development

Strain	Cells Depleted	Caveats
Irf8$^{-/-}$ (Schiavoni et al., 2002; Aliberti et al., 2003)	cDC1s, B cells, monocytes, eosinophils, basophils	Myeloid neoplasia eventually results with age Conditional deletion with *Itgax-cre* or *Zbtb46-cre* can prevent depletion of other cell types seen in *Irf8*$^{-/-}$ mice
Id2$^{-/-}$ (Hacker et al., 2003)	cDC1s, NK cells, ILCs	
Nfil3$^{-/-}$ (Kashiwada et al., 2011)	cDC1s, NK cells, ILCs	
Batf3$^{-/-}$ (Hildner et al., 2008)	cDC1s	In certain genetic backgrounds residual cDC1-like cells remain in skin-draining LNs
Relb$^{-/-}$ (Burkly et al., 1995)	CD11b^{+}ESAM^{+} cDC2s in spleen	Fatal multiorgan inflammation
Ltbr$^{-/-}$ (Weih et al., 1995)	CD11b^{+}ESAM^{+} cDC2s in spleen	Loss of lymph nodes, can overcome with conditional deletion with *Itgax-cre*
Notch2$^{f/f}$ ***Itgax-cre*** (Lewis et al., 2011; Satpathy et al., 2013)	CD11b^{+}ESAM^{+} cDC2s in spleen, CD103^{+}CD11b^{+} cDC2s in small intestine lamina propria	Transcriptional changes also evident in cDC1s and CD11b+ cDC2s
Klf4$^{f/f}$ ***Itgax-cre*** (Tussiwand et al., 2015)	Subset of cDC2s similar to CD24^{-}CD11b^{-} cDC2s in skin-draining LNs	
Irf4$^{f/f}$ ***Itgax-cre*** (Bajana et al., 2012) (Schlitzer et al., 2013) (Persson et al., 2013) (Gao et al., 2013)	~50% CD11b^{+} cDC2s in lung, ~50% CD103^{+}CD11b^{+} cDC2s in small intestine lamina propria, complete absence of cDC2s in lymph nodes	cDC2s cannot migrate, failure to migrate may explain many observed phenotypes May also affect M2 macrophages or GM-CSF activated monocytes
Tcf4$^{f/f}$ ***Itgax-cre*** (Cisse et al., 2008)	pDCs	
Zeb2$^{f/f}$ ***Itgax-cre*** (Scott et al., 2016)	pDCs	