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Genetic Dissection of Dendritic Cell Homeostasis and Function: Lessons from Cell Type–Specific Gene Ablation

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Abstract

Dendritic cells (DCs) are a heterogeneous cell population of great importance in the immune system. The emergence of new genetic technology utilizing the CD11c promoter and Cre recombinase has facilitated the dissection of functional significance and molecular regulation of DCs in immune responses and homeostasis *in vivo*. For the first time, this strategy allows observation of the effects of DC-specific gene deletion on immune system function in an intact organism. In this review, we present the latest findings from studies using the Cre recombinase system for cell type–specific deletion of key molecules that mediate DC homeostasis and function. Our focus is on the molecular pathways that orchestrate DC life span, migration, antigen presentation, pattern recognition, and cytokine production and signaling.

Keywords

Dendritic cell; Homeostasis; Cytokine; Pattern recognition; Cre recombinase

Introduction

Dendritic cells (DCs) act as sentinel immune cells at sites exposed to the environment, such as the gut, lung, and skin. Upon activation by innate stimuli, such as those mediated by Toll-like receptors (TLRs), DCs capture antigens, migrate to draining lymph nodes (LNs), and present them to responding T cells to initiate adaptive immunity. DCs are the most potent antigen-presenting cells (APCs) for the activation of naïve CD4⁺ and CD8⁺ T cells [1] but are also crucial in mediating immune tolerance under steady state [2]. DCs provide three main signals that dictate the ensuing T cell responses. Signal 1 involves antigens presented by DC surface molecules such as major histocompatibility complex (MHC) I and II. Signal 2 provides co-stimulatory signals; the most notable example is the B7 molecules expressed on DCs (CD80 and CD86) that interact with CD28 on T cells. Signal 3 is mainly attributed to the production of cytokines by DCs that activate or inhibit effector responses.

Ablation of DCs by diphtheria toxin A (DTA) or diphtheria toxin receptor (DTR) has provided important insight into the *in vivo* role of DCs. Jung et al. [3] introduced the DTR gene under the transcriptional control of the CD11c (*itgax*) promoter to ablate DCs after the

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administration of diphtheria toxin, and this temporally controlled short-term elimination of DCs impaired CD8⁺ T cell priming. Additionally, Ohnmacht *et al.* [4] and Birnberg *et al.* [5] independently generated mice that express DTA in CD11c⁺ cells for constitutive ablation of DCs. This led to disrupted immune homeostasis, including the development of autoimmunity [4] and myeloproliferative disorders [5]. Since the initial descriptions of these models, additional studies have further advanced our understanding of the roles of DCs in tolerance induction, inflammation, and protective immunity. For example, ablation of DCs under steady state leads to a loss of regulatory T (T_{reg}) cells and overproduction of inflammatory cytokines such as interferon gamma (IFN γ) and interleukin 17 (IL-17) from T cells [6]. In experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis, the loss of DCs reduces T_{reg} numbers in a programmed cell death 1 (PD-1)–dependent manner and aggravates EAE pathogenesis [7]. These studies illustrated DC-mediated maintenance of the T_{reg} pool and immune tolerance. In contrast, depletion of lung DCs in a model of ovalbumin-induced airway hyperreactivity reduces Th₂ responses and the asthma-like phenotype [8]. In models of pathogen challenge, DC depletion impairs pathogen clearance and protective immunity, as observed in the infection by influenza [9], herpes simplex virus type 1 [10], *Mycobacterium tuberculosis* [11], *Listeria monocytogenes*, and *Plasmodium yoelii* [3]. In addition to altering DC-mediated T cell responses, DTR-mediated DC depletion causes neutrophilia, which enhances host antibacterial immune defense against *Yersinia enterocolitica* [12, 13]. Overall, these cell depletion studies highlight the importance of DCs in mediating T cell-mediated tolerogenic and immunogenic responses, as well as homeostasis of the myeloid compartment. However, because CD11c can be expressed by plasmacytoid DCs (pDCs) and non-DCs such as monocytes, macrophages and NK cells, caution should be applied when interpreting CD11c-based cell depletion studies (reviewed in [14]). The recently developed zDC-DTR mice, which capitalizes on the unique expression of the transcription factor zDC (Zbtb46 or Btd4) in conventional DCs (cDCs) and committed cDC precursors, allows selective ablation of cDCs, while sparing other CD11c expressing cells that contributed significantly to immune defense against pathogen and tumor challenge [15].

For mechanistic studies of molecular pathways in DCs, Cre recombinase driven by the CD11c promoter has been instrumental in uncovering the roles of DC-expressed genes by allowing their specific deletion in DCs. Two laboratory teams have reported the generation of genetic models with CD11c promoter-driven expression of Cre recombinase [16, 17]. Reizis' group generated the CD11c-Cre line from a mouse genomic bacterial artificial chromosome (BAC) library containing the entire CD11c (encoded by *itgax*) gene, with the first exon replaced by Cre recombinase [16]. Chervonsky's group generated transgenic mice expressing Cre driven by a ~5 kb genomic CD11c promoter/enhancer fragment [17]. These powerful genetic systems have been widely adopted over the past few years, leading to the identification of multiple key regulators of DC biology. However, there are certain limitations of the cre-lox system, including the efficiency of target gene deletion and Cre-mediated toxicity. Also, undesired deletion has been observed in other cells, including T cells [18] and alveolar macrophages [19], and such off-target deletion appears to be allele-specific (unpublished observations). Therefore, it is important to verify cell type-specificity

and efficiency of gene deletion in DCs and subsets, and to include Cre-expressing mice to control for Cre-mediated toxicity wherever possible [20].

Here we discuss the molecular pathways underlying DC homeostasis and function, based on the findings mainly derived from DC-specific genetic ablation systems, particularly CD11c-Cre mouse models. Our focus is on the pathways that regulate DC survival, migration, antigen presentation, pattern recognition, and cytokine production and recognition. Since we focus mainly on DC homeostasis and function, we refer the readers to excellent reviews describing the origin and development of DCs [21–23].

Regulation of DC survival and homeostasis

As described above, constitutive depletion of DCs alters immune homeostasis by inducing myeloproliferation and autoimmunity [4, 5], whereas acute DC depletion diminishes the T_{reg} population [6]. Conversely, blocking DC apoptosis via DC-specific overexpression of the baculovirus caspase inhibitor p35 causes DC accumulation, chronic lymphocyte activation, and autoimmunity [24]. Thus, proper control of DC viability and pool size are critical for prevention of adverse immune reactions. Recent studies using the CD11c-Cre system have revealed several proteins in the cell apoptosis and necroptosis machineries that contribute to DC survival. In addition, certain cell surface receptors, signaling pathways, and transcription factors regulate DC survival (Figure 1). All together, the concerted actions of these mechanisms maintain DC homeostasis and immune function.

Factors in the apoptosis and necroptosis machineries

Apoptotic cell death is mediated by intrinsic apoptotic signaling pathways (controlled by the balance of pro- and anti-apoptotic BCL-2 family members) and extrinsic apoptotic signaling pathways (controlled by signals delivered from surface death receptors and effector molecules release by cytotoxic immune cells). The BCL-2 family proteins have been widely studied for their roles in apoptosis. Activation of the pro-apoptotic Bax and Bak proteins induces mitochondrial outer membrane permeabilization and cytochrome C release from the mitochondria, leading to downstream caspase activation, DNA cleavage, and apoptotic cell death [25]. Deletion of Bax (germline) and Bak (DC-specific) reduces spontaneous death of DCs. Consequently, activated DCs accumulate with age and trigger spontaneous T cell activation and development of autoimmunity. In addition to exhibiting a defect in spontaneous cell death, Bak^{-/-}Bax^{-/-} DCs are resistant to killing mediated by T_{reg} cells [26]. These studies show that active control of DC apoptosis is important for regulation of DC turnover and prevention of autoimmunity.

Fas (also known as CD95 and Apo-1), the receptor for Fas ligand (FasL), is expressed on the surface of immune cells and functions as a cell death-inducing receptor. DC-specific deletion of Fas results in age-dependent DC accumulation and systemic autoimmunity characterized by lymphoid hyperplasia and high-titer anti-nuclear antibodies [17]. Since Fas is upregulated on DCs after they encounter maturation stimuli, Fas-dependent removal of activated DCs serves as an important mechanism to diminish T cell responses and prevent autoimmunity [17]. Fas-FasL interactions also act in concert with other proteins, such as perforin and granzyme B, to induce cell death [27, 28]. Killer cells (e.g., cytotoxic T

lymphocytes) release perforin, which forms a pore complex in the plasma membrane of target cells, and granzyme B, which induces apoptosis of target cells by proteolytic cleavage of BID and Caspase-3. Specifically, perforin-mediated pore complex facilitates the transfer of cytolytic granules containing granzymes from killer to target cells that include activated immune cells [29]. In Fas^{-/-} DCs, additional germline loss of perforin further potentiates DC accumulation and autoimmune inflammation over that seen in Fas^{-/-} DCs alone [30]. Therefore, Fas and perforin-dependent killing systems synergize to mediate extrinsic maintenance of DC homeostasis to limit T cell activation.

Fas-associated death domain (FADD), a signaling molecule that directly interacts with Fas, triggers caspase-8 (CASP8) activation. CASP8 activation in turn leads to downstream activation of BID and Caspase-3 via proteolytic cleavage and engagement of apoptotic cell death. However, loss or inhibition of either FADD or CASP8 induces RIP1- and RIP3-dependent necroptosis, an alternative cell death pathway [31]. Loss of FADD in DCs reduces absolute number of cDCs in the spleen, mainly due to the loss of the CD8⁺ DC subset, as well as CD103⁺ DCs in Peyer's patches and mesenteric LNs (MLNs). With age, mice with FADD deficiency in DCs develop systemic inflammation, splenomegaly, and lymphadenopathy with increased myeloid and B cell numbers. Treatment with broad-spectrum antibiotics (to eliminate gut microbiota), germline (but not DC-specific) loss of MyD88, or loss of RIP3 rescues the inflammatory phenotype initiated by reduction of CD8⁺ and CD103⁺ DCs. Overall, these findings indicate that gut microbiota induce TLR-mediated RIP3-dependent necroptosis of DCs in the absence of FADD signaling [32]. Thus, a shift in the balance from apoptotic to necrotic signaling perturbs DC homeostasis by rendering DCs more sensitive to cell death caused by innate stimulation.

Receptors and transcription factors in DC survival

Notch signaling regulates cell differentiation, proliferation, survival, and development. The transcription factor RBP-J is the main nuclear transducer of Notch signaling. DC-specific deletion of RBP-J results in selective loss of splenic CD8⁻ (CD11b⁺) cDCs associated with increased apoptosis and loss of expression of Deltex1, a Notch signaling target. Other splenic DC subsets and LN and tissue DCs remain largely unaffected [16]. Among the Notch receptors, Notch2 appears to play a dominant role. DC-specific deletion of Notch2, but not Notch1, decreases the number of splenic CD11b⁺ cDCs by specifically ablating a population marked by high expression of the adhesion molecule Esam. Notch2 deletion also results in the loss of CD103⁺CD11b⁺ DCs in the intestinal lamina propria. Consequently, deficiency of Notch2 in DCs attenuates priming of CD4⁺ T cells and development of Th₁₇ responses in the intestine [33]. More recently, these Notch2-dependent CD103⁺CD11b⁺ intestinal DCs were shown to provide an obligatory source of IL-23 required for antimicrobial responses to *Citrobacter rodentium* [34]. Therefore, RBP-J activity and Notch2 signaling are mainly required for the differentiation and survival of CD11b⁺ DCs.

Another factor involved in regulating DC-mediated Th₁₇ cell differentiation is interferon regulatory factor 4 (IRF4). IRFs are a group of transcription factors with important roles in the activation of type I interferon responsive genes and thus regulate many facets of innate and adaptive immunity. Loss of IRF4 in DCs causes a survival defect of CD103⁺CD11b⁺

but not CD103⁺CD11b⁻ DCs in the mesenteric LNs. Importantly, CD103⁺CD11b⁺ DC-derived IL-6 supports Th₁₇ differentiation in the MLN [35]. Another independent study reported that CD11b⁺IRF4⁺ mucosal DCs produce IL-6 and IL-23, which act to promote the differentiation and stabilization of mucosal Th₁₇ cells, respectively [36]. Therefore, IRF4 is necessary for the survival of CD103⁺CD11b⁺ mucosal DCs to mediate Th₁₇ responses. In addition, IRF4 function in DCs is important for Th₂ responses, by orchestrating the differentiation of a novel PDL2⁺CD301b⁺ DC subset [37]. Thus, IRF4 expression in DCs contributes to both Th₁₇ and Th₂ responses.

Signaling pathways regulating DC survival

Transforming growth factor beta-activated kinase 1 (TAK1) integrates signals from multiple receptors in innate and adaptive immune cells, and signals to activation of downstream pathways including JNK, p38 and NF- κ B. Loss of TAK1 in DCs causes increased apoptosis of DCs, in particular, lymphoid CD8⁺ and non-lymphoid CD103⁺ DCs and pDCs. Associated with defective DC survival, mice harboring TAK1-deficient DCs develop a myeloproliferative disorder characterized by the accumulation of neutrophils and inflammatory monocytes. Mechanistically, TAK1 integrates pro-survival signals from innate receptors to activate NF- κ B and Akt-Foxo pathways [38]. Furthermore, loss of TAK1 in DCs impairs T cell priming in vivo [38] and T cell activation and expansion, as well as IFN γ production in a contact hypersensitivity model [39]. Thus, TAK1 signaling maintains DC survival that in turn prevents myeloproliferation and orchestrates T cell immunity.

The mechanistic target of rapamycin (mTOR) kinase pathway comprises two distinct complexes, namely mTORC1, defined by the adaptor protein Raptor, and mTORC2, defined by Rictor. mTOR regulates multiple basic cellular functions, including cell growth and metabolism, thereby dictating cell fate decisions. Phosphatase and tensin homologue (PTEN) is a negative regulator of mTOR kinase, by inhibiting the upstream activator PI3K [40]. Recently mTOR has garnered much attention in the immune system due to its important role in lymphocyte function [41]; however, its role in DCs is just beginning to be appreciated. Loss of either PTEN or Raptor expands splenic CD8⁺ DCs [18, 42]. However, PTEN deletion in DCs expands CD103⁺ cDCs in the intestine and other tissues [18], while Raptor deletion increases intestinal CD11b⁺ DCs [42]. Mechanistically, while loss of PTEN increases responsiveness to the DC growth factor Flt3 ligand [18], increased numbers of Raptor^{-/-} DCs are ascribed to a pro-survival effect arising from enhanced AKT phosphorylation or a reduction of TLR-mediated cell death [42]. Furthermore, loss of Raptor in Langerhans cells (LCs), which are specialized DCs found in the skin, enhances apoptosis and reduces epidermal and dermal LCs. Raptor deficiency also affects migration of these cells [43]. These findings suggest that increased and decreased mTOR activation affects DC survival via different mechanisms, thereby contributing to DC pool size and subset composition.

In summary, both the basic cell death machinery and the pathways regulating its activity impinge upon DC survival. While specific subsets of DCs may require a unique set of intracellular signals to survive (i.e., IRF4 for CD103⁺CD11b⁺ DCs), genetic manipulation of the cell death machinery in general affects all DCs. Increased DC death diminishes DC

regulatory activities under steady state and often leads to myeloproliferation, whereas elevated DC survival may cause abnormal T cell activation and autoimmunity. Altogether, cell death in DCs plays an important role in immune system homeostasis and function.

Migration of DCs

Immune surveillance by DCs in non-lymphoid tissue is important for immune defense. A critical aspect of the DC sentinel function is migration from the site of antigen capture to the draining LN via the afferent lymphatic vessel. Within the draining LN, DCs present acquired antigens to lymphocytes and induce their activation, thereby eliciting antigen-specific adaptive immune responses. Chemokine receptors expressed on DCs and downstream signaling pathways are important regulators of DC migration [44].

Regulation of migration by surface receptors

Before immune activation, DC subsets express multiple chemokine receptors at varying levels. One of the crucial receptors regulating DC migration is CCR7. Semi-mature steady-state DCs constitutively travel to the LNs in a CCR7-dependent manner. In the LNs, DC-T cell interactions occur and prolong the time T cells spend in the LNs. The presence of these steady state DCs induces the expression of CCL21 by T-zone fibroblastic reticular cells. CCL21 is a ligand for CCR7 on T cells and, as a consequence, attracts T cells to the LNs, supporting their retention. This retention is also partially mediated by binding of stromal cell-derived CCL21 to the surface of DCs, and the production of VEGF by DCs to promote high endothelial venule formation [45]. Overall, the expression of CCR7 on DCs plays an important role in DC migration that further impacts T cell homeostasis in the LNs.

In addition to resident DCs in non-lymphoid tissues, splenic DC populations have sentinel functions by entering the splenic marginal zone between white and red pulp and capturing and presenting blood-borne antigens [46]. Migration of CD4⁺ DCs to the spleen is mediated by the chemotactic receptor EBI2 (also known as GPR183), which binds the endogenous ligand 7 α ,25-dihydroxycholesterol. DC-specific loss of EBI2 reduced the numbers of CD4⁺ DCs in the spleen. In addition, EBI2^{-/-} DCs show a severe impairment of germinal center responses due to reduced induction of T and B cell responses [47]. These experiments suggest that EBI2 and its ligand are essential for the positioning of splenic DCs in the marginal zone and the initiation of specific immune responses.

Role of intracellular signaling in migration

As described above, signaling via mTORC1 but not mTORC2 is required for LC migratory homeostasis. Raptor^{-/-} LCs initially seed the epidermis equally well to their wild-type counterparts. However, progressively, Raptor^{-/-} LCs show an increased tendency to leave the skin, leading to the loss of LCs in the skin but increased frequency of migratory DCs in the skin-draining LNs. Concordantly, the loss of Raptor reduces expression of E-cadherin and β -catenin, both involved in adhesion, as well as CCR7 [43]. Overall, Raptor ablation in LCs increases their migration and leads to loss of these sentinel DCs in the skin [43]. Further, deletion of the adaptor molecule p14, which is part of the upstream 'Ragulator'

complex involved in the activation of mTORC1, in DCs ablates the epidermal LC population early after birth due to impaired proliferation and increased apoptosis [48].

Cdc42, a Rho-family GTPase, also controls migration of LCs. DC-specific deletion of Cdc42 reduces LC migration. In response to FITC skin painting and during homeostasis, Cdc42^{-/-} LCs fail to leave the skin or enter the skin-draining LNs. In addition, Cdc42^{-/-} DCs elicit suboptimal T cell responses [49]. Although Cdc42 has been implicated in mTOR activation in certain systems [50], the opposite effects of Raptor and Cdc42 deficiencies highlight mTOR-independent activity of Cdc42 in LC migration.

Sirtuins are a family of NAD⁺-dependent protein deacetylases. Of these, sirtuin 1 (Sirt1) is activated in response to increased intracellular levels of NAD⁺ and increased cAMP levels/protein kinase A activation [51]. The absence of Sirt1 in DCs reduces the migration of antigen-loaded DCs from the lung to the draining LNs. At least in part, this effect can be attributed to the increased PPAR γ activation as a result of Sirt1 deletion. In an OVA-induced asthma model, Sirt1 deletion in DCs attenuates the severity of inflammation [52]. Therefore, Sirt1 expression in DCs is necessary for DCs after antigen capture, and the loss of Sirt1 in DCs reduces Th₂ inflammatory responses.

Together, proper regulation of DC migration is necessary for the homeostasis of the immune system and induction of antigen-specific responses. Migration of DCs is dependent on surface expression of chemokine receptors and adhesion molecules. Such process is further programmed by intracellular signaling pathways, in particular environmental sensing pathways, and this is consistent with the sentinel functions of DCs.

Antigen Presentation

DC are superior APCs partly due to their high expression of MHC, which present peptide antigens for activation of conventional T cells [1] and expression of CD1d, which presents lipid antigens to activate natural killer T (NKT) cells [53]. Further, antigen presentation by resting and activated DCs has profoundly different consequences, revealing the role of DCs in orchestrating both tolerance and immunity [2]. In addition to antigen presentation, the acquisition, intracellular shuttling, and processing of antigens are important functions of DCs and can dictate the outcome of an ensuing immune response [54].

Antigen presentation to CD4⁺ T cells

Antigen presentation by MHC II is necessary for CD4⁺ T cell priming by DCs. DCs lacking MHC II are unable to present antigens to naïve T cells and elicit T cell responses. In the OVA-asthma model, MHC II^{-/-} DCs are unable to generate the pathogenic Th₂ response for airway inflammation [55]. Emerging evidence highlights an important role for the autophagy pathway in antigen presentation and other immune functions [56]. In DCs, autophagy mediated by ATG5 is necessary for exogenous antigen presentation by MHC II, while antigen cross-presentation on MHC I remains intact in the absence of ATG5. In response to herpes simplex virus infection, CD4⁺ T cell priming is impaired in ATG5-deficient DCs, and the host is unable to control infection. Mechanistically, assembly of the autophagy machinery is required for phagosome-lysosome fusion and MHC II loading of

processed antigens [57]. Thus, antigen presentation by MHC II on DCs requires the autophagy machinery to mediate CD4⁺ T cell responses.

Antigen presentation by CD1d and elicitation of NKT cells

Unlike the MHC molecules that present peptide antigens, CD1d displays glycolipid antigens, allowing DCs to activate NKT cells, a non-conventional T cell subset recognizing these antigens through semi-invariant T cell receptors [58]. Although less is known about mechanisms of CD1d-restricted than MHC-restricted antigen presentation, recent studies indicate an important role of DC-expressed CD1d in iNKT activation. The absence of CD1d on DCs attenuates the OVA-induced asthma phenotype after previous sensitizations with OVA and the CD1d ligand PBS57, exemplified by the reduction of eosinophils and T cells in the airway and serum IgE and IgG1 titers [59]. Further, after initial interactions with CD1d-expressing DCs, NKT cells provide cognate help to B cells to promote antibody responses [60]. Interestingly, differential induction of Th₁- or Th₂-like NKT cell responses is contingent upon lipid antigen structures and types of APCs involved, with the former responses selectively requiring CD1d expression on DCs [61]. Recent studies have further identified negative pathways affecting DC-mediated iNKT activation. For instance, adenosine receptor A_{2A}R agonists tolerize DCs and suppress DC-mediated activation of NKT cells in kidney ischemic reperfusion injury [62]. Altogether, DCs are potent mediators of CD1d-antigen presentation and activation of specific NKT cells.

Pattern Recognition and DC Activation and Maturation

Role of TLRs and MyD88 signaling in DCs in host defense and autoimmunity

The prototypical pattern recognition receptors are the TLRs, which recognize a wide array of pathogen-associated molecular patterns (PAMPs) derived from bacteria, fungi, protozoa, and viruses. MyD88 is an important adaptor protein to transduce signals from various TLRs (except for TLR3) as well as IL-1R, leading to the activation of downstream pathways NF- κ B, AP-1, and IRF [63]. Due to its ability to recognize PAMPs, TLR and Myd88 activation is important for host defenses against pathogens. For instance, lack of MyD88 in DCs, but not macrophages or neutrophils, results in high susceptibility to *Toxoplasma gondii* infection. MyD88^{-/-} DCs produced less IL-12, which resulted in delayed NK responses and less activation of inflammatory monocytes [64].

Moreover, specific loss of MyD88 in DCs ameliorates autoimmune inflammation in several genetic models, including a strong protective effect from the development of colitis in IL-10^{-/-} mice [65], but a partial effect at reducing inflammation at the environmental surfaces (skin, lungs, and gut) in Foxp3^{-/-} mice [66]. Interestingly, in the MRL.Fas^{lpr} murine lupus model, MyD88 signaling in DCs and B cells orchestrates separate mechanisms for disease pathogenesis. MyD88 deficiency in B cells ameliorates nephritis, whereas MyD88 ablation in DCs does not affect nephritis but reduces dermatitis, suggesting distinct mechanisms of action of Myd88 signaling in B cells and DCs in lupus [67]. Further, MyD88-dependent and independent pathways drive population expansion of T cells and activation of DCs and T cells, respectively, in the autoimmune disease model arising from the lack of the ubiquitin editing protein A20 in DCs (see below for details) [68]. These

results suggest that DC-specific MyD88 signaling exerts specific effects in mediating inflammation in discrete genetic autoimmune models.

In addition to autoimmunity induced by genetic modification, several chemically induced autoimmune models also depend upon MyD88s signaling. The dextran sulfate sodium (DSS) colitis model induces uncontrolled dissemination of gut microbiota. Germline loss of MyD88 exacerbates the colitis and results in mortality. Whereas the loss of MyD88 in DCs only partially contributes to DSS-induced mortality, B cell-specific loss nearly phenocopies DSS-induced colitis mortality as observed in germline MyD88^{-/-} mice [69]. Imiquimod (IMQ) is a synthetic TLR7 agonist that, when applied to the skin of mice, causes a psoriasis-like skin inflammation. MyD88 expressed only in DCs is sufficient for psoriasis-like skin inflammation induced by IMQ [70]. Moreover, DC-specific deletion of ABIN-1 exacerbates IMQ-induced experimental psoriasis, which can be reversed by simultaneous deletion of MyD88 in ABIN1^{-/-} DCs [71].

Overall, these studies show that MyD88-dependent signaling on DCs exerts profound effects on immune defense and autoimmune inflammation. Nonetheless, MyD88-independent pathways in DCs are also important contributors, whereas in certain disease models, MyD88 signaling in other cells especially B cells can play a more dominant role.

Negative regulation of TLR signaling and DC activation

The transcription factor NF- κ B plays an important role in various aspects of DC function [72]. Recent studies have identified how negative regulators of NF- κ B activity contribute to DC functional regulation. A20 restrains NF- κ B activation from multiple innate immune receptors by inhibiting ubiquitination on key signal-transducing molecules (including TRAF2 and TRAF6) [73]. Ablation of A20 in DCs results in spontaneous DC maturation, excessive T cell activation and development of autoimmunity manifested as lymphocyte-dependent colitis, arthritis, and enthesitis [68]. The ubiquitin sensor ABIN-1 encoded by *Tnip1* restricts TNF α -induced cell death signaling [74]. DC-specific deletion of ABIN-1 causes autoimmunity marked by myeloproliferation and lymphoproliferation, associated with increased splenic pDC but not cDC numbers. The loss of ABIN-1 in DCs increases NF- κ B and MAPK signaling, leading to greater IL-23 production in response to TLR stimulation [71]. Overall, NF- κ B signaling of DCs is actively regulated to maintain DC homeostasis.

Another negative regulator of DC activation is the nonreceptor protein-tyrosine phosphatase Shp1, which functions downstream of multiple receptors including TLRs, integrins, receptor tyrosine kinases, and cytokine receptors. The *Ptpn6* gene encodes the Shp1 protein, and deletion or mutation of *Ptpn6* causes the *motheaten* phenotype, characterized by skin lesions and reduced viability due to autoimmune disease [75]. DC-specific Shp1 deletion results in spontaneous DC maturation, activation of T and B cells, expansion of various immune cells, and development of autoimmunity [19, 76]. This phenotype depends, at least in part, on TLR signaling as concomitant deletion of MyD88 partially rescues the Shp1^{-/-} DC phenotype [19]. Recently, the Src kinase Lyn was found to restrict DC responses to TLR stimulation. Loss of Lyn in DCs causes spontaneous lymphocyte activation and tissue inflammation, which can be reversed by the additional loss of MyD88 in DCs [77]. Overall,

negative regulation of TLR and other signaling by multiple factors is necessary to maintain functional homeostasis of DCs.

Fc receptors and downstream kinase Syk

Aside from TLRs, other receptors expressed by DCs are also involved in DC activation and maturation. Fc receptors (FcRs), which are comprised of different types recognizing specific antibody isotypes, bind the constant region of antibodies and thus allow DCs to interact with antibodies produced by B cells. While FcR γ I, FcR γ III, and FcR γ IV are positive regulators of immune responses, FcR γ IIb provide an inhibitory signal [78]. Loss of FcR γ IIb on DCs exacerbates cytokine responses to antigen-antibody immune complex (IC). Furthermore, DCs deficient in FcR γ IIb exhibit increased basal and IC-induced maturation, and potentiate induction of CD8⁺ T cell immunity [79]. Thus, FcRs play important roles in regulating the responses of DCs to IC by altering various facets of their function.

Upon FcR ligation on DCs, the kinase Syk is activated, facilitating antigen internalization and presentation. DC-specific loss of Syk reduces the incidence of diabetes [80], and impairs Th₂ responses in asthma [81]. Aside from FcRs, activation of Syk can be mediated by the DC-specific receptor DNGR-1 that recognizes dead cells. The loss of DNGR-1 on DCs reduces cross-priming of dying vaccinia virus (VACV)-infected cells. DNGR-1^{-/-} DCs induce diminished CD8⁺ T cell responses to VACV, similar to Syk^{-/-} DCs described above, resulting in impaired clearance of VACV [82]. Overall, these studies establish a critical role for Syk activation in mediating FcR and DNGR-1 functions in DCs.

Cytokine production and signaling

The cytokine milieu during the interaction of DCs and T cells plays a crucial role in immune responses. DCs contribute to the cytokine milieu, thus emphasizing the importance of properly regulating DC cytokine production. Conversely, DCs receive inputs from their environment, including cytokines that signal through their receptors to dictate DC function (Table 1). Below, we discuss cytokine production from DCs and the responsiveness of DCs to cytokines, as well as signaling pathways downstream of cytokine receptors.

Roles of the anti-inflammatory cytokines IL-10 and TGF- β and their receptors

Although IL-10 and TGF- β have multiple functions, they are mostly recognized for their potent anti-inflammatory properties [83]. IL-10 is both produced and recognized by DCs. LCMV infection induces IL-10 production in CD8⁺ DCs, and DC-specific ablation of the IL-10 gene significantly diminishes the serum amount of IL-10 after LCMV infection. However, targeted deletion of the IL-10 receptor (IL-10R) in DCs does not change their ability to mount an LCMV-specific T cell response [84]. In contrast to the response to LCMV infection, IL-10R expressed on DCs plays a dominant role in dermal allergy. Specifically, the loss of IL-10R on DCs leads to increased pro-inflammatory cytokine production in response to oxazolone ear painting. Furthermore, mice with DC-specific loss of IL-10R show exacerbated hapten-induced allergic T cell effector responses. In addition to the effects seen in the allergic model, mice with DC-specific deficiency of IL-10R

experience splenomegaly and increased DC frequencies [85]. These results reveal the potent anti-inflammatory properties of IL-10 signaling in DCs.

Deletion of TGF- β 1 or TGF- β receptor II (TGF- β RII) in Langerhans cells (LCs) (by Langerin-Cre) or TGF- β RI in all DCs (by CD11c-Cre) induces spontaneous cutaneous DC migration and increases DC maturation [86, 87]. LC-specific loss of TGF- β signaling does not alter cytokine milieu [86]. In contrast, deletion of TGF- β RII in all DCs by CD11c-Cre results in multi-organ autoimmune disease, accompanied by spontaneous T and B cell activation. These mice die before 15 weeks due to immunopathology, associated with decreased Foxp3 expression, abnormal expansion of T_{reg} and excessive IFN γ production by DCs [88], highlighting the crucial effects of TGF- β signaling in DCs.

Several recent studies have focused on the role of the $\alpha_v\beta_8$ integrin in DCs, which converts TGF- β precursor molecule into its active form. Travis et al [89] reported that loss of the β_8 chain of $\alpha_v\beta_8$ on DCs leads to systemic autoimmune disease. In vitro, the mutant DCs fail to induce T_{reg}, an effect that depends upon TGF- β activity [89]. In addition, Lacy-Hulbert et al [90] described that deletion of the α_v chain of $\alpha_v\beta_8$ in hematopoietic cells causes colitis and systemic inflammation [90]. Although $\alpha_v\beta_8$ function in DCs is important for maintaining immune homeostasis under steady state, loss of $\alpha_v\beta_8$ signaling in DCs protects mice from EAE [91, 92] and asthma [93], due to impairments in TGF- β activation and the ensuing Th₁₇ responses. Additionally, loss of $\alpha_v\beta_8$ in DCs protects mice from infection with the parasite *Trichuris muris*, associated with enhanced Th₂ responses [94].

Overall, these studies show that signals by IL-10 or TGF- β in DCs are necessary for immune homeostasis and function. DCs devoid of the production, recognition, or activation of these cytokines contribute to the altered balance between tolerance and inflammation.

Roles of pro-inflammatory cytokines and cytokine receptors

A number of studies employing DC-specific deletion of cytokines receptors have revealed important roles of intrinsic cytokine signaling in DCs, even though these receptors are expressed by many cell types. IL-15 receptor alpha (IL-15R α) is a pleiotropically expressed molecule that trans-present IL-15 to NK and T cells. Using mice lacking IL-15R α in distinct cell types, Mortier et al [95] reported that IL-15R α expressed by DCs selectively supports central memory T cell maintenance via *trans*-presentation of IL-15. In contrast, IL-15R α expressed on macrophages is important for both central and effector memory CD8⁺ T cells, as well as early transition from effector to memory cells. Furthermore, IL-15R α expressed by both DCs and macrophages contribute to NK cell homeostasis and activation [95]. IL-4 is a canonical Th₂ cytokine involved in the immune responses to *Leishmania major* infection. Vaccination of mice with *L. major* antigen-loaded DCs dampens tissue damage at the site of *L. major* infection. This DC vaccination requires IL-4 receptor alpha (IL-4R α) expression on DCs to control *L. major* infection and associated tissue damage, as demonstrated by DC-specific deletion of IL-4R α [96]. Recognition of lymphotoxin- β (LT β) via its receptor (LT β R) is required to effectively control *C. rodentium* infection. Loss of LT β R in DCs results in heightened susceptibility to *C. rodentium* infection. Mechanistically, LT β R^{-/-} DCs produce less IL-23, which is important in the induction of IL-22 by innate lymphoid cells for immune defense against *C. rodentium* [97]. Type I IFNs are important for anti-viral

and anti-tumor immunity. Type I IFNs are recognized by IFN α/β receptor 1 expressed by CD8⁺ DCs to support rejection of immunogenic tumors, via cross-priming of CD8⁺ T cells [98]. Loss of IFN receptors on DCs also affects the production of IL-10 in response to LCMV infection [84], and dsRNA-mediated suppression of CNS autoimmunity [99].

The cytokine IL-27 consists of two subunits: p28 and EBI3. The deletion of the p28 subunit in DCs was recently reported. In the B16 tumor model, loss of p28 in DCs leads to impaired recruitment of NK and NKT cells into the tumor microenvironment, associated with diminished induction of CXCL10 expression by myeloid-derived suppressor cells [100]. In the concanavalin A-induced liver injury, p28^{-/-} DC mice experience increased liver injury due to greater IFN γ production by CD4⁺ T cells [101]. Together, these studies show that a spectrum of pro-inflammatory cytokines is involved in DC function, and the loss of cytokine receptors or production can exert drastic effects on immune responses.

Signaling pathways controlling DC cytokine production

Given the crucial roles of cytokines and cytokine receptors in DCs, it is not surprising that cytokine production and signaling in DCs are controlled by intricate mechanisms (Table 2). Multiple intracellular pathways are important regulators of this process. Many of the signaling pathways involved in positive and negative regulation of pattern recognition and DC activation and maturation, as described above, also play an important role in DC cytokine production. For instance, deletion of Shp1 causes elevated basal and LPS-induced expression of pro-inflammatory cytokines DCs, as well as increased IL-10 but decreased IL-12 production [76]. Deletion of A20 in DCs results in increased IL-6, TNF α , IL-12, and IL-10 production [68], whereas the loss of ABIN-1 increases IL-6, TNF α , IL-23, and IL-12p70 production [71]. Furthermore, Syk is required for TNF α production after CpG stimulation [102], and IFN β production after Dectin-1 stimulation [103], and Raptor/mTORC1 from intestinal DCs is important for IL-10 production [42]. In addition to these molecules described above, additional pathways, including those distal to receptor stimulation, are also implicated in DC-dependent cytokine production and immune function.

Among the central pathways activated by innate receptors is p38 MAPK. Although p38 MAPK is comprised of four family members encoded by distinct genes, p38 α is the predominant form in DCs. Activation of p38 α integrates multiple innate receptors including TLR and C-type lectin receptors [104]. Loss of p38 α in DCs attenuates IL-6 but elevates IL-27 expression, thereby reducing Th₁₇ responses. Consequently, loss of p38 α in DCs (but not macrophages or T cells) partially protects mice from EAE [104]. Moreover, p38 α activity in intestinal CD103⁺ DCs is important for the expression of TGF- β II and retinaldehyde dehydrogenase (an important enzyme for retinoic acid synthesis), and loss of p38 α attenuates DC-mediated T_{reg} generation and induction of oral tolerance [105]. Moreover, germline deletion of MKP-1, a key negative regulator of p38 MAPK, results in dysregulated expression of both pro- and anti-inflammatory cytokines in DCs [106], although DC-specific deletion of MKP-1 has not been reported. These results indicate that the p38 α and MKP-1 pathway in DCs contributes to both autoimmune responses and immune tolerance.

β -catenin is crucial in transducing signals from Wnt. β -catenin signaling is constitutively active in intestinal DCs [107]. The absence of β -catenin in DCs causes spontaneous pro-inflammatory cytokine production while reducing the levels of IL-10, TGF- β and retinaldehyde dehydrogenase. Consequently, mice harboring the mutant DCs exhibit spontaneous T cell activation and exacerbation of experimental colitis [107]. Therefore, β -catenin signaling programs the tolerogenic activity of DCs.

The signaling molecule TRAF6 is important for TLR-mediated activation of DCs. Unexpectedly, deletion of TRAF6 in DCs results in the loss of mucosal tolerance and spontaneous development of Th₂ responses in the intestine [108]. This is due to defective IL-2 production from the mutant DCs and the impaired generation of T_{reg} cells [108]. Deletion of another TRAF molecule, TRAF3, in DCs results in impaired production of type I IFN, TNF α and IL-10 but unaltered IL-6 production [109]. The TRAF3 function in cytokine regulation is cell context-specific, as TRAF3-deficient B cells show elevated production of various cytokines and type I IFN [109]. Overall, these findings illustrate the complex control of DC cytokine production and immune function by various signaling molecules.

Transcription factors involved in DC cytokine production

Emerging evidence indicates that many transcription factors with important roles in adaptive immunity are also implicated in DC functional regulation. Nuclear factor of activated T cells (NFAT) encompasses a group of transcription factors that were traditionally associated with T cell activation. When NFATc1 is specifically deleted in DCs and further bred onto an NFATc2-deficient (germline) background, DC production of IL-10 and IL-12 following TLR stimulation is diminished [110]. Deletion of Blimp-1, a key regulator of T cell differentiation, in DCs results in increased IL-6 production and preferential induction of follicular helper T cells, thereby increasing lupus-like autoantibody production *in vivo* [111]. Estrogen receptor alpha (ER α), a nuclear receptor, is required for pDC-mediated pro-inflammatory cytokine responses in response to estradiol [112]. Together, perturbations in transcription factor expression in DCs can lead to imbalanced immune homeostasis to a great extent by altering DC cytokine production.

The JAK-STAT pathway is activated by a large number of cytokine receptors, eventually leading to STAT-mediated transcriptional changes. Stat3 is activated by multiple cytokines including the immunosuppressive IL-10. The loss of Stat3 in DCs increases production of inflammatory cytokines, including IL-6, TNF α , IL-12, and IL-23, and causes mucosal inflammation [113]. In addition, Stat3-deficient DCs are resistant to IL-21-induced apoptosis [114]. Loss of Stat1, which is involved in IFN signaling, in DCs impairs immune responses against *L. monocytogenes* by enhancing antigen-specific T_{reg} proliferation [115]. Stat5 function in DCs is important for Th₂ responses in the skin and lungs [116]. Loss of Stat5 in DCs ablates the ability of DCs to respond to thymic stromal lymphopoietin (TSLP), which upregulates expression of costimulatory molecules and chemokines. Importantly, Th₂ responses in mice containing Stat5-deficient DCs resemble those observed in mice deficient in the TSLP receptor, thereby highlighting the crucial role of TSLP-Stat5 axis in DCs in driving Th₂ responses [116]. Overall, the identification of the signaling and transcriptional

pathways important for DC cytokine production and signaling has further advanced our understanding of molecular mechanisms of DC biology.

Concluding remarks

In conclusion, recent studies involving genetic engineering of DCs via the Cre-lox recombination system have provided important insights into the molecular regulation of DC homeostasis and function. These elegant studies highlight various facets of DC functional impacts, and the sensitivity of DCs to modulation and their dependence on individual genes for proper function. Given the potential for therapeutic intervention, it is of particular interest to understand molecular pathways that shape DC-mediated immune tolerance and activation of distinct types of adaptive immunity (Figure 2). The genetic dissection of DC homeostatic and functional pathways has revealed new insight into the molecular mechanisms that impact DC biology at multiple levels, including extrinsic factors, immune receptors, signaling pathways and transcription factors. Such knowledge holds great promises for the identification of innovative targets and strategies to modulate DC function for therapeutic intervention of infectious, inflammatory and malignant disorders.

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Figure 1. Factors Controlling DC Survival

Several factors positively and negatively control the generation of various subsets of DCs, including DC found in the lymphoid tissue: CD11b⁺ (CD4⁺), CD8⁺, CD4⁻CD8⁻, and DC found in non-lymphoid tissue: CD11b⁺CD103⁺ DC and LCs. The non-lymphoid CD103⁺ DC are thought to be related to the lymphoid CD8⁺ DC. In addition, pDC exist, which are thought have developmental differences to conventional DC.

Figure 2. Signaling and transcriptional pathways in DC-mediated immunogenic and tolerogenic responses
Multiple molecular pathways act in DC to instruct the decision between immunity and tolerance by dictating effector and regulatory T cell responses.

Table 1

Summary of deletion of DC-specific cytokine receptors via CD11c-Cre

Target Gene	Effect in gene-deleted DCs	References
IL-10R	↑ Proinflammatory cytokines ↑ Hapten-induced allergic reaction	[85]
TGF- β RI	↑ DC migration and maturation	[86, 87]
TGF- β RII	Multiorgan autoimmune disease Spontaneous T and B cell activation ↓ Ability to generate T _{reg}	[88]
IL-15R α	↓ NK cell homeostasis and activation ↓ Central memory T cell proliferation	[95]
IL-4R	↑ <i>Leishmania</i> -induced tissue damage after DC vaccination	[96]
LT β R	↓ Anti- <i>Citrobacter</i> immune response ↓ IL-22, IL-23p19 production	[97]
IFNAR1	↓ Anti-tumor immunity and CD8 ⁺ responses Loss of dsRNA-mediated suppression of CNS autoimmunity	[98] [91]

Table 2

Summary of DC signaling and transcriptional pathways involved in the production of cytokines and other factors

Target Gene	Effect in the gene-deleted DCs	References
Signaling pathways		
Raptor	↓IL-10	[42]
A20	↑IL-6, TNFα, IL-12, IL-10	[68]
ABIN-1	↑IL-6, TNFα, IL-23, IL-12p70	[71]
Shp1	↑TNFα, IL-1β, IL-6, IL-10, and IFNβ ↓IL-12β	[76]
Syk	↓TNFα ↓IFNβ	[102] [103]
p38α	↓IL-6, CD86 ↑IL-27 ↓TGF-βII	[104] [105]
β-catenin	↑IL-6, IL-23 ↓IL-10, TGF-β	[107]
TRAF6	↓IL-12, IL-2	[108]
TRAF3	↓IL-6, TNFα, IL-10 ↑IL-12	[109]
Transcription factors		
NFATc1	↓IL-10, IL-12 (in the NFATc2 ^{-/-} background)	[110]
Blimp1	↑IL-6	[111]
ERα	↑IFNα, TNFα, IL-12p40	[112]
Stat3	↑IL-6, TNFα, IL-12, and IL-23	[113]
Stat5	TSLP-induced CCL17 and co-stimulatory molecules	[116]