Genetic models of human and mouse dendritic cell development and function

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Abstract | Dendritic cells (DCs) develop in the bone marrow from haematopoietic progenitors that have numerous shared characteristics between mice and humans. Human counterparts of mouse DC progenitors have been identified by their shared transcriptional signatures and developmental potential. New findings continue to revise models of DC ontogeny but it is well accepted that DCs can be divided into two main functional groups. Classical DCs include type 1 and type 2 subsets, which can detect different pathogens, produce specific cytokines and present antigens to polarize mainly naive $CD8^+$ or $CD4^+$ T cells, respectively. By contrast, the function of plasmacytoid DCs is largely innate and restricted to the detection of viral infections and the production of type I interferon. Here, we discuss genetic models of mouse DC development and function that have aided in correlating ontogeny with function, as well as how these findings can be translated to human DCs and their progenitors.

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Dendritic cells (DCs) are essential regulators of innate and adaptive immune responses. A diversity of functionally distinct DC subsets has been described, and emerging models have incorporated variability across tissues, species and genetically diverse individuals. Classical DCs (cDCs), also known as conventional DCs, have been defined by their discrete functions and transcriptional identities and include two major subsets, known as type 1 cDCs (cDC1s) and type 2 cDCs (cDC2s). The distinction between cDC1 and cDC2 subsets is supported by genetic models of DC development, which have been essential to define the specialized functions of cDC subsets[1]. For the most part, cDC1s present exogenous, cell-associated antigens to $CD8^+$ T cells, thereby regulating cytotoxic T lymphocyte (CTL) responses to intracellular pathogens and cancer. By contrast, the cDC2 subset mainly presents soluble antigens to $CD4^+$ T cells, thereby regulating immune responses to extracellular pathogens, parasites and allergens[2]. Plasmacytoid DCs (pDCs) are well established as a source of type I interferon during viral infections. However, the ontogeny and function of pDCs remain active areas of debate[3]. Genetic models of cellular functions are most developed for cDCs, and so this Review does not cover pDC function in great depth. Here, we review genetic models and in vivo descriptions that have led to the identification of human and mouse DCs and their progenitors, and the use of these models to advance our understanding of DC functions.

Transcriptional regulation of development

The specification of DC progenitors to discrete DC subsets occurs after the restriction of multipotent progenitors (MPPs) to the myeloid or lymphoid lineage[4]. Early work identified progenitors of the myeloid lineage that have DC, monocyte, macrophage and granulocyte potential. Recently, multiple DC-restricted progenitors have been identified that can give rise to the major DC subsets[5–8]. In this section, we review the current models of monocyte and DC development in mice (FIG. 1). These mouse models, in turn, provide a framework to highlight similarities and differences between mouse and human DC ontogeny[9].

Lineage restriction of multipotent progenitors. The primary function of haematopoietic stem cells (HSCs) in mice and humans is to generate all blood cell types. The capacity of HSCs to generate blood cells must be balanced by mechanisms to maintain stemness during an entire lifespan[4,9,10]. In the lifetime of a mouse, HSCs divide only a few times, giving rise to MPPs that are highly proliferative and populate the entire haematopoietic system[11]. Although HSCs can theoretically give rise to all haematopoietic lineages, methods of genetic barcoding and indexed cell sorting have uncovered patterns of bias towards one lineage or another, known as lineage imprinting or transcriptional priming[12–14]. So far, evidence for imprinting or priming of HSCs is mainly limited to correlations of transcriptional signatures with the developmental fate of individual clones. In the case of DC development, it has

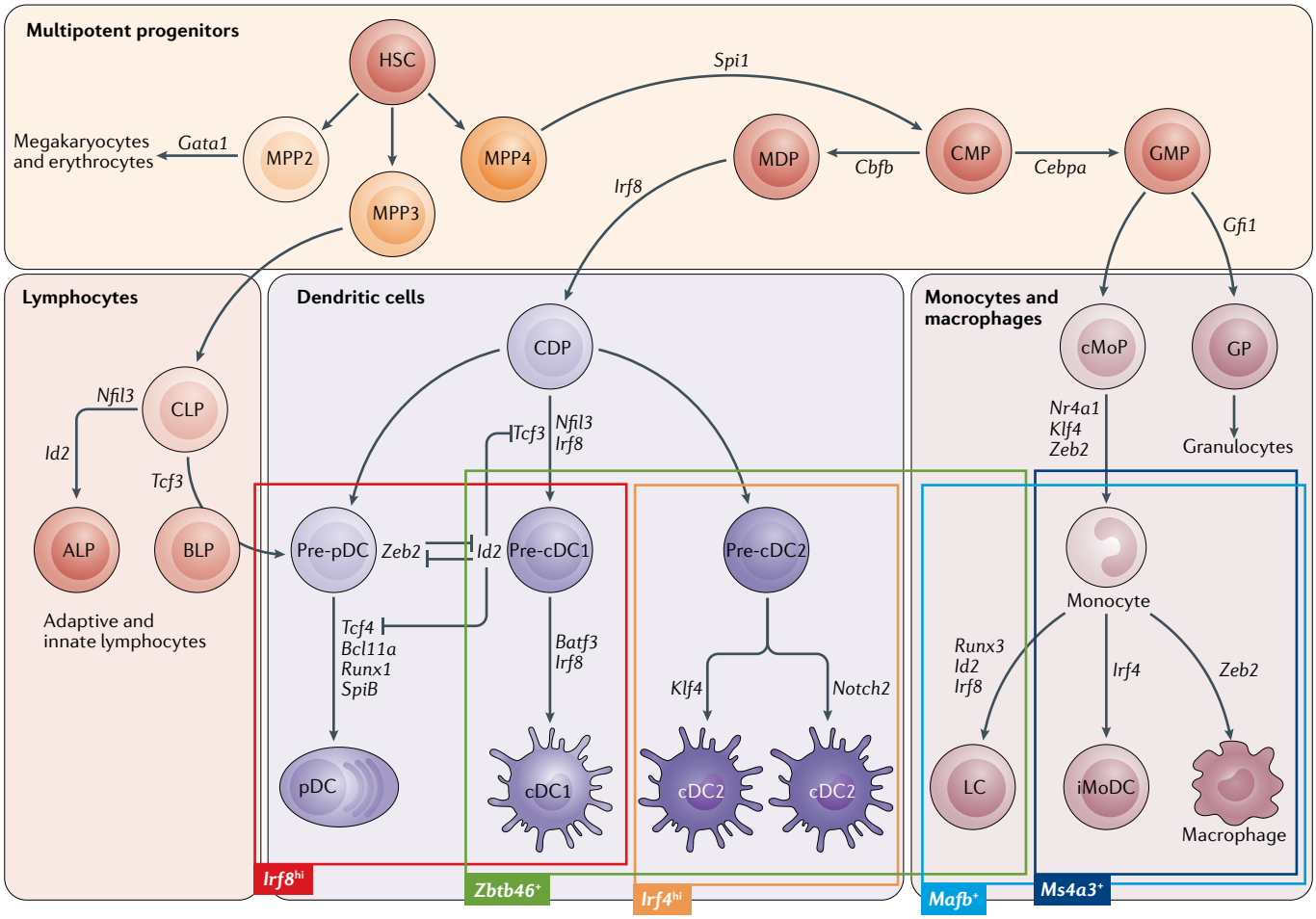

Fig. 1 | **Genetic models of mouse dendritic cell development and lineage restriction.** Hierarchical models of haematopoiesis are based on the developmental deficiencies that are observed in mice with mutations in transcription factor-encoding genes; these genes are shown adjacent to the associated stages of lineage restriction and specification. The figure highlights the development of mouse dendritic cell (DC), monocyte and macrophage subsets from shared progenitors. Haematopoietic stem cell (HSC)-derived multipotent progenitors (MPPs) undergo stages of differentiation to produce lineage-restricted progenitors of lymphocytes and myeloid cells — common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). The CLP population can be separated into two subsets on the basis of Ly6D expression. The all-lymphoid progenitor (ALP) is $Ly6D^-$ and has B cell, T cell and innate lymphoid cell potential. The *Tcf3*-dependent B cell-biased lymphoid progenitor (BLP) is $Ly6D^+$ and gives rise to B cells and plasmacytoid DCs (pDCs). High levels of expression of interferon regulatory factor 8 (*Irf8*) are associated with the specification of pDCs and type 1 classical DCs (cDC1s). Expression of *Zbtb46* is a specific marker of cDC specification that is induced first in pre-cDC1s and precursor type 2 classical DCs (pre-cDC2s). cDC2 subsets are also characterized by high levels of *Irf4* expression. Langerhans cells (LCs), monocytes and macrophages are all marked by *Mafb*-driven lineage tracing. Although LCs are not thought to be derived from the common DC precursor (CDP) population, given their embryonic origin, they do express *Zbtb46* when they migrate to lymphoid organs from the skin. Lineage tracing based on *Ms4a3* marks monocytes and monocyte-derived macrophages, as opposed to macrophages of embryonic origin. cMoP, common monocyte progenitor; GMP, granulocyte–macrophage progenitor; GP, granulocyte progenitor; iMoDC, immature monocyte-derived dendritic cell; MDP, monocyte–dendritic cell progenitor.

been suggested that specification occurs early in HSCs or MPPs that express high levels of interferon regulatory factor 8 (IRF8)[10,15]. However, although transcriptional signatures within early stem cells may correlate with granulocyte, monocyte or DC potential, they are not sufficient to recapitulate the chronologically ordered specification events that have been well established genetically across haematopoietic lineages[16,17]. Recently, single-cell analysis of clonal bias among sibling stem cells showed that they can have divergent developmental trajectories that are independent of transcriptional priming[18].

Lymphoid and myeloid lineage divergence. The common myeloid progenitor (CMP) and the common lymphoid progenitor (CLP) were initially described as cell populations that are committed to myeloid and lymphoid lineages, respectively[19,20]. Early models suggested that CMPs gave rise to granulocyte–monocyte progenitors (GMPs), which have lost megakaryocyte–erythroid progenitor (MEP) potential but can give rise to all myeloid lineages[21]. Deficiency in the transcription factor GATA1 results in embryonic lethality as a result of failed differentiation of MEPs, and PU.1 is expressed in progenitors that have excluded MEP potential[22,23]. Therefore, mutual repression between PU.1 and GATA1 was proposed as a mechanism to regulate the divergence of MEPs from CMPs. However, this remains an active area of debate because PU.1 is necessary for the development of both lymphoid and myeloid lineages, and so alone cannot enforce myeloid cell identity[24]. In addition, analysis of

Box 1 | **Lymphoid origin of plasmacytoid dendritic cells**

Early work on dendritic cell (DC) development suggested that classical DCs (cDCs) and plasmacytoid DCs (pDCs) could develop from both the common lymphoid progenitor (CLP) and the common myeloid progenitor[30,230–232]. Evidence reported over the past two decades, such as *Rag1* expression, *Il7ra* expression and V(D)J recombination during pDC development, continues to support a lymphoid origin of pDCs[216,230,233–235]. As classically defined, both CLPs (Lineage$^-$CD135$^+$CD115$^-$CD127$^+$) and common DC precursors (CDPs; Lineage$^-$CD135$^+$CD117^{int}CD115$^+$CD127$^-$) can give rise to pDCs[20,41,232,234]. This is supported by lineage tracing driven by expression of the lymphoid and myeloid markers, *Il7ra* and *Csf1r*, respectively[216,219]. pDCs are marked in both models in vivo.

Recently, single-cell RNA sequencing analysis revealed heterogeneity within populations of CLPs and CDPs that was used to identify clonogenic progenitors of pDCs, referred to as pre-pDCs[5,7,236]. One study found that bone marrow progenitors that represent a fraction of the CLP population (Lineage$^-$CD127$^+$CD135$^+$CD117$^{int/low}$ Ly6D$^+$SiglecH$^+$) give rise exclusively to pDCs[7]. An independent group reported similar pDC potential within the Ly6D$^+$ CLP population and could exclude pDC potential from the CDP population on the basis of CD81 expression[5]. The former group suggested that CDPs give rise to a distinct subset of *Zbtb46*–GFP-expressing pDCs in the bone marrow and spleen with functional characteristics of cDCs[7]. This conclusion is limited by the reliance on co-expression of *Zbtb46* with canonical pDC markers that are known to be expressed by all DC progenitors at different stages[8]. Genetic models of pDC development, such as *Bcl11a* and *Zeb2* deficiency, could help to resolve the ontogeny of pDCs in vivo[55,56,79]. Loss of pre-pDCs from both CLP and CDP populations in *Zeb2*-deficient mice, for example, could indicate that there is no genetic basis to model pre-pDCs as two ontogenetically unrelated populations. Alternatively, examination of *Tcf3*-deficient mice, which have a reduction in the Ly6D$^+$ fraction of the CLP population, could support such a distinction[237].

PU.1 and GATA1 protein expression did not identify a population that co-expresses these factors[25,26]. With the recent discovery that MEPs can be derived directly from a biased MPP population, mutual repression between PU.1 and GATA1 may not be necessary to explain the divergence of MEPs from a myeloid-restricted progenitor such as the CMP[9,27].

Although the development of lymphoid lineages is beyond the scope of this Review, some discussion of the CLP is relevant to observations that DCs can also be derived from populations that are classically defined as lymphoid-restricted progenitors. CLPs induce expression of the transcription factors GATA3, TCF1 (also known as TCF7) and BCL-11B, and develop into T cells after encountering Notch ligands in the thymus. By contrast, an absence of Notch signalling in the bone marrow leads to the development of B cells, which requires the induction of TCF3 (also known as E2A), EBF1 and PAX5 (REF.[17]). pDCs have been reported to develop from a fraction of the CLP population that has excluded T cell potential but retained B cell potential (BOX 1).

Restriction of granulocyte potential. GMPs were originally proposed to give rise to monocyte–DC progenitors (MDPs), which lack granulocyte potential[28–30]. However, this model requires revision in the light of recent findings by multiple groups. Single cells within the GMP and MDP populations were shown to have surface marker heterogeneity that correlates with fate bias[30–33]. These studies suggest that monocytes and granulocytes can develop from intermediate progenitors in the bone marrow that do not retain DC potential. A restricted granulocyte progenitor and a common monocyte progenitor (cMoP) are now proposed to develop from a common GMP-like progenitor. A GMP lineage tracing model based on restricted expression of *Ms4a3* identified granulocyte progenitors and cMoPs as GMP-derived populations[31]. However, a small percentage of monocytes in this study were untraced, which is consistent with the results of other groups that suggest monocytes can be derived from a progenitor not shared with granulocyte progenitors[33]. Lineage tracing driven by *Mafb* expression also supports a model whereby monocytes and macrophages are separate lineages from DCs and granulocytes[34]. *Cbfb* and *Cebpa* are required for monocyte and granulocyte development, respectively[35,36]. CBFβ has been shown to support the expression of IRF8, which is in turn associated with the exclusion of granulocyte potential[35,37]. Deletion of *Irf8* results in unrestrained C/EBPα activity, a myeloproliferative disorder of expanded granulocyte progenitor and granulocyte populations, and defective development of Ly6C$^+$ monocytes[37–39].

Dendritic cell specification. Initial work on the development of DCs from bone marrow progenitors identified a population referred to as the common dendritic cell precursor (CDP). Developing from MDPs, CDPs give rise to pDCs, cDC1s and cDC2s (REFS[40,41]). A mechanism to explain loss of monocyte and macrophage potential on transition from MDPs to CDPs has not so far been identified. Although CDPs are markedly reduced in *Irf8*$^{-/-}$ mice, pDCs and cDC2s can still develop despite significant surface marker, transcriptional and functional changes[42]. cDC1 and cDC2 subsets are functionally and transcriptionally more similar to each other than to pDCs[43]. Therefore, it was proposed that pDC progenitors diverge from CDPs before the specification of individual cDC subsets, which are derived from a putative precursor population known as pre-cDCs[44–46]. However, it has since been recognized that pre-cDCs are a heterogeneous population of cDC1-specified or cDC2-specified progenitors[6,8,44]. Pre-pDCs have also been identified recently and are thought to originate from CLPs as well as CDPs[5,7] (BOX 1). After specification in the bone marrow, cDC-restricted progenitors may continue to undergo additional rounds of cell division in lymphoid organs and peripheral tissues, ultimately exiting the cell cycle on activation or homeostatic maturation[46,47].

Mutual repression of cDC1 and pDC specification. Recent advances have closely linked the genetic mechanisms of cDC1 and pDC specification. Development of cDC1s from bone marrow progenitors requires *Irf8*, *Batf3*, *Id2* and *Nfil3* (REFS[48–50]). Deficiencies in *Batf3*, *Id2* or *Nfil3* can be rescued by the ectopic expression of *Irf8*, which indicates that high levels of IRF8 expression are central to the genetic programme that controls cDC1 development[51,52]. Steady-state cDC1 development is impaired in *Batf3*$^{-/-}$ mice after specification occurs in the bone marrow as a result of insufficient maintenance of IRF8 expression levels[6,53] (BOX 2; FIG. 2).

Models of cDC1 development have been further expanded by a recent study that examined heterogeneity in the CDP population on the basis of in vivo expression of *Zbtb46*, *Id2*, *Nfil3* and *Zeb2* (REF.[54]). In the bone marrow, *Zbtb46*–GFP expression within the CDP population

Lineage tracing
A method to identify cells that are expressing or have expressed a gene of interest at any point during their development, which enables the study of progenitor–progeny relationships.

Box 2 | Regulation of dendritic cell development by enhancer switching

Enhancers surrounding transcription factor-encoding genes are dynamically regulated during haematopoiesis, which is necessary for lineage divergence and the maintenance of cell identity[238]. Lineage-defining transcription factors can enforce cellular identity through autoactivation, a mechanism by which a factor positively regulates its own expression. One example of a transcription factor that uses autoactivation during myeloid cell development is PU.1 (encoded by *Spi1*), which was shown to bind to an enhancer 14 kb upstream of *Spi1* and increase its own expression by 100-fold[239]. Reminiscent of this model of PU.1 autoactivation, enhancers of interferon regulatory factor 8 (*Irf8*) were recently shown to mediate IRF8 autoactivation in cooperation with BATF3 (REF.[6]). Close examination of IRF8-binding sites in the *Irf8* locus revealed three novel enhancers that are +41 kb, +32 kb and −50 kb from the transcription start site and are necessary to maintain high levels of *Irf8* expression[53]. Germline deletion of the +41-kb enhancer resulted in reduced *Irf8* expression in the monocyte–dendritic cell progenitor population and in plasmacytoid dendritic cells (pDCs), and blocked precursor type 1 classical dendritic cell (pre-cDC1) specification in the common dendritic cell precursor population. It was proposed that early activation of this enhancer depends on TCF3 (also known as E2A), which is consistent with TCF3 deficiency resulting in a failure of pDC and cDC1 development. The +32-kb enhancer is active in cDC1s and is bound by BATF3 but not TCF3. This suggested that a switch from IRF8–TCF3-mediated autoactivation at the +41-kb enhancer to IRF8–BATF3-mediated autoactivation at the +32-kb enhancer occurs on cDC1 specification. Pre-cDC1s develop normally in *Irf8* $+32^{-/-}$ and *Batf3*$^{-/-}$ mice, but a failure to maintain high levels of IRF8 results in cDC1 deficiency in secondary lymphoid organs and peripheral tissues. Although deletion of the −50-kb enhancer had no effect on DC development, it resulted in reduced IRF8 levels and functional defects in monocytes. Studies of autoactivation by lineage-defining transcription factors have thus revealed novel mechanisms by which non-coding genetic elements may regulate haematopoiesis[240].

marks a $CD11C^{-}$MHC class $I^{-}CD117^{int}$ population of cDC1-specified progenitors, which give rise to a previously defined $CD11C^{+}$MHC class $I^{int}CD117^{int}$ pre-cDC1 population[6,8]. This early pre-cDC1 population expresses increased levels of *Id2* and *Nfil3* and decreased levels of *Zeb2* when compared with bulk CDPs, and its development depends on *Id2* and *Nfil3*. These results revealed the stage at which these factors function during cDC1 development. When cultured ex vivo, $ZEB2^{low}$ CDPs are biased towards cDC1s, which suggests that cDC1 specification involves the inhibition of *Zeb2* (REF.[54]). Because ZEB2 regulates pDC development, it was hypothesized that early induction of *Nfil3* functions to block pDC potential[54–56]. In support of this hypothesis, pre-cDC1s are restored in *Nfil3*$^{-/-}$*Zeb2*$^{-/-}$ mice, which shows that the functions of NFIL3 are dispensable in the absence of ZEB2 (REF.[54]).

The E protein transcription factor TCF4 (also known as E2-2) is also required for pDC development[57,58]. ID2 heterodimerizes with E proteins to block their activity by preventing binding to DNA[59]. This early observation is the basis for the hypothesis that ID2 functions during cDC1 specification to inhibit TCF4, thus blocking pDC development. Recently, it was shown that *Id2* expression marks a subset of pre-cDC1s in the CDP population, further suggesting that it functions during cDC1 development to block E protein activity and pDC potential[54]. Therefore, it was hypothesized that *Id2* would be dispensable for cDC1 development in a genetic background where pDC specification cannot occur. Accordingly, cDC1 development occurs normally in *Id2*$^{-/-}$*Zeb2*$^{-/-}$ mice[54]. Although cDC2s also express *Id2* and *Zeb2*, they do not require these genes for development. To shed light on the potential gene targets of ZEB2, whole transcriptome analysis of *Zeb2*-deficient cDC2s revealed increased expression of *Id2*, which suggests that ZEB2 might directly inhibit *Id2* transcription[54–56].

Human progenitors of dendritic cells and monocytes. The study of patients with combined immunodeficiencies has provided insight into the genes that regulate human haematopoiesis. For example, deficiency in GATA2 has been associated with a human syndrome known as DC, monocyte, B cell and natural killer cell lymphoid (DCML) deficiency[60]. It is not clear whether GATA2 functions in a shared progenitor of these lineages or whether it functions independently after the divergence of myeloid and lymphoid lineages. Humans with *IKZF1* haploinsufficiency have a deficiency in pDC development that correlates with an increase in the size of cDC1 populations[61]. It has been suggested from a survey of chromatin immunoprecipitation followed by sequencing data that IKZF1 represses *ID2*, thereby promoting TCF4 activity and pDC specification[62]. Mutations in *TCF4* are associated with a deficiency in pDC development in patients with Pitt–Hopkins syndrome[57]. Similar to the broad defects in the haematopoietic compartment that are associated with *Irf8* deficiency in mice, several reports have linked human mutations in *IRF8* to DC deficiencies, as well as defects in the development and function of granulocytes, B cells, T cells and natural killer cells[63–65].

Several human counterparts of the mouse DC and monocyte progenitors have been identified on the basis of surface marker expression and developmental potential (TABLE 1). Although human DCs can develop from CMPs, early work suggested that human DCs can also be derived from CLPs[66–69]. This led to the conclusion that human DC ontogeny does not strictly conform to models of myeloid and lymphoid lineage divergence in mice[69–71]. A human CMP-derived granulocyte–monocyte–DC progenitor (GMDP) was recently reported to have combined potentials of mouse MDPs and GMPs[72]. Alternatively, heterogeneity in a GMDP-like population suggests that the DC potential of human GMPs is the result of contamination by a separate MDP population[73]. Human granulocyte progenitors, cMoPs and CDPs have also been identified but their ontogeny remains an active area of debate[72,74–77].

Outstanding questions in DC development. Compared with cDC1s, the genetic mechanisms that regulate pDC and cDC2 specification are less well understood. The recent identification of clonogenic pDC progenitors, known as pre-pDCs, provides a new population with which CLPs and CDPs can be compared to identify the stages at which TCF4, ZEB2 and BCL-11A function to regulate pDC development[5,7,55,56,78,79]. Mechanisms of cDC2 specification are poorly understood and so are not discussed in this Review. Although pre-cDC2s that exclude cDC1 and pDC potential have been identified in the bone marrow, no genes necessary for pre-cDC2 development have so far been identified[6,8]. Notwithstanding, several genes that regulate cDC2 subset diversification in lymphoid organs and peripheral tissues have been identified and are described later in this Review.

E protein
A member of a family of transcription factors, including TCF3 (also known as E2A), TCF4 (also known as E2-2) and TCF12 (also known as HEB), that are essential for the development of several haematopoietic lineages and that bind conserved DNA motifs known as E-boxes.

Functional specialization of DC subsets

Human and mouse DCs have been organized into functional subsets based on various lines of evidence. In mice, genetic models of ontogeny have aided in the unification of functionally redundant subsets across tissues that are otherwise distinct on the basis of surface marker expression[1] (TABLE 2). Similarly, these models have helped to separate phenotypically similar populations that have non-redundant functions. Advances along these lines have also been made for human DCs, in particular through the use of high-throughput single-cell analysis of transcriptomes and surface protein expression. However, independent groups have provided disparate definitions for seemingly overlapping human DC subsets, which necessitates a unification of the human DC subsets that have so far been described (TABLE 3). In this section, we review the diversity of human and mouse DC subsets, discuss the ontogenetic and functional similarities that are well established and highlight some unresolved differences (FIG. 3).

Non-redundant functions of cDC1s in mice. It has been appreciated for more than three decades that the DC lineage is specialized to prime antigen-specific T cells and that cDC1s are probably superior inducers of $CD8^+$ CTL responses in vivo[80,81]. Initial work carried out ex vivo showed that cDC1s mediate CTL responses through a process known as cross-priming or cross-presentation, which involves the uptake of exogenous, cell-associated antigens, loading of processed peptides onto MHC class I molecules and their presentation to $CD8^+$ T cells in lymphoid tissues[82–84]. The first in vivo demonstration that cDC1s mediate CTL responses was accomplished through the analysis of *Batf3*$^{-/-}$ mice. These mice have a deficiency in cDC1 development, which results in diminished CTL responses, susceptibility to viral infections and uncontrolled tumour growth[49]. This essential function of *Batf3*-dependent cDC1s in inducing CTLs has since been shown in a wide variety of model systems by numerous independent groups[85–91].

With regard to the use of *Batf3*$^{-/-}$ mice as a genetic model to define the non-redundant functions of cDC1s in vivo, multiple groups have described a phenomenon in which cDC1 development can be restored, albeit partially, in some tissues of *Batf3*$^{-/-}$ mice. Infection with mycobacteria, IL-12 administration and irradiation can restore cDC1 development through compensation by other BATF factors, such as BATF and BATF2 (REFS[51,92]). Deletion of an enhancer bound by BATF3 blocks this compensation, suggesting that BATF and BATF2 function to support *Irf8* expression at this site in the absence of BATF3 (REF.[53]) (BOX 2). By restoring cDC1 populations in *Batf3*$^{-/-}$ mice through transgenic overexpression of *Irf8*, it was recently shown that a restricted set of genes regulated by BATF3 is required for cDC1-intrinsic rejection of immunogenic tumours[52,93]. The genetic targets of BATF3 that regulate cDC1 identity and function remain an active area of investigation.

cDC1s can regulate innate immune responses through functions that are independent of cross-presentation to CTLs. cDC1s uniquely express Toll-like receptor 11 (TLR11), a pattern recognition receptor for the *Toxoplasma gondii* antigen profilin[43]. Early work identified IL-12 as a target of signalling through TLR11, and later analysis of *Batf3*$^{-/-}$ mice showed that cDC1s are a non-redundant source of IL-12 necessary to mediate resistance to acute infection with *T. gondii*[94]. It has been

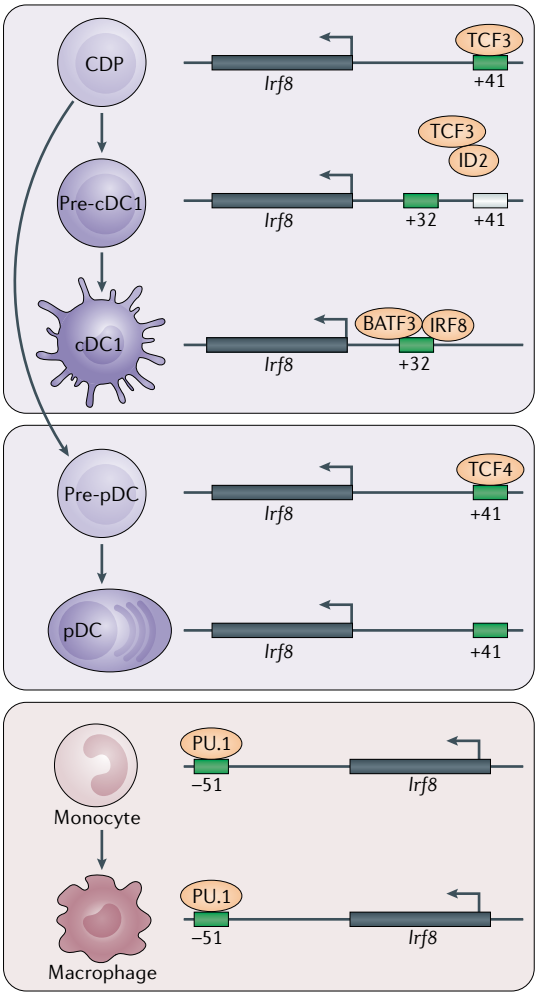

Fig. 2 | **Stage-specific enhancer activation regulates *Irf8*-dependent specification of dendritic cell and monocyte progenitors.** One mechanism for the development of type 1 classical dendritic cells (cDC1s) and plasmacytoid DCs (pDCs) from bone marrow progenitors is the strict regulation of interferon regulatory factor 8 (*Irf8*) expression by distal, evolutionarily conserved enhancer elements. E proteins — such as TCF3 (also known as E2A) and TCF4 (also known as E2-2) — support *Irf8* expression through their actions at an E-box motif-containing enhancer located 41 kb upstream of the *Irf8* transcription start site. An inactivating mutation at this locus impairs *Irf8* expression in pDCs and blocks the specification of precursor cDC1s (pre-cDC1s) in the common DC precursor (CDP) population. On pre-cDC1 specification, *Nfil3* is induced upstream of *Id2*. By blocking E protein activity, ID2 imposes a requirement for an alternative enhancer located 32 kb upstream of *Irf8* to promote *Irf8* expression. In turn, *Irf8* expression and cDC1 specification are supported by BATF3 and by IRF8-dependent autoactivation. An alternative enhancer located 51 kb downstream of the *Irf8* locus is required for *Irf8* expression regulated by PU.1 in monocytes and macrophages.

Table 1 | **Markers of human and mouse dendritic cell and monocyte progenitors**

Progenitor population	Human cell surface markers	Mouse cell surface markers
Pre-cDC1	CD135, CD117, CD116, CD123$^{low/int}$, CD45RA, CADM1 (Lineage negative[a])	CD135, CD115, CD117^{int}, CD11C, CD226, MHC class II$^{low/int}$ (Lineage negative[b,c])
Pre-cDC2	CD135, CD117, CD116, CD123^{low}, CD45RA, CD172^{hi}, CD1C (Lineage negative[a])	CD135, CD117^{low}, CD11C, Ly6C, ZBTB46 (Lineage negative[b,c])
Pre-cDC	CD135, CD117, CD116, CD123, CD45RA, CD172^{int}, CD22 (Lineage negative[a])	CD11C, CD135, CD172^{int} (Lineage negative[b,c])
CDP	CD34, CD38, CD135, CD117, CD116, CD123^{hi}, CD45RA (Lineage negative[a])	CD135, CD115, CD117^{int} (Lineage negative[b,d])
MDP	CD34, CD38, CD135, CD117, CD115, CD123 (Lineage negative[a])	CD135, CD115, CD117^{hi} (Lineage negative[b,d])
cMoP	CD34, CD38, CD45RA, CD135, CLEC12A, CD64	CD135^{-}, CD117, CD115, Ly6C (Lineage negative[b,d])
GMDP/GMP	CD34, CD38, CD135, CD117, CD123^{int} (Lineage negative[a])	CD117, CD34^{-}, CD16/CD32^{low} (Lineage negative[b,d])
CMP	CD34, CD38, CD135 (Lineage negative[a])	CD117, CD34, CD16/CD32^{low} (Lineage negative[b,d])
CLP	CD34, CD45RA, CD10 (Lineage negative[a])	CD135, CD127, CD117$^{int/low}$ (Lineage negative[b,d])
Pre-pDC	ND	CD135, CD117$^{int/low}$, CD127, Ly6D, SiglecH, CD81 (Lineage negative[b])

cDC, classical dendritic cell; cDC1, type 1 classical dendritic cell; cDC2, type 2 classical dendritic cell; CDP, common dendritic cell precursor; CLP, common lymphoid progenitor; cMoP, common monocyte progenitor; CMP, common myeloid progenitor; GMDP, granulocyte–monocyte–dendritic cell progenitor; GMP, granulocyte–monocyte progenitor; MDP, monocyte–dendritic cell progenitor; ND, not determined; pDC, plasmacytoid dendritic cell. [a]Lineage negative for CD3, CD19, CD20, CD56, CD14, CD66B, CD303, CD335, CD10, CD123, CD1C. [b]Lineage negative for CD3, CD19, Ly6G, Ter-119, NK1.1, CD105, B220, MHC class II. [c]Lineage negative for SiglecH, CD127. [d]Lineage negative for CD5, CD11B, CD8α, CD4, CD127.

shown that IL-12 produced by cDC1s activates the production of interferon-γ (IFNγ) by natural killer cells, which in turn primes regulatory functions in developing monocytes[95]. TLR3 is another pattern recognition receptor that is selectively expressed by the cDC1 subset[43]. Stimulation of TLR3 with double-stranded RNA, including the model antigen poly(I:C), is sufficient to induce cDC1 maturation and promote antiviral and antitumour immune responses[96,97].

Multiple models of conditional gene deletion have been developed to study the in vivo functions of cDC1s. Among these, the deletion of genes on the basis of *Xcr1* expression is the most recently developed model and provides the best specificity for cDC1s (REFS[98,99]). XC-chemokine receptor 1 (XCR1) is expressed exclusively by the cDC1 subset in mice and humans[86,100–102]. These models have been used most recently to identify novel innate immune functions of cDC1s. For example, specific deletion of *Vegfa* in cDC1s results in decreased numbers of infiltrating neutrophils and reduced skin inflammation at sites infected with *Propionibacterium acnes*[103]. By contrast, CLEC9A (also known as DNGR1) was shown to inhibit the production of CXC-chemokine ligand 2 (CXCL2) by *Batf3*-dependent cDC1s, thus limiting inflammation associated with neutrophil recruitment[104]. These studies have highlighted previously unknown crosstalk between cDC1s and neutrophils, whereby the regulatory functions of cDC1s seem to be context dependent.

Several cell surface receptors have been identified as being necessary for the regulation of adaptive immune responses by cDC1s. XCR1 and its ligand XCL1 are required for the appropriate localization of cDC1s and CD8$^+$ T cells within lymphoid organs. Mice deficient in *Xcr1* or *Xcl1* have marked reductions in size and altered cellular localization of CD8$^+$ and CD4$^+$ T cell populations in the spleen, lymph nodes and intestines both at steady state and during inflammation[99,102,105]. Although its expression is not unique to the cDC1 subset, the receptor LY75 (also known as DEC-205) has been targeted to deliver antigens to cDC1s to improve T cell responses and can also function as a receptor to internalize CpG nucleotides for recognition by intravacuolar TLR9 (REFS[106–108]). CLEC9A has also been targeted for antibody-mediated delivery of model antigens to cDC1s, which can improve the efficacy of vaccines against model tumours and viral infections[109–111]. Similar strategies have been applied to target antigens to cDC1s through XCR1 (REFS[112,113]).

Mouse models of cDC1 function have highlighted the potency of this cell type in modulating cancer immunotherapy through the activation of tumour-specific CD8$^+$ T cells. Therapeutic responses to checkpoint blockade in mice involving antibodies to TNFRSF9 (also known as CD137), PD1, PDL1 or CTLA4 require *Batf3*-dependent DCs[88,114,115]. Similarly, certain types of adoptive T cell therapy require cDC1s to induce tumour rejection[89]. Intratumoural injection of adjuvants can lead to a reduction in tumour mass and promote tumour rejection in a *Batf3*-dependent manner[116–118]. Similarly, administration of the growth factor FLT3L expands DC populations and promotes cDC1-mediated tumour rejection when combined with immunotherapy[119–121]. Intratumoural administration of XCL1 has also been shown to promote tumour rejection[122]. This is in line with observations in mice that natural killer cells recruit cDC1s to tumours through expression of XCL1 (REF.[123]).

Despite compelling evidence that cDC1s are the main cell type that mediates cross-presentation of cell-associated antigens in vivo, most studies of the molecular mechanisms of cross-presentation have been carried out using DCs derived in vitro from monocytes and bone marrow with granulocyte–macrophage colony-stimulating factor (GM–CSF) and IL-4 (known as GMDCs)[124]. Although these studies have been useful to advance our understanding of antigen processing and presentation, they do not necessarily recapitulate the cell-intrinsic functions of cDC1s in vivo (BOX 3). Therefore, we limit discussion here to a few recent studies that have identified genes that regulate cross-presentation by cDC1s in vivo. It was recently shown that an integral membrane protein, WDFY4, is specifically expressed by cDC1s and is required for the cross-presentation of cell-associated antigens to CD8$^+$ T cells. cDC1s develop normally in *Wdfy4*$^{-/-}$ mice, but these mice have many of the same immunological defects as *Batf3*$^{-/-}$ mice[125]. It was hypothesized that WDFY4

Checkpoint blockade
A type of immunotherapy that inhibits immune signalling cascades that are normally engaged to prevent autoimmunity and uncontrolled inflammation but that can also prevent effective immune responses to cancer, for example.

Type II immune responses
On recognition of parasites or activation by allergens, cytokines such as IL-4, IL-5, IL-13 and IL-10 are produced, and naive CD4+ T cells are polarized to T helper 2 cells.

Type III immune responses
On recognition of extracellular bacterial pathogens, cytokines such as IL-6, IL-17, IL-21, IL-22, IL-23 and transforming growth factor-β are produced, and naive T cells are polarized to T helper 17 cells.

regulates vesicular trafficking necessary for antigen processing, but the large size of the *Wdfy4* gene has so far limited genetic, biochemical and structural analyses of its function. Another protein that is putatively involved in vesicular trafficking and that is required for cross-presentation by cDC1s in vivo is RAB43 (REF.[126]). Notably, GMDCs derived from *Rab43*$^{-/-}$ mice have no defect in cross-presentation, which further underscores the importance of distinguishing cross-presentation by cDC1s from that by GMDCs. Along these lines, the vesicular trafficking protein SEC22B was initially studied in GMDCs and shown to be required for cross-presentation through the regulation of phagosome maturation[127]. Conflicting results between groups have made it difficult to establish whether SEC22B is essential for cross-presentation in vivo, but preliminary results suggest that it may be necessary for antitumour immune responses[128,129].

Non-redundant functions of cDC2s in mice. Multiple functional subsets of cDC2s have been identified, all of which are characterized by high relative levels of IRF4 expression[130]. cDC2s are an ontogenetically separate fraction of cDCs with subset-specific functions that are unable to compensate for cDC1 deficiencies. Here, we review various cDC2-intrinsic genetic deficiencies that have aided in the separation of cDC2s into discrete subsets. The broad functions of cDC2s have been revealed through the analysis of *Irf4*-deficient mice. A major caveat of this model is that it differentially affects cDC2 subsets in a tissue-specific manner[131,132]. Notwithstanding this caveat, extensive analysis of the immunological defects associated with *Irf4* deficiency has highlighted the importance of cDC2s in the regulation of type II immune responses to allergens and parasites, type III immune responses to extracellular pathogens and the gut microbiota, and humoral immune responses to blood-borne antigens[133–139].

Table 2 | **Useful models for the definition of dendritic cell ontogeny and function**

Gene	Expression by				Genetic mouse model					Refs
	cDC1s	cDC2s	pDCs	DC progenitors	Reporter	Knockout	Floxed	Cre recombinase	Diphtheria toxin receptor	
Markers										
Itgax	+	+	+	+	No	No	No	Yes	Yes	198–203
Clec9a	+	–	+	+	Yes	Yes	No	Yes	Yes	45,204
Cd207	+	+	–	–	No	No	No	Yes	Yes	205–208
SiglecH	–	–	+	+	No	No	No	Yes	Yes	204,209,210
Xcr1	+	–	–	–	Yes	Yes	Yes	Yes	Yes	99,211
Karma/Gpr141b	+	–	–	–	No	No	No	No	Yes	212
Clec4a4	–	–	–	–	No	No	No	No	Yes	213
Mgl2	–	+	–	–	No	No	No	No	Yes	134
Clec4c	–	–	+	–	No	No	No	No	Yes	152
Cx3cr1	–	+	–	+	Yes	Yes	No	No	Yes	214,215
IL7r	–	–	–	+	Yes	Yes	Yes	Yes	No	216
Csf2r	–	–	–	+	Yes	Yes	Yes	Yes	No	217–219
Transcription factors										
Zbtb46	+	+	–	+	Yes	Yes	No	Yes	Yes	6,179,220
Batf3	+	+	–	+	No	Yes	No	No	No	49
Tcf3	–	–	–	+	Yes	No	Yes	No	No	221
Tcf4	–	–	+	+	No	No	Yes	No	No	57
Irf4	–	+	–	–	Yes	Yes	Yes	No	No	222
Nfil3	+	–	–	+	No	Yes	Yes	No	No	50,223
Id2	+	+	–	+	No	No	Yes	No	No	48,224,225
Klf4	–	+	–	–	No	No	Yes	No	No	144,226
Zeb2	–	+	+	+	No	No	Yes	No	No	55,56,227
Irf8	+	+	+	+	No	Yes	Yes	No	No	228,229
Irf8 + 32 kb	+	–	–	+	No	Yes	No	No	No	53
Irf8 + 41 kb	+	–	+	+	No	Yes	No	No	No	53

cDC1, type 1 classical dendritic cell; cDC2, type 2 classical dendritic cell; DC dendritic cell; pDC, plasmacytoid dendritic cell.

Table 3 | **Markers of human and mouse dendritic cell subsets**

DC subset	Human cell surface markers (Lineage negative[a,b]; Lineage positive[c])	Mouse cell surface markers (Lineage positive[d])
cDC1	XCR1, CD45, CADM1, CLEC9A, CD141	XCR1, CD24 (Lineage negative[b,e])
Context-dependent cDC1	CAMK2D, XCR1, CCR7, CD103, CD11C	CD103, CD207, CD326, CD8A, CLEC9A
cDC2	CD45, CD1C, FcεR1A, CD172A	CD172A (Lineage negative[b,e])
cDC2A	CD5	ESAM, CD4, CD103, CD11B
cDC2B	CD14, CD163	CD24, MGLL
Context-dependent cDC2	CCR7, CD103, CD11B, CD11C, CD80, CD86	CCR7, CD80, CD86, F4/80
pDC	CD45RA, CD123, CD2	B220, SiglecH, CD317, Ly6D, CCR9, Ly6C (Lineage negative[e])

Markers listed as 'context-dependent' can vary across tissues and physiological settings and so are separated from markers that generally have consistent patterns of expression for the given subsets. cDC1, type 1 classical dendritic cell; cDC2, type 2 classical dendritic cell; DC dendritic cell; pDC, plasmacytoid dendritic cell; XCR1, XC-chemokine receptor 1. [a]Lineage negative for CD3, CD19, CD20, CD56. [b]Lineage negative for B220, SiglecH, Bst2. [c]Lineage positive for HLA-DR, CD45. [d]Lineage positive for CD11C. [e]Lineage negative for CD3, CD19, Ly6G, Ter-119, NK1.1, CD64.

More recently, analysis of cDC-intrinsic transcription factor deficiencies has enabled functions to be assigned to discrete cDC2 subsets. *Notch2*-dependent cDC2s are found in the spleen, lung and gut-associated lymphoid tissue[140]. This subset was also shown to be dependent on *Ltbr* and *Rbpj*[141]. *Notch2*-dependent cDC2s in the gut regulate type III immune responses to extracellular pathogens, such as *Citrobacter rodentium*[140]. cDC2-intrinsic expression of IL-23 activates group 3 innate lymphoid cells, which in turn confer resistance to infection through the production of IL-22 and activation of gut epithelial cells[142]. In the spleen, a *Notch2*-dependent cDC2 subset surveys the circulation for soluble antigens and is required for germinal centre formation and antibody production. In the absence of this cDC2 subset, immunization with sheep red blood cells or heat-killed *Listeria* fails to induce the proliferation of T follicular helper cells or germinal centre B cells[143]. By contrast, the *Klf4*-dependent cDC2 subset migrates from barrier surfaces to draining lymph nodes, where these cells regulate type II responses to parasites and allergens[144].

Antitumour immune responses and the effects of checkpoint blockade require intact $CD8^+$ and $CD4^+$ T cell responses[114,145]. Although a role for cDC1-mediated antigen presentation on MHC class II molecules and priming of $CD4^+$ T cells has not been ruled out, non-redundant roles for cDC2s in priming $CD4^+$ T cells are well established[146]. Consistent with these observations, tumour antigen-bearing cDC2s isolated from the tumour microenvironment can prime antigen-specific $CD4^+$ T cells ex vivo. Ablation of $CD11B^+$ cDC2s in tumour-draining lymph nodes of *Cx3cr1*$^{LSL–DTR}$*Itgax*^{Cre} mice (in which diphtheria toxin treatment specifically depletes $CX_3CR1^+CD11C^+$ cells, which include $CD103^-CD11B^+$ cDC2s) results in reduced priming and differentiation of naive $CD4^+$ T cells[147]. To that end, various anticancer immunotherapies may require cDC2s for their efficacy, as reviewed recently by others[148].

Diversity and function of mouse and human pDCs. The non-redundant functions of mouse pDCs have been difficult to uncover owing to a lack of in vivo infection models that require pDCs for survival. Notwithstanding, descriptive studies of pDC activities during immune responses in mice have shown that they are poised for the recognition of viruses and production of type I interferon. The rapid response of pDCs to viral infection is enabled by their expression of pattern recognition receptors, such as TLR7 and TLR9, which recognize single-stranded RNA and CpG dinucleotides, respectively[149,150]. However, these receptors are also expressed by other immune cell lineages, such as monocytes, B cells and cDCs, which may provide a redundant source of innate recognition during viral infection[43]. It is unclear whether a redundant source of type I interferon exists in humans, as patients with Pitt–Hopkins syndrome have a deficiency in pDC development that correlates with impaired IFNα production[57].

Several models have been developed to specifically ablate pDCs (TABLE 2), which show that pDCs have a role in the control of acute and chronic viral infections. Conditional deletion of the transcription factor *Tcf4* in *Itgax*^{Cre} mice results in steady-state loss of pDCs in vivo[57]. Mice lacking pDCs in this model fail to control acute viral hepatitis or infection with chronic lymphocytic choriomeningitis virus[151]. A widely used model of pDC ablation is based on pDC-specific expression of the human gene encoding CLEC4C (also known as BDCA2 and CD303). Although this gene is not conserved in mice, transcriptional activation by its promoter drives pDC-intrinsic expression of diphtheria toxin receptor (DTR) when inserted as a transgene[152]. Several studies using this model of pDC deficiency have shown that pDCs contribute to the control of viral infection by regulating the population expansion of natural killer cells and antigen-specific CTLs and provide a local source of type I interferon that can induce cDC1 maturation[105,152,153]. *CLEC4C*-based or *Tcf4*-based models have provided strong evidence to validate long-standing claims that pDCs contribute to autoimmunity, most notably in models of systemic lupus erythematosus[154–157]. Unlike cDCs, which exit the cell cycle in peripheral tissues and secondary lymphoid organs, pDCs undergo terminal differentiation before exiting the bone marrow, which depends on the transcription factor RUNX2 (REFS[41,158,159]). The maintenance of pDC function as measured by type I interferon production is, in turn, regulated by IRF8 in terminally differentiated cells[42].

The role of cross-presentation by pDCs and translation of mouse models to human pDC function remain active and unresolved areas of debate[160]. In mice and humans, both cDC1s and pDCs have been shown to cross-present antigens[161–164]. However, in vivo immune responses in mice that require cross-presentation for survival do not depend on an intact pDC population[165]. Furthermore, when human and mouse cDC1s are compared side by side with pDCs, cDC1s are far superior in terms of their ability to cross-present cell-associated antigens to CTLs[100,166]. Reports that have shown the ability of pDCs to cross-present have used soluble peptides of varying lengths as model antigens[164,167]. It is known that

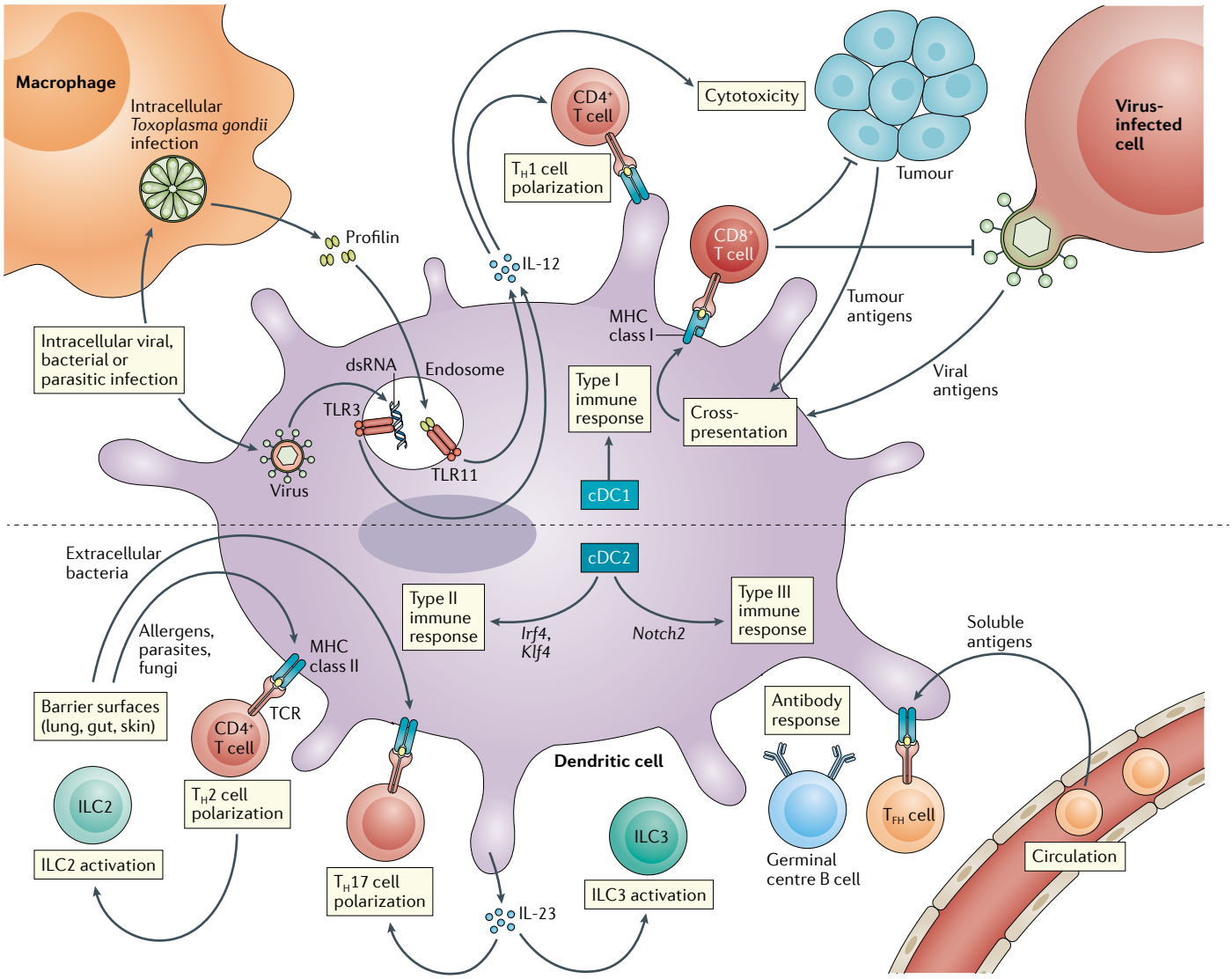

Fig. 3 | **Specialized functions of mouse classical dendritic cell subsets.** Type 1 classical dendritic cells (cDC1s) are specialized in the regulation of type I immune responses through the priming and activation of cytotoxic $CD8^+$ T cells and $CD4^+$ T helper 1 (T_H1) cells. Relative to other DC subsets, cDC1s specifically express Toll-like receptor 3 (TLR3) and TLR11, which recognize double-stranded RNA (dsRNA) and the *Toxoplasma gondii* antigen profilin, respectively. cDC1s are an essential source of IL-12 and are necessary for resistance to intracellular viral, bacterial and parasitic infections. cDC1s are uniquely capable of acquiring antigens associated with host cells, through a process known as cross-presentation, which is essential for pathogen clearance and antitumour immune responses. Type 2 classical dendritic cells (cDC2s) regulate type II and type III immune responses and antibody responses to soluble antigens. cDC2s at barrier surfaces, such as in the lung, gut and skin, regulate type II immune responses to parasites, fungi and allergens and are required for the expansion of $CD4^+$ T helper 2 (T_H2) cell populations and activation of group 2 innate lymphoid cells (ILC2s). The regulation of such responses depends on cDC-intrinsic expression of interferon regulatory factor 4 (*Irf4*) and has been attributed to the *Klf4*-dependent cDC2 subset. Type III immune responses are regulated by a distinct subset of *Notch 2*-dependent cDCs, which are a necessary source of IL-23 during acute infection with *Citrobacter rodentium*. IL-23 is necessary to activate group 3 innate lymphoid cells (ILC3s) and to induce differentiation of $CD4^+$ T helper 17 (T_H17) cells. cDC2s have also been shown to regulate antibody responses though the induction of germinal centre responses to soluble antigens in lymphoid organs. Deficiency in *Notch2*-dependent cDCs results in a failure to induce $CD4^+$ T follicular helper (T_{FH}) cells and germinal centre B cells in the spleen, for example. TCR, T cell receptor.

the molecular mechanisms for the cross-presentation of soluble antigens are distinct from those for the cross-presentation of cell-associated antigens. The latter requires direct transfer of antigens from infected, apoptotic or necrotic cells to the antigen-presenting cell, followed by processing and presentation[162]. Despite the controversies regarding non-redundant functions of pDCs, several groups are working to harness the ability of pDCs to produce type I interferon and internalize exogenous antigens for novel vaccines and immunotherapies[168–171].

Most recently, the discovery of heterogeneity in the classically defined human pDC population ($HLA\text{-}DR^+CD45RA^+CD123^+$) has called into question whether the functions that have been attributed to human pDCs could be the result of contamination with a functionally distinct DC subset with cDC-like characteristics. Two independent groups identified a

Box 3 | **Cross-presentation by BMDCs, GMDCs and MoDCs**

Significant effort has been made to identify the cell types and molecules that regulate cross-presentation. Whereas the focus of this Review is limited to the ontogeny and function of classical dendritic cells (cDCs) and plasmacytoid DCs, various cell types, such as monocytes and bone marrow-derived DCs (BMDCs), can be cultured in vitro with granulocyte–macrophage colony-stimulating factor (GM–CSF) alone or plus IL-4 to generate GMDCs with cross-presentation capacities[241,242]. Similarly, it has been suggested that monocytes can differentiate into DCs known as monocyte-derived DCs (MoDCs) that have the capacity to cross-present antigens in vivo[243]. However, when compared side by side with type 1 classical dendritic cells (cDC1s), GMDCs and MoDCs are phenotypically distinct and inferior at priming T cells[244]. Unlike cDC1s, they do not require *Batf3* for development but depend on alternative factors, such as *Irf4*, *Ahr* and *Mafb* in mice and humans[126,245–247]. The putative functions attributed to GMDCs, BMDCs and MoDCs have been recently reviewed by others and remain a matter of contentious debate[248,249]. Regardless of such controversy, monocytes remain a target antigen-presenting cell population for the development of novel immunotherapies[250].

DC population, referred to here as AS-DC, that was $AXL^+SIGLEC6^+CD123^+CD11C^{low}$ (REFS[172,173]). Another group identified a similar $CD33^+CX_3CR1^+$ population that was shown to contaminate classically defined pDCs[174]. This population had phenotypical similarities to a previously described human pre-DC progenitor, leading the last group to conclude that AS-DCs are actually DC progenitors with a phenotype similar to pre-cDCs[76,119]. When this population was excluded, functions that were formerly attributed to pDCs, such as priming of naive T cells and IL-12p40 production, could not be recapitulated[164,175]. Subsequently, a putative mouse homologue of the AS-DC was reported and named a transitional DC, although with limited genetic evidence to support its ontogeny[176]. Regardless of AS-DC ontogeny, the identification of phenotypical and functional heterogeneity within human pDC populations revives the question of whether they are a non-redundant subset of antigen-presenting cells.

Diversity and function of human cDC1s. Only one functional subset of cDC1s has so far been identified in mice and humans. Markers used to identify human cDC1s in vivo and in vitro include XCR1, CADM1, thrombomodulin (also known as CD141), CLEC9A and aminopeptidase N (also known as CD13). The expression of lineage-defining transcription factors is conserved between mouse and human cDC1s, including *IRF8*, *BATF3*, *ID2* and *ZBTB46* (REFS[174,177–180]). The requirement for IRF8 in cDC1 development and survival is evolutionarily conserved between mice and humans, as supported by the observation that rare mutations in the human *IRF8* locus result in cDC1 deficiency[64]. As discussed for mice, the effects of *IRF8* deficiency in humans extend beyond the cDC1 lineage, resulting in multilineage defects and immunodeficiency[63]. The ability to generate cDC1-like cells from human fibroblasts using a protocol that involves the overexpression of *SPI1*, *IRF8* and *BATF3* lends further support to the assertion that cDC1 development and ontogeny are conserved between mice and humans[181].

The major in vivo functions attributed to cDC1s in mice are consistent with the descriptions of human cDC1 activities so far reported, although there is some divergence in cytokine expression profiles. For example, several groups have observed that human cDC1s produce less IL-12 than cDC2s, which is the opposite to mouse models in which cDC1s have been shown to be the obligate source of IL-12 for certain infections[94,166,182,183]. However, there is no effect on the production of IL-12 by leukocytes from patients with *SPPL2A* mutations, who lack cDC2s (REF.[184]). This result suggests that IL-12 can be produced outside the cDC2 compartment. However, these patients have a defect in T helper 1 cell-mediated responses to mycobacteria, highlighting the possibility that cDC2s can regulate type I immune responses, a function that is restricted to cDC1s in mice[184]. The superior ability of cDC1s to cross-present cell-associated antigens seems to be conserved between mice and humans[86,100,182,185,186]. In both mice and humans, $CD8^+$ T cells and natural killer cells produce XCL1, which supports a role for $XCR1^+$ cDC1s in CTL responses[187]. This is consistent with observations in humans that the size of cDC1 populations positively correlates with CTL-dependent antitumour and antiviral immune responses[123,185,188–190].

Diversity and function of human cDC2s. Defining cDC2 subset diversity is complicated by the highly variable expression of surface markers across tissues in mice and humans. Heterogeneity in surface marker expression within human cDC2s has been dissected using high-dimensional flow cytometry and single-cell RNA sequencing by various groups. Subsets of human cDC2s can be identified using CD1A, CD1C, CD1D, CD172A, dipeptidyl peptidase 4 (also known as CD26), CLEC4C, FcεRIα, HLA-DQ and CD163 (REFS[130,172,174,191,192]). Multiple studies have documented overlapping surface phenotypes between cDC2s and monocytes, particularly for CD115, CD1C, CD14, CD64, CCR2 and CX_3CR1 expression[193]. Exclusive expression of C5AR1 (also known as CD88) and FcαR (also known as CD89) by human monocytes was recently shown to allow for their separation from human cDC2s, which express FcεRIα uniformly[191].

Although CD14 has been used as a lineage marker for monocytes, cDC2s were recently shown to include $CD14^+$ and $CD14^-$ subsets in some tissues[173,191]. Two subsets exist within the $CD14^-$ fraction of cDC2s on the basis of CD5 expression, referred to here as cDC2A ($CD5^+$) and cDC2B ($CD5^-$). Comparison of their transcriptomes indicates that cDC2A and cDC2B subsets correspond to populations that have been identified independently and named DC2 and DC3, respectively[173]. However, the extent to which these populations phenotypically and functionally overlap remains to be examined, and these studies do not provide sufficient evidence to determine the genetic basis for functional and phenotypical heterogeneity in human cDC2 subsets. It should be noted that the identity of a putative DC population, named DC4, has since been revised to a subset of $CD16^+$ monocytes[191,194]. Functionally, cDC2A and cDC2B subsets were equally capable of inducing type I responses ex vivo, as shown by IFNγ production and the proliferation of naive $CD4^+$ T cells[173]. cDC2As were poor inducers of type II and type III responses. Under inflammatory conditions, an additional $CD14^+CD163^+$ population clustered with $CD5^-$ cDC2Bs was identified and correlated with increased production of IL-4 and IL-17A by T helper 2 cells and T helper 17 cells, respectively[191].

Type I immune responses
On recognition of viral or intracellular bacterial pathogens, cytokines such as IL-2, IL-12 and interferon-γ are produced, and naive $CD8^+$ and $CD4^+$ T cells are polarized to cytotoxic T lymphocytes and T helper 1 cells, respectively.

These results are consistent with previous studies in mice and humans showing that cDC2s regulate type II and type III immune responses[195]. Although a specific cDC2 subset was not identified, human cDC2s can polarize naive $CD4^+$ T cells to a T follicular helper cell phenotype by direct priming and production of polarizing cytokines, such as transforming growth factor-β[196].

Recent work that compared the transcriptomes of cDC2s suggests that at least two subsets are ontogenetically conserved between mice and humans[197]. Reporter analysis of *Tbx21* (which encodes the transcription factor T-bet) and *Rorc* (which encodes nuclear receptor RORγ) expression revealed two cDC2 subsets that were phenotypically and functionally distinct with respect to their induction of polarizing cytokine production by naive $CD4^+$ T cells[197]. Notably, markers associated with *Notch2*-dependent and *Klf4*-dependent cDC2s in mice, such as ESAM and MGLL (also known as MGL2), are also expressed by the human cDC2A and cDC2B subsets, respectively[140,141,144]. NOTCH2 and KLF4 expression were recently reported to correlate with two subsets of IRF4-expressing human cDC2s, which were $CD1C^+CD14^-$ ($NOTCH2^{low}KLF4^{hi}$) and $CD1C^-CD14^+$ ($NOTCH2^{hi}KLF4^{low}$)[191]. However, more detailed analyses are required to establish functional differences between human cDC2s on the basis of NOTCH2 and KLF4 expression.

Conclusions

Advances in our understanding of DC development have been essential to the elucidation of specialized DC functions. There is significant overlap between humans and mice in terms of DC development, but caution must be used in interpreting results as the molecular mechanisms that regulate DC physiology may not be evolutionarily conserved. Along these lines, the discovery of human mutations in genes that regulate DC development and function has provided novel links to blood disorders, immunodeficiencies and autoimmunity[62]. Mouse models of DC development have established cDC1s as potent regulators of type I immune responses through interactions with the innate and adaptive immune systems. Therefore, the functions of cDC1s are central to the development of immunotherapies that modulate CTL responses to prevent and treat viral infections and cancer[148]. In mice and humans, cDC2s can regulate humoral, type I, type II and type III immune responses through antigen presentation and polarizing cytokine production. There are several differences between mice and humans in terms of the behaviour of cDC2s, particularly with respect to type I immune responses. Recent advances in the description of human cDC2 diversity should provide novel insights into the non-redundant functions of human cDC2s. Although the non-redundant functions of human and mouse pDCs and GMDCs remain controversial, substantial efforts have been made to harness their functions ex vivo. Continued research in the field of DC development and function should advance models of the underlying cellular and molecular mechanisms that are used by DCs to maintain homeostasis and regulate immune responses to diverse pathogens and cancers.

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