Review

THE EMBO JOURNAL

Dendritic cell maturation: functional specialization through signaling specificity and transcriptional programming

Marc Dalod[1,2,3,†], Rabie Chelbi[1,2,3], Bernard Malissen[1,2,3,†] & Toby Lawrence[1,2,3,*,†]

Abstract

Dendritic cells (DC) are key regulators of both protective immune responses and tolerance to self-antigens. Soon after their discovery in lymphoid tissues by Steinman and Cohn, as cells with the unique ability to prime naïve antigen-specific T cells, it was realized that DC can exist in at least two distinctive states characterized by morphological, phenotypic and functional changes—this led to the description of DC maturation. It is now well appreciated that there are several subsets of DC in both lymphoid and non-lymphoid tissues of mammals, and these cells show remarkable functional specialization and specificity in their roles in tolerance and immunity. This review will focus on the specific characteristics of DC subsets and how their functional specialization may be regulated by distinctive gene expression programs and signaling responses in both steady-state and in the context of inflammation. In particular, we will highlight the common and distinctive genes and signaling pathways that are associated with the functional maturation of DC subsets.

Keywords dendritic cells; homeostasis; immunity; tolerance
DOI 10.1002/embj.201488027 | Received 27 January 2014 | Revised 17 March 2014 | Accepted 19 March 2014 | Published online 15 April 2014
The EMBO Journal (2014) 33: 1104–1116

Dendritic cell heterogeneity

Dendritic cells are cells of the mononuclear phagocyte system and develop in the bone marrow from common DC precursors that give rise to plasmacytoid DCs (pDCs) and to intermediate cells known as pre-conventional DC (pre-cDC). After exiting the bone marrow, pre-cDC transiently circulate in the bloodstream and migrate into lymphoid and non-lymphoid tissues where they differentiate into cDC, pDC also enter tissues from the blood stream, constitutively in the case of secondary lymphoid organs but only upon inflammation in the case of non-lymphoid tissues. Analysis of secondary lymphoid organs (spleen, lymph nodes) and of non-lymphoid tissues (skin, intestine, lung, skeletal muscle and liver) has led to the identification of several distinct populations of cDC. Regardless of their anatomical location or species of origin, cDC can be grouped into two major subsets based on their phenotype, gene expression program, functional specialization and the transcription factors that specify their development (Guilliams *et al*, 2010) (Fig 1).

Xcr1$^+$ and CD11b$^+$ cDC

The expression of CD8α or CD103 at the cell surface was originally used to classify a subset of cDC, often referred to as CD8α-type cDC. However, CD8α expression does not constitute a "universal" marker of this subset and CD103 is also expressed by intestinal cDC belonging to a second subset known as CD11b$^+$ cDC (see below). In contrast, the chemokine receptor Xcr1 has been recently shown to be strictly specific for CD8α-type cDC (Bachem *et al*, 2012; Crozat *et al*, 2011). The C-type lectin Clec9a (also known as Dngr1) is also selectively expressed on CD8α-type cDC, but also on pDC and on a subset of DC progenitors (Schraml *et al*, 2013). On that basis, CD8α-type cDC will be denoted as Xcr1$^+$ cDC for the purpose of this review. The human DC subset often referred to as CD141 (BDCA3)$^+$ cDC, is the equivalent of mouse Xcr1$^+$ cDC (Robbins *et al*, 2008; Haniffa *et al*, 2012) and can also be identified by its specific expression of XCR1 (Bachem *et al*, 2010; Crozat *et al*, 2010; Yamazaki *et al*, 2010) and CLEC9A (Caminschi *et al*, 2008; Huysamen *et al*, 2008; Sancho *et al*, 2008).

The other major population of cDC are the CD11b$^+$ cDC. The phenotypic overlap between CD11b$^+$ cDC and other cells of the mononuclear phagocyte system—primarily monocyte-derived DC (moDC) and tissue-resident macrophages—has confounded their analysis. All cDC—including CD11b$^+$ cDC—have a short half-life (approximately 3–5 days in LT and slightly longer in NLT such as the lung or kidneys (Ginhoux *et al*, 2009)) and are continuously replaced from bone marrow progenitors in a manner that depends on the cytokine Flt3L, but that is independent of the CCR2 chemokine receptor. In contrast, the development and maintenance of moDC and tissue-resident macrophages from bone marrow progenitors occurs independently of Flt3L but requires CCR2 expression. Such distinctive developmental requirements allowed the

1 Centre d'Immunologie de Marseille-Luminy (CIML), Aix-Marseille University, UM2, Marseille, France
2 Institut National de la Santé et de la Recherche Médicale (INSERM), U1104, Marseille, France
3 Centre National de la Recherche Scientifique (CNRS), UMR7280, Marseille, France
*Corresponding author. Tel: +33 491269166; Fax: +33 491269430; E-mail: lawrence@ciml.univ-mrs.fr
†Senior authors sharing equal contribution.

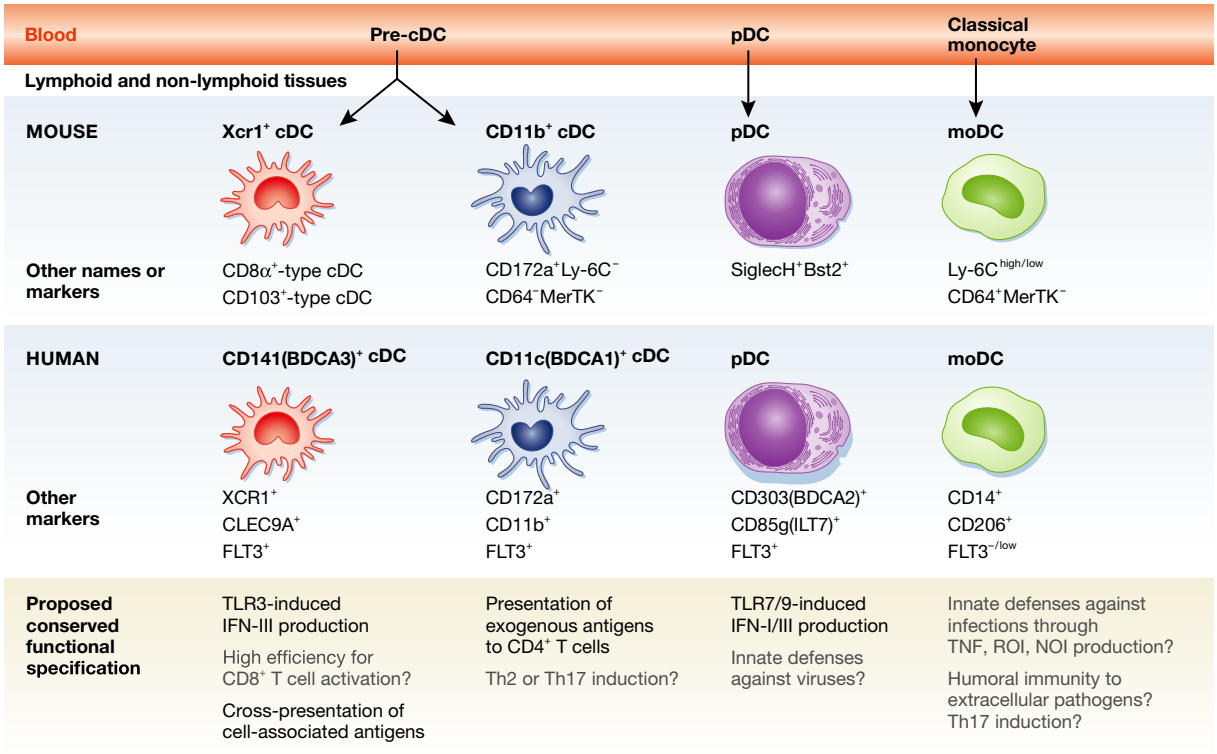


Figure 1. Major subsets of DC in mouse and human.
Human and mouse DC subsets can be aligned into four major subsets irrespective of their location in secondary lymphoid tissues or in the parenchyma of non-lymphoid organs. They correspond to Xcr1$^+$ cDC, CD11b$^+$ cDC, pDC and moDC. The precursors that are found in the blood and give rise to the four major DC subsets are shown. Alternative markers or names used to identify those subsets are also indicated, as well as the proposed conserved functional specialization of these subsets.

unambiguous identification of CD11b$^+$cDC as CD11b$^+$Ly6C$^-$ CD64$^-$ MerTK$^-$ cells, which distinguished them from moDC and macrophages (Gautier *et al*, 2012; Tamoutounour *et al*, 2012, 2013). Note that CD64 corresponds to the high-affinity IgG receptor FcγRI. MerTK is a receptor protein-tyrosine kinase that recognizes apoptotic cells and promotes their uptake by phagocytic cells, but inhibits their activation, in particular by antagonizing signaling through the receptor for type I interferons (IFN-I) (Rothlin *et al*, 2007). Human cDC expressing CD1c (BDCA1) have a gene signature that resembles that of mouse CD11b$^+$ cDC and are likely to represent functional homologs (Robbins *et al*, 2008; Schlitzer *et al*, 2013).

pDC

Mouse pDC are functionally characterized by their unique ability to produce high levels of IFN-I upon stimulation with viruses (Asselin-Paturel *et al*, 2001; Bjorck, 2001; Nakano *et al*, 2001). Under steady-state conditions, their morphology resembles that of a plasma cell as their name indicates. However, they develop dendrites after activation. Mouse pDC can be unequivocally identified as CD11b$^-$CD11c^{int} Bst2^{hi} or as CD11b$^-$ SiglecH$^+$. Of note, some pDCs express CD8α under steady-state conditions, and the expression of this marker is further induced on activated pDCs (Asselin-Paturel *et al*, 2001; Dalod *et al*, 2002). Thus, care must be taken not to erroneously include CD8α$^+$ pDC into the population of Xcr1$^+$cDC, as may happen when using only CD11c and CD8α to define the later.

Lymphoid tissue (LT) and non-lymphoid tissue (NLT) cDC

DCs can be also classified according to their anatomical location. For instance, both Xcr1$^+$ and CD11b$^+$ cDC can either spend their whole life in secondary lymphoid tissues (LT-cDC), or reside first in the parenchyma of non-lymphoid tissues (NLT-cDC). These interstitial NLT-cDC have the ability to migrate via afferent lymphatics to the draining lymph nodes (LN), where they are classified as migratory NLT-cDC (mig-NLT-cDC). Note that the LT-cDC that permanently reside in the spleen are also endowed with migratory properties, in that they are originally located in the red pulp or in the marginal zone where they sense antigens or pathogens that are transported in the blood, and subsequently migrate to the T-cell zone of the periarteriolar lymphoid sheats.

Monocyte-derived DC

During inflammation, monocyte-derived inflammatory DC (Inf-moDC) develop in inflamed tissues from extravasated Ly6C^{hi} (classical) blood monocytes and disappear once the inflammation resolves. Inf-moDC have been identified in pathological conditions in both humans (Segura *et al*, 2013) and mice (Serbina *et al*, 2003). In some inflammatory settings or during infections by viral, bacterial, fungal and parasitic agents, moDC differentiate into tumor necrosis factor (TNF) and inducible NO synthase (iNOS)-producing DC (Tip-DC) with potent antimicrobial effector functions (Serbina *et al*, 2003; Aldridge *et al*, 2009; Tamoutounour *et al*, 2012; Bain *et al*, 2013).

Under steady-state, non-inflammatory conditions, Ly6C^{hi} blood monocytes are also capable of extravasating into tissues such as the dermis, the intestinal lamina propria and the lung, where they can eventually give rise to moDC that have a short half-life and are continuously renewed (Gautier *et al*, 2012; Tamoutounour *et al*, 2012, 2013; Bain *et al*, 2013; Jakubzick *et al*, 2013). Therefore, moDC and Inf-moDC likely represent alternative context-dependent fates of the same Ly6C^{hi} blood monocyte precursors (Bain *et al*, 2013). Inf-moDC and moDC are both characterized by their CD11b^{+} CCR2^{+} CD64$^{lo/+}$MerTK^{-} phenotype and show transcriptomic features reminiscent of CD11b^{+} cDC on the top of their monocytic signature, both in mice (Gautier *et al*, 2012; Tamoutounour *et al*, 2012, 2013) and humans (Segura *et al*, 2013). Although moDC represented the major DC subset in the lungs and the dermis upon exposure to house dust-mite and a contact allergen, respectively, they remained the smallest subset among migratory cells within draining LNs, indicating that moDCs migrate poorly as compared to cDC subsets (Plantinga *et al*, 2013; Tamoutounour *et al*, 2013).

DC maturation

One of the most critical features of DC biology is their functional maturation. This is a complex process characterized by the acquisition of a number of fundamental properties: antigen processing and presentation, migration and T-cell co-stimulation (Mellman & Steinman, 2001). However, as will be detailed later, DC maturation is a heterogeneous process that can confer distinctive functional properties.

In steady-state, NLT-cDC and LT-cDC are described as being resting or immature, a phenotype characterized by a low surface expression of major histocompatibility complex (MHC) class II molecules and co-stimulatory molecules (e.g. CD80, CD86 and CD40). In response to activation by infection, injury or vaccination, NLT-cDC and LT-cDC undergo a program of maturation, which renders them capable of inducing the clonal expansion of antigen-specific naïve T cells and their concomitant differentiation into effector T cells. In this context, immunogenic maturation of cDC leads to the upregulation of MHC class II and co-stimulatory molecules at the cell surface, the CCR7-dependent migration to T-cell-rich zones in LNs, and endows the capacity to release cytokines promoting the differentiation of naïve antigen-specific T cells into effector cells, as well as the activation of various other types of immune cells (Probst *et al*, 2003; Sporri & Reis e Sousa, 2005; Gatto *et al*, 2013). Depending on the nature of the stimuli they sense, cDC can produce distinct cytokines and trigger the differentiation of different types of effector T cells, thereby permitting the adaptation of T-cell polarization to the specific nature of the threat.

Importantly, in steady-state, a fraction of LT-cDC and NLT-cDC undergo a constitutive maturation, which we also refer to as "homeostatic maturation" (Lutz & Schuler, 2002). As a result, they upregulate expression of MHC class II molecules at the cell surface, to almost identical levels found under inflammatory conditions, and they migrate to draining LNs and to T-cell zones in a CCR7-dependent manner (Ohl *et al*, 2004). The presence of high levels of MHC class II molecules on these cDC is likely to enhance their capacity to engage T cells. However, this homeostatic maturation program confers tolerogenic rather than immunogenic properties to cDC, in that they lack the ability to drive the differentiation of naïve self-reactive T cells into effectors and thus result in abortive T-cell responses (Probst *et al*, 2003; Sporri & Reis e Sousa, 2005). Tolerogenic cDC are thought to display processed self-peptide for purging the peripheral T-cell repertoire from those excessively self-reactive T cells that have escaped central tolerance. In addition, these cDC maintain T-cell tolerance to innocuous environmental antigens through the generation of induced regulatory T cells (iTreg). Thus, steady-state cDC maturation is likely to play an important role in peripheral tolerance. As discussed below, the nature of the sterile triggers that result in homeostatic cDC maturation remains an enigma.

DC maturation induced by pattern-recognition receptors (PRR)

All DC subsets, and other antigen-presenting cells such as macrophages, are equipped with a battery of pattern-recognition receptors (PRRs), which can detect molecular patterns of invading microorganisms or endogenous "danger" signals and alert the immune response. These PRRs are expressed both on the cell surface and inside the cell and are extremely diverse, detecting a wide range of molecular species including proteins, carbohydrates, lipids and nucleic acids (Takeuchi & Akira, 2010). The most widely studied family of PRRs on DC are the Toll-like receptors (TLRs). Triggering of TLRs on DC is thought to be critical for their functional maturation to immunogenic DC and the priming of naïve T cells in response to infection, and therefore coupling innate and adaptive immunity. Importantly, some TLRs also recognize host molecules, such as the nucleic acid sensing TLRs (TLR3, TLR7 and TLR9) (Akira *et al*, 2006). Inappropriate DC activation by these endogenous TLR agonists may be linked to the development of autoimmune diseases such as rheumatoid arthritis (RA), psoriasis and systemic lupus erythematosus (SLE). TLR-mediated recognition of commensal microorganisms may also have an important role tissue homeostasis, the most notable example being the intestine where blockade of TLR signaling leads to impaired barrier function and inflammation (Rakoff-Nahoum *et al*, 2004), and it was recently shown that TLR signaling in DC was required to maintain immune homeostasis and tolerance to gut microbiota (Han *et al*, 2013).

Functional specialization of DC through selective TLR expression

Different DC subsets express distinct repertoires of TLRs (Fig 2), which is likely to contribute to their functional specialization. For example, pDC express high levels of TLR7, TLR9 and TLR12. TLR7 and TLR9 detect single-stranded RNA (ssRNA) and unmethylated CpG DNA, respectively. These motifs are found in both bacteria and viruses. TLR12 expression on pDC recognizes the profilin from the intracellular parasite *Toxoplasma gondii* (Koblansky *et al*, 2013). Several studies in mice have shown the importance of TLR7 and TLR9 in the immune response to bacterial and viral pathogens (Akira *et al*, 2006), and of TLR11 and TLR12 in immune responses against *T. gondii* (Yarovinsky *et al*, 2005; Koblansky *et al*, 2013). Mouse Xcr1^{+} cDC uniquely express TLR11 and high levels of TLR3, but do not express TLR4, TLR7 and TLR8, or only very low levels compared to other cell types (Fig 2, (Crozat *et al*, 2009); http://biogps.org/). However, rigorous

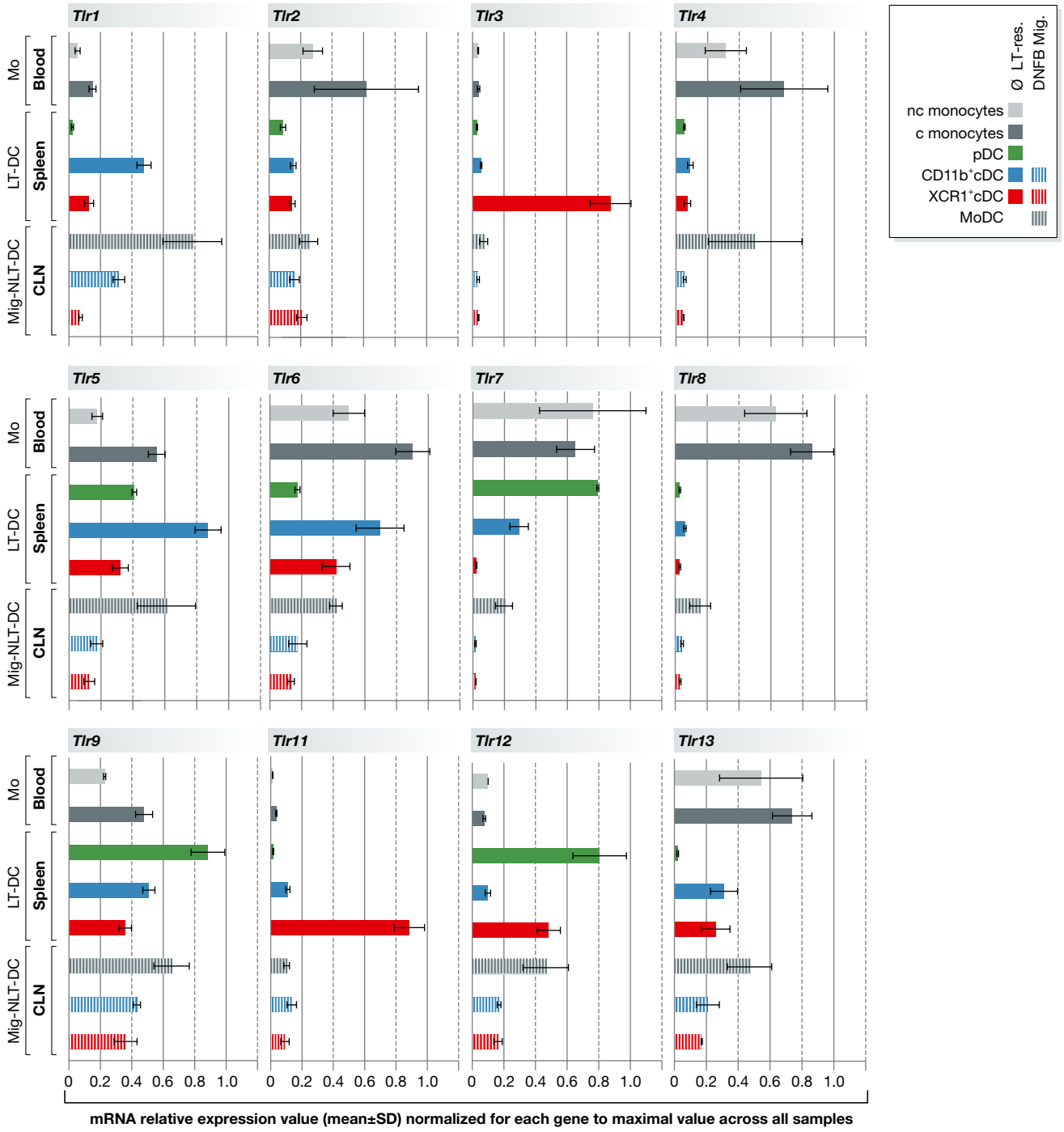


Figure 2. Comparison of the expression patterns of TLRs across DC and monocyte subsets in mice.
The bar graphs show relative gene expression (mean ± SD) for individual TLRs across blood monocyte subsets (gray bars), spleen LT-DC subsets from untreated animals (plain color bars) and cutaneous LN (CLN) mig-NLT-DCs from DNFB skin-painted animals (hatched color bars). These data are compiled from our own publically available datasets and those of the Immgen consortium (Heng *et al*, 2008; Tamoutounour *et al*, 2013). Key: c monocytes, classical blood monocytes characterized as CD11b^{+} Ly6c^{hi} MHCII^{-} cells; nc monocytes, non-classical monocytes characterized as CD11b^{+} Ly6C^{lo} MHCII^{-} cells. For each gene, expression values are normalized to maximal expression across all samples and the mean of 2 to 5 replicates for each cell subset is shown.

examination of the cell-intrinsic responses to TLR4 triggering by XCR1^{+} DC will require pure populations of these cells stimulated *in vitro* with LPS, or comparing *in vivo* responses of WT versus *Tlr4*$^{-/-}$ cells in mixed bone marrow chimeras. Although one report suggests TLR11 expression is low on Xcr1^{+} cDC (Koblansky *et al*, 2013), several reports have shown that TLR12/TLR11 heterodimers are required for *T. gondii* profilin recognition by Xcr1^{+} cDC (Yarovinsky *et al*, 2005; Andrade *et al*, 2013; Raetz *et al*,

2013). TLR3 recognizes viral double-stranded RNA (dsRNA) (Alexopoulou *et al*, 2001), and its expression by DC is particularly important for promoting cross-priming in the context of viral infection (Schulz *et al*, 2005), which is a defining property of the Xcr1$^+$ cDC subset (Guilliams *et al*, 2010). Interestingly, a recent study showed that commensal bacteria in the gut produce dsRNA, which distinguishes them from pathogenic bacteria, and the TLR3-mediated recognition of gut microflora was shown to be important in maintaining immune homeostasis (Kawashima *et al*, 2013). It would therefore be interesting to examine the role of TLR3 signaling in Xcr1$^+$ cDC for tolerance to commensal bacteria. The TLRs involved in the recognition of components of bacterial cell walls and flagella (TLR1, 2, 4, 5 and 6) or bacterial rRNA (TLR13) are most strongly expressed on monocytes, neutrophils and to some extent on moDC and CD11b$^+$ cDC (Fig 2). However, TLR13 is also expressed in Xcr1$^+$ DC (Fig 2) and allows them to respond to bacterial 23S rRNA (Oldenburg *et al*, 2012). Strikingly, while it recognizes ssRNA, TLR8 expression appears to be even more restricted to monocytes and neutrophils.

Overall, with regard to their TLR expression pattern, pDC and Xcr1$^+$ cDC appear to be more specifically equipped for detection of intracellular pathogens, either directly or through phagocytosis of material from infected cells, while CD11b$^+$ cDC and moDC appear to be more specialized for detection of extracellular pathogens. The analysis of expression patterns of other PRRs across immune cells types has confirmed the notion of functional specialization by DC subsets in the recognition of different pathogens or in the detection of specific danger signals. In particular, moDC, monocytes and neutrophils, but also to some extent CD11b$^+$ cDC, express higher levels of cytosolic sensors involved in the recognition of productive intracellular infection by bacteria or viruses (Crozat *et al*, 2009; Luber *et al*, 2010). Of note, both homeostatic and PRR-induced maturation can lead to dramatic changes in TLR expression on DC, in particular TLR3 and TLR11 are downregulated upon maturation of XCR1$^+$ cDC (Fig 2). On the contrary, PRR-induced maturation can induce TLR3 expression on CD11b$^+$ cDC (not shown). This emphasizes the dynamic nature of TLR expression in DC subsets and thus of their response to a given stimulus. The expression pattern of PRRs across DC subsets is rather well conserved between mouse and human, with a few exceptions, such as TLR9, which is broadly expressed in the mouse but mainly restricted to pDC in the human (Crozat *et al*, 2009), and TLR8, which in human is not only expressed on monocytes and neutrophils but also on moDC, CD11b$^+$ cDC and XCR1$^+$ cDC but still absent from pDC (Hornung *et al*, 2002; Lindstedt *et al*, 2005).

Specificity of TLR-signaling pathways in DC

There are three major signaling pathways that dictate the functional consequences of DC activation by TLRs: mitogen-activated protein kinase (MAPK), nuclear factor-κB (NF-κB) and interferon regulatory factors (IRFs) (Akira *et al*, 2006). Different TLRs are coupled to distinct downstream signaling pathways by the selective use of different TIR-domain signaling adaptor molecules: MYD88, TRIF, TRAM and TIRAP (MAL). All TLRs, except TLR3, utilize the MYD88 adaptor to trigger activation of TAK1 (TGFβ Activated Kinase 1; MAP3K7), which is responsible for downstream activation of both MAPK and NF-κB, which cooperatively mediate pro-inflammatory cytokine production, such as TNFα, IL-12 and IL-6. These cytokines have an important role in the polarization of T helper cell subsets during priming by DC, and thus the functional consequences of DC activation. It appears that TAK1 signaling is particularly important in DC maturation and survival, since mice with a specific deficiency of TAK1 in the DC lineage have severe defects in DC development and function (Wang *et al*, 2012). However, specific kinases downstream of TAK1, such as the MAPKs p38 and JNK, and IκB Kinase (IKK)—which regulates activation of NF-κB—may have more specific functions in DC maturation. For example, activation of p38α in DC is particularly important for Th17-mediated immune responses (Huang *et al*, 2012), but not DC survival and homeostasis. However, p38α signaling was also shown to be important for Treg conversion by migratory CD103$^+$ DC in the intestine and mucosal tolerance (Huang *et al*, 2013), indicating that its role is not exclusive to the immunogenic maturation of DC. The specific roles of JNK and IKK signaling in DC maturation have not yet been determined, but it would be interesting to see how they are related to those of TAK1 and p38α.

TLR3 and TLR4 recruit a specific adaptor called TRIF (Yamamoto *et al*, 2002, 2003a; Hoebe *et al*, 2003), which activates TANK-binding kinase 1 (TBK1) and IκB kinase ε (IKKε) (Hemmi *et al*, 2004), leading to the activation of IRF3 and induction of IFN-I expression. In the case of TLR4, another adaptor called TRAM is required to recruit TRIF for IRF3 activation (Yamamoto *et al*, 2003b), while TIRAP couples TLR4 and TLR2 signaling to MYD88 (Fitzgerald *et al*, 2001; Horng *et al*, 2002). TLR7 and TLR9 also trigger IFN-I induction, selectively in pDC, through a distinct MYD88-dependent signaling pathway that activates IRF7 directly (Honda *et al*, 2005a,b). In pDC, IRF7 is constitutively expressed and forms a complex with TLR7/9 and MYD88 (Honda *et al*, 2005a), sequestered in distinct endosomes where IFN-I production is initiated upon appropriate stimulation. This distinctive expression pattern, and subcellular co-localization of TLR7, TLR9, MyD88 and IRF7 in pDC probably, has an important role in conferring their specific function as a major source of IFN-I upon viral infection.

Although TLR3 does not signal through MYD88, TRIF recruitment can also activate NF-κB and MAPK through RIP1 or TAK1 (Cusson-Hermance *et al*, 2005). Therefore, NF-κB and MAPK activation is conserved among all TLR receptors, as well as the induction of pro-inflammatory cytokines such as TNFα and IL-1β. However, the ability to trigger IRF3 or IRF7 activation, and consequently IFN-I expression, is restricted to TLR4 and the nucleic acid sensing TLRs (TLR3, 7, 8, and 9). IRF3 also directly regulates expression of other genes that have important functions in DC for T-cell priming, including the expression of IRF7, to amplify IFN-I production, and IL-12p40/p35 (Ramirez-Carrozzi *et al*, 2009)—a critical cytokine for Th1-cell polarization. Furthermore, autocrine and paracrine IFN-I signaling in DC is important for the amplification of IL-12p40/p35 expression (Gautier *et al*, 2005). Thus, IRF3 activation may be a critical factor for IL-12 induction in DC and consequently is likely to be important for the polarization of Th1 cell response during naïve T-cell priming. TLR7, TLR9 and TLR3 agonists also selectively promote cross-presentation in DC (Datta *et al*, 2003; Le Bon *et al*, 2003; Schwarz *et al*, 2003; Schulz *et al*, 2005), suggesting that the triggering of IFN-I production by DCs is particularly important for optimal priming of Th1 cells and induction of strong cytotoxic CD8 T-cell responses (Manicassamy & Pulendran, 2009). Therefore, at

least in the context of Th1 and CD8 T-cell priming, IFN-I induction represents an important functional distinction between different TLR agonists by DC in their role in the immune response. Much less is known about the role of specific TLR-signaling pathways in other polarized T-cell responses. As mentioned above, p38 MAPK activation in DC has been specifically implicated in Th17-cell polarization (Huang *et al*, 2012), which may be due to the important role for p38 in MYD88-dependent IL-6 expression.

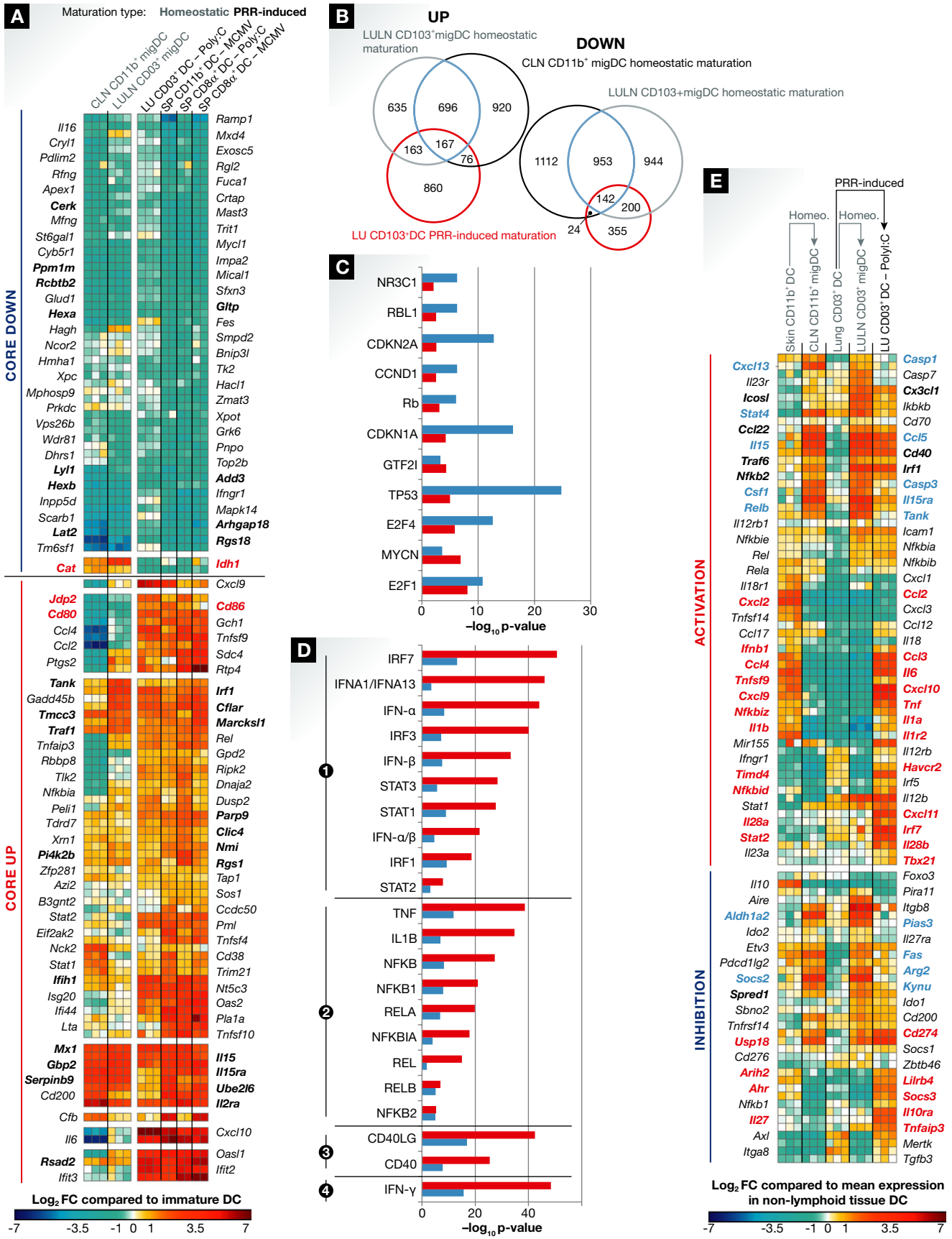
Besides the distinct expression pattern of different TLRs by DC subsets, there is also selective use of specific signaling pathways by different DC. For example, while TLR9 engagement in pDC leads to direct activation of IRF7 (Honda *et al*, 2005a,b), and downstream induction of IFN-I (IFN-α and IFN-β) and IFNλ, in moDC and cDC TLR9 triggers a distinct pathway leading to activation of IRF1 and specific induction of IFN-β (Schmitz *et al*, 2007; Hoshino *et al*, 2010). In addition to the differential induction of IFNs, other important differences between the genes regulated by IRF1 and IRF7 may have functional consequences for pDC versus cDC activation by TLR9. Interestingly, TLR7- and TLR9-mediated induction of IFN-I in both pDC and cDC is dependent on the same upstream kinase; IKKα (IKK1; CHUK) (Takeda *et al*, 1999). In Flt3-derived pDC stimulated with TLR7 or TLR9 agonists, IKKα directly phosphorylates IRF7 leading to induction of IFN-I (Hoshino *et al*, 2006). Similarly, in Flt3-derived cDC stimulated with CpG DNA, IKKα is also responsible for IRF1 activation and induction of IFN-β (Hoshino *et al*, 2010). Furthermore, we recently showed that IKKα is required for TRIF-dependent IRF3 activation and IFN-β induction in GMCSF-derived DC (Mancino *et al*, 2013), which represent moDC. These studies suggest the role of IKKα in IFN-I induction by DC is conserved among DC subsets and different TLR ligands.

Another conserved element in IFN-I induction by TLR3, TLR7 and TLR9 is the signaling adaptor TRAF3 (Hacker *et al*, 2006; Oganesyan *et al*, 2006). The TRAF signaling adaptors are important regulators of innate immune signaling pathways, they act as ubiquitin ligases and couple receptor signaling to downstream kinases such as TBK1/IKKε in the case of TRAF3, and TAK1 in the case of TRAF6—which is required for both MAPK and IKK/NF-κB activation by TLRs (Akira *et al*, 2006). Interestingly, while TRAF3 is required for MYD88-dependent and TRIF-dependent IFN-I induction, TRAF3 negatively regulates MYD88-mediated NF-κB activation and pro-inflammatory cytokine expression, at least in the context of TLR4 signaling (Hacker *et al*, 2006). By regulating the balance between pro-inflammatory cytokine production and IFN-I expression, TRAF3 could have a key role in modulating DC activation. IKKα has also been shown to inhibit pro-inflammatory cytokine production in macrophages (Lawrence *et al*, 2005), suggesting a similar role for IKKα in modulating cytokine production in response to TLR signaling in favor of IFN-I production. We have shown that IKKα activation in DC is critical for Th1 cell priming and acquired immunity to intracellular pathogens (Mancino *et al*, 2013). But the specific role of TRAF3 in DC activation *in vivo* has yet to be determined.

Homeostatic DC maturation

While the mechanisms of TLR-induced DC maturation have become increasingly well characterized, we still understand little about the signaling pathways and upstream regulators that drive steady-state DC maturation. A concept of "limited," or "partial," maturation has been proposed to distinguish the homeostatic (tolerogenic) DC maturation in steady-state, from TLR-induced (immunostimulatory) DC maturation (Lutz & Schuler, 2002). Recent studies suggest that genes, which regulate the NF-κB signaling pathway, play critical roles in restraining DC maturation in steady-state (Dissanayake *et al*, 2011; Hammer *et al*, 2011; Kool *et al*, 2011). In the absence of such factors, steady-state DC maturation becomes immunostimulatory, leading to inappropriate priming of naïve T cells and autoimmunity. A20, the product of the *Tnfaip3* gene, is a negative regulator of NF-κB and MAPK signaling and restricts pro-inflammatory signals through the TNF receptor, TLRs, nucleotide-binding oligomerization domain protein (NOD) and CD40 (Ma & Malynn, 2012). Targeted deletion of A20 in DC, using CD11c-Cre transgenic mice, led to marked splenomegaly and lymphadenopathy within 3 weeks of birth associated with the accumulation of large numbers of myeloid and lymphoid cells (Hammer *et al*, 2011; Kool *et al*, 2011). A20 was shown to restrict MyD88-dependent signals that drive IL-6 and TNF secretion by DCs and subsequent T-cell expansion. But, A20 also restricted MyD88-independent signals in DC that upregulate T-cell co-stimulatory molecules, and blockade of CD80 and CD86 in mice with A20-deficiency in DC significantly antagonized the increased activation of adoptively transferred naive T cells. These studies suggested that the absence of A20 expression in DC led to unrestrained MyD88-independent signals that increase co-stimulatory molecule expression and cause spontaneous T-cell activation. In addition, enhanced MyD88-dependent signals in DC increase secretion of cytokines such as IL-6 that drive T-cell and myeloid expansion. The A20-binding protein, ABIN-1 (encoded by the *Tnip1* gene), has also been shown to be an important negative regulator of TLR-induced NF-κB and MAPK activation. Deletion of *Tnip1* in CD11c-expressing cells leads to exaggerated DC activation in a mouse model of psoriasis, associated with increased MyD88-dependent IL-23 expression (Callahan *et al*, 2013). One should make a cautionary note here as to the DC-specific nature of these observations, as with any Cre-transgenic system there is often off-target deletion of the "floxed" gene, in the case of CD11c, this promoter is also expressed prominently in macrophages, NK cells and even in activated T cells. In fact, the targeted deletion of A20 in myeloid cells, using lysozyme M (*Lyz2*)-Cre mice, also leads to spontaneous autoinflammatory disease (Matmati *et al*, 2011), although Lyz2 is not strongly expressed in cDC.

In another study, the NF-κB protein p105 (NFKB1) was found to be crucial for maintaining the resting state of cDC (Dissanayake *et al*, 2011). Upon adoptive transfer, self-antigen-pulsed, bone marrow-derived DC lacking Nfkb1 were able to activate $CD8^+$ T cells directed toward self-antigens and trigger autoimmunity in the absence of TLR signals. NFKB1 is the precursor for the p50 subunit of NF-κB, which lacks a transactivation domain, and is therefore transcriptionally inactive. The formation of p50 homodimers has been shown to repress NF-κB-dependent expression of pro-inflammatory genes and is thought to be a particularly important mechanism for endotoxin (LPS) tolerance in chronically activated macrophages (Bohuslav *et al*, 1998). The ability of p50 to inhibit IFN-β expression in this context has been shown to be critical for the tolerogenic phenotype (Cheng *et al*, 2011). Although not tested by Dissanayake and colleagues, it would be interesting to evaluate



the role of enhanced IFN-β production in the immunostimulatory phenotype of Nfkb1-deficient DC, given the strong association between IFN-I signaling networks and TLR-induced DC maturation (Baranek *et al*, 2012; Vu Manh *et al*, 2013; Pantel *et al*, 2014).

Gene expression signatures of steady-state and TLR-induced DC maturation

To investigate the immunogenic maturation of DC subsets in different contexts, we recently performed a genome-wide analysis of gene expression signatures in several DC subsets stimulated with various TLR agonists. We then applied bioinformatics tools to identify signaling pathways and transcription factors associated with DC maturation under these conditions. A major conclusion of our analysis was that different DC subsets undergo a convergent transcriptional reprogramming during TLR stimulation. Furthermore, the signaling pathways regulating activation of NF-κB and IRF3, or IRF7, were strongly implicated in this common program of TLR-induced DC maturation (Vu Manh *et al*, 2013). Here, we have extended this strategy to investigate the differences between TLR-induced "immunogenic" DC maturation and homeostatic "tolerogenic" DC maturation. We analyzed how the expression of the core sets of genes, identified as modulated by TLR-induced immunogenic DC maturation, was affected during homeostatic—tolerogenic— DC maturation. For this, we compared NLT-cDC with their migratory counterparts in tissue-draining LNs (mig-NLT-cDC) (Miller *et al*, 2012). At first glance, the corresponding heatmap shows that most of the core genes that were downregulated upon TLR-induced DC maturation ("CORE DOWN" genes), such as in splenic DC from mice infected with mouse cytomegalovirus (MCMV) or in lung CD103$^+$ DC from mice challenged with dsRNA (PolyI:C), were also strongly down-modulated during homeostatic DC maturation (Fig 3A). Furthermore, many of the core genes that were upregulated upon TLR-induced DC maturation ("CORE UP" genes) were also strongly induced upon homeostatic DC maturation. These data show that converging changes occur in gene expression between homeostatic and TLR-induced DC maturation, suggesting that overlapping instructing signals are driving both processes. Next, we used ingenuity pathway analysis to determine whether the gene sets regulated upon homeostatic DC maturation were enriched for targets of the same transcription factors, activation receptors or cytokines previously identified as major regulators of TLR-induced DC maturation (Fig 3B–C). The genes down-modulated both upon TLR-induced and homeostatic DC maturation were strongly enriched for targets of the E2F1 network (Fig 3C) and for annotations linked to positive regulation of mitosis (not shown), showing that homeostatic DC maturation is characterized by the repression of cell cycle genes, as we previously reported for the maturation of spleen DC subsets upon MCMV infection *in vivo* (Baranek *et al*, 2012). As expected, the genes induced in lung CD103$^+$ DC from PolyI:C-treated mice were very strongly enriched for targets of the IRF/IFN-I network (Fig 3D, ❶), as well as the NF-κB/TNF/IL-1β network (❷) and the CD40/CD40LG (❸) or IFNγ (❹) networks. Surprisingly, this was also the case for the genes commonly induced during homeostatic maturation. However, many genes within these networks were induced specifically during either TLR-induced or homeostatic maturation (Fig 3D and Supplementary Table S1). This suggests that the outcome of IRF, NF-κB, CD40 or IFNγ signaling during DC maturation is dictated by instructive signals depending on the tissue localization and the inflammatory context, which must ultimately determine the functional consequences of DC maturation.

What distinguishes "tolerogenic" from "immunogenic" DC maturation?

Certain "CORE UP" genes encoding for immune activation molecules (*Mx1*, *Rsad2*, *Il15*, *Il15ra*, *Irf1*), thought to be selectively upregulated during TLR-induced immunogenic DC maturation, were also strongly induced during homeostatic maturation of tolerogenic DC (Fig 3A). Conversely, the *Tnfaip3* gene, encoding the A20 protein previously reported to preserve immune homeostasis by preventing DC activation in steady-state, was highly induced under all conditions of TLR-induced maturation but was down-modulated upon homeostatic maturation of skin CD11b$^+$ cDC. These observations prompted us to further examine the changes in the expression of genes involved in activation versus inhibition of immune responses during homeostatic or TLR-induced DC maturation (Fig 3E). A

Figure 3. Comparison of homeostatic versus PRR-induced DC maturation.

(A) Converging changes in gene expression between homeostatic and PRR-induced DC maturation. Heatmap showing the fold change between immature and mature DC for two sets of genes previously reported to be, respectively, decreased ("CORE DOWN") or increased ("CORE UP") upon TLR-induced maturation; irrespective of DC subset, stimuli and species of origin. For homeostatic maturation, fold change in gene expression levels was computed by comparing mature DC having migrating in cutaneous or lung lymph nodes under steady-state conditions (CLN CD11b$^+$ mig-NLT-DC and LULN CD103$^+$ mig-NLT-DC) to their immature counterparts from skin or lung. For TLR-induced maturation, fold change in gene expression levels was computed by comparing TLR-stimulated DC (lung CD103$^+$ DC isolated from PolyI:C-treated animals, spleen CD11b$^+$ DC and CD8α$^+$ DC isolated from MCMV-infected mice, and spleen CD8α$^+$ DC isolated from PolyI:C-treated animals) to their immature, unstimulated, counterparts from the same tissue. Genes that showed a statistically significant and similar regulation in their expression in homeostatic maturation and in PolyI:C-induced maturation of lung CD103$^+$ DC are shown in bold, black font. Genes that showed a statistically significant regulation in these conditions but with a reciprocal change between homeostatic maturation versus PolyI:C-induced maturation of lung CD103$^+$ DC are highlighted in bold, red font. (B-D) Overlapping instructive signals drive homeostatic and PRR-induced DC maturation. Venn diagrams were drawn for comparing the sets of genes significantly induced (UP) or repressed (DOWN) upon TLR-induced maturation of lung CD03$^+$ DC isolated from PolyI:C-treated animals, and upon homeostatic maturation of CLN CD11b$^+$ mig-NLT-DC or in LULN CD103$^+$ mig-NLT-DC. Ingenuity pathway analysis was used to search whether resulting gene lists were enriched for targets of known transcription factors, activation receptors or cytokines for repressed (C) genes or induced (D) genes. The results of the most significant enrichments obtained are shown as bar graphs, with regulators regrouped by functional network according to IPA classification (❶, IRF/IFN-I network; ❷, NF-κB/TNF/IL-1β network; ❸, CD40/CD40LG network; ❹, IFN-γ network). Red bars indicate p-values for TLR-induced maturation (red circle of the Venn diagrams) and blue bars for homeostatic maturation (specifically for the genes commonly regulated upon both conditions of homeostatic maturation, area circled by a blue line on the Venn Diagrams). The corresponding gene lists are given in Supplementary Table S1. (E) Changes in the expression of genes involved in activation versus inhibition of immune responses upon homeostatic or PRR-induced DC maturation. The heatmap shows the relative expression of individual genes in immature versus mature DC, normalized to the mean expression levels in immature DC. Genes that showed a statistically significant and similar induction in their expression in homeostatic maturation and in TLR-induced maturation are shown in bold, black font. Genes that showed a statistically significant induction in TLR-induced maturation reaching levels higher than those observed in homeostatic maturation are highlighted in bold, red font. Conversely, genes that showed a statistically significant induction upon homeostatic maturation reaching levels higher than those observed in TLR-induced maturation are highlighted in bold, blue font.

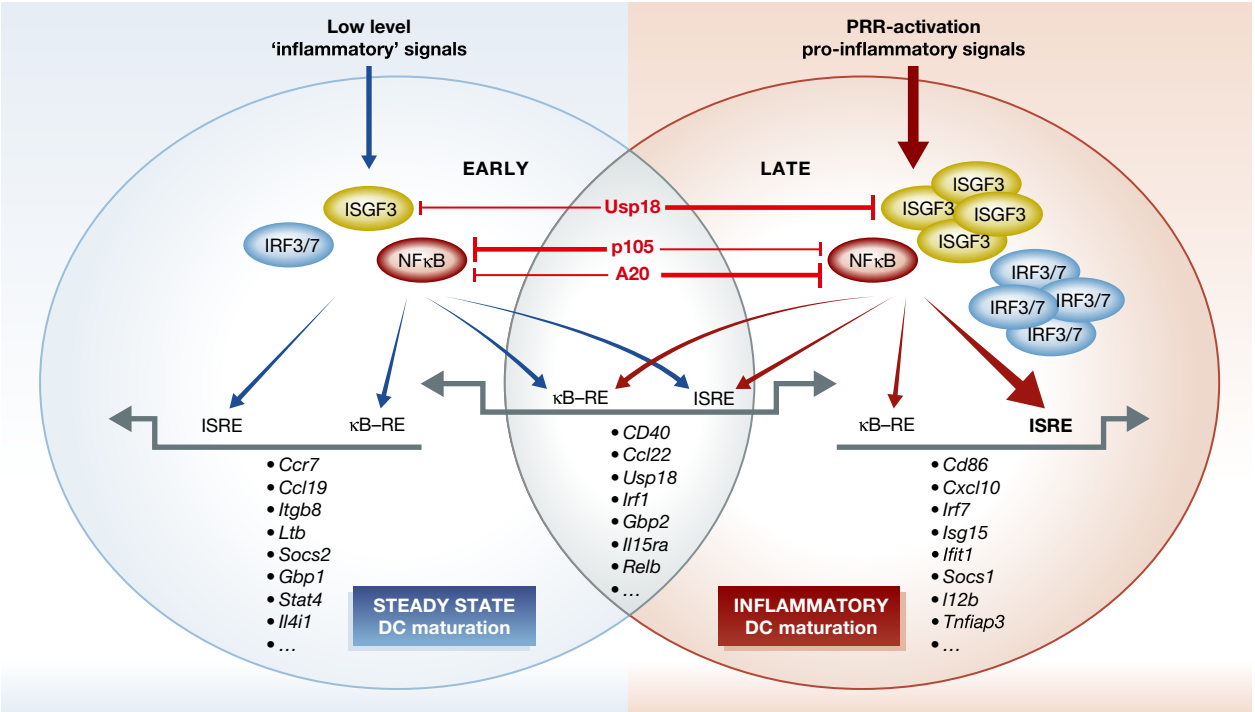


Figure 4. Key components of homeostatic versus PRR-induced DC maturation.
The scheme illustrates commonalities and differences in the signaling pathways and downstream regulation of gene expression triggered in DC during homeostatic (tolerogenic) versus TLR-induced (immunogenic) maturation. The stronger and broader activation of signaling by the IRF/IFN-I and NFκB/TNF/IL-1β networks in TLR-induced maturation might not only result from activating receptors on DC but also from differences in the kinetics of the activation of negative regulators including A20 and p105, with a very early induction during homeostatic DC maturation contrasting with a delayed, although even stronger induction occurring during TLR-induced activation, as a late negative feedback loop in response to autocrine/paracrine inflammatory cytokine signaling.

number of "activation" genes, shown in red on the upper heatmap of Fig 3E, were expressed to higher levels in TLR-stimulated DC. However, many of these genes were induced equally, or even more strongly, in DC during homeostatic maturation (shown in bold black and in blue, respectively, on the upper heatmap of Fig 3E). Some "inhibitory" genes, shown in blue on the lower heatmap of Fig 3E, were expressed to higher levels upon homeostatic DC maturation. However, many of these genes were induced in TLR-stimulated DC to similar, or even higher levels (shown in bold black and red, respectively, on the lower heatmap of Fig 3E). For example, Itgb8 was induced to similar levels upon homeostatic maturation in migratory dermal CD11b$^+$ cDC found in CLN and upon PolyI:C challenge in CD103$^+$ lung DC, although it was induced to even higher levels upon homeostatic maturation in migratory CD103$^+$ DC from lung-draining lymph nodes (LULN). The strong induction of inhibitory genes during TLR-induced DC maturation are likely to reflect the development of potent negative feedback loops that limit DC activation even under strong inflammatory conditions to tightly control the intensity and duration of DC activation and thus prevent unbridled, deleterious immune responses.

In the course of this analysis, differences were observed between the two conditions of homeostatic maturation and even between steady-state immature DC subsets, for example *Il12b* was induced upon homeostatic maturation of lung CD103$^+$ but not skin CD11b$^+$ DC, and immature skin CD11b$^+$ DC expressed the highest levels of *Il10*, compared to lung resident or splenic DC. These observations suggested that the polarization of DC functions toward tolerance, during homeostatic maturation, versus immunogenicity upon TLR-induced maturation, is very complex. It might not simply correlate with the respective induction of a universal set of immunosuppressive versus pro-inflammatory genes as previously proposed (Miller *et al*, 2012). Rather, a complex array of molecules involved in the activation or inhibition of immune responses is induced in DC both during homeostatic and TLR-induced maturation, but differences in their nature and kinetics of induction might contribute to distinct functional outcomes. Indeed, it appears that the consequences of signaling through the IRF/IFN-I and NF-κB/TNF/IL-1β networks differ between homeostatic and TLR-induced maturation, with a stronger and broader activation in the later condition, especially of IRF/IFN-I network genes (Fig 4). Further studies integrating more complex and better-controlled datasets, including kinetic studies of models of TLR-induced versus homeostatic DC maturation, will be necessary to address these issues in more depth. Finally, it is important to consider post-translational control of gene expression and subcellular localization of molecules during DC maturation. It is well documented that MHC and CD86 expression in DC are not only controlled at the transcriptional level but also through regulation of their intracellular trafficking to the plasma membrane by molecules such as MARCH1 (De Gassart *et al*, 2008; Tze *et al*, 2011), and it is likely that other important aspects of DC cross-talk with T cells are controlled at the post-transcriptional level. For example, a recent report has shown that TLR-driven early glycolytic reprogramming in

DC is necessary to promote *de novo* synthesis of fatty acids for the expansion of the endoplasmic reticulum and Golgi, required for the production and secretion of proteins that are integral to DC activation. Blockade of glycolytic reprogramming in TLR-stimulated DC selectively inhibited the translation but not the transcription of genes encoding cytokines and co-stimulatory molecules (Everts *et al*, 2014). Hence, it would be interesting to examine whether the "activation" genes induced at the mRNA level upon homeostatic DC maturation are actually translated or not, and if so whether this is correlated with differences in metabolic reprogramming between TLR-induced and steady-state DC maturation.

Perspectives

Our review highlights the complex processes leading to the functional maturation of DC that integrate the intrinsic properties of different DC subsets, dictated by their specific gene expression programs, and contextual signals received from the tissue microenvironment. It is clear that different DC subsets are equipped to respond to specific signals by virtue of their distinctive gene expression programs. However, there are also some common "core" elements to the maturation pathways of different DC subsets that are triggered irrespective of the tissue or inflammatory context.

In our analyses reported here, we have uncovered a reciprocal regulation of new candidate genes (*Idh1*, *Cat* and *Jdp2*) between PRR-induced versus homeostatic DC maturation. It will be interesting to examine the potential roles for these genes in the functional switch between tolerance and immunogenicity during DC maturation. For example, *Idh1* encodes for the metabolic enzyme isocitrate dehydrogenase 1 and gain-of-function mutants are frequently found in glioma, acute myeloid leukemia (AML), melanoma, thyroid cancer and chondrosarcoma patients where they are proposed to overproduce 2-hydroxyglutarate (2HG) leading to inhibition of epigenetic regulators and consecutive aberrant histone- and DNA-hypermethylation. While nothing is known about the function of *Idh1* in DC, it is tempting to speculate that its downregulation upon PRR stimulation might play an important role in the regulation of DC metabolism and the epigenetic landscape regulating a switch in cellular functions from tolerance to immunogenicity.

To further delineate the specific genes that differentiate steady-state "tolerogenic" DC maturation from PRR-induced "immunogenic" DC maturation, irrespective of DC subset, tissue localization or animal species, it will be important to generate broader gene expression datasets comparing tolerogenic versus immunogenic DC subsets isolated from the same tissue in steady-state and after PRR-challenge, respectively. The lack of these direct comparisons is a major limitation of the existing data and analysis presented here.

The implication of NF-κB and IRF-signaling pathways in both steady-state and inflammatory DC maturation is intriguing. It is of no surprise that these pathways are important regulators of genes induced during TLR-induced DC maturation, and this is supported by a wealth of the literature. However, the role of these pathways in steady-state DC maturation is less obvious. Recent studies suggest that negative regulators of NF-κB activation, A20, TNIP and NFKB1, have an important role in restraining DC activation in both steady-state and during inflammatory conditions, to prevent the inappropriate or uncontrolled activation of DC and the subsequent unfolding of pathological immune responses. Indeed, these pathways also appear to be important in the development of autoimmune and autoinflammatory diseases, as genome-wide association studies in humans have strongly linked polymorphisms in the A20 and TNIP1 loci to the development of SLE and psoriasis, respectively (Ma & Malynn, 2012). However, expression of these genes does not distinguish steady-state and TLR-induced DC maturation.

While NF-kB- and IRF-regulated genes feature strongly in both steady-state and PRR-induced DC maturation, the strong induction of IRF-dependent genes appears to be a distinguishing feature of PRR-induced "immunogenic" DC maturation, at least in mice (Fig 4). This is in keeping with the potent adjuvant properties of IFN-I inducing TLR agonists for priming T-cell responses. However, the specific roles of these pathways in steady-state versus PRR-induced DC maturation have yet to be ascertained.

Supplementary information for this article is available online: http://emboj.embopress.org

Acknowledgements

The studies performed in the laboratories of MD, BM and TL are supported by institutional funding from INSERM, CNRS and the Aix-Marseille-Universite, and grants from L'Agence Nationale de la Recherche (ANR); ANR-08-MIEN-008–02 and ANR-09-BLAN-0073–02 to MD, ANR-09-MIEN-029-01 and ANR-10-BLAN-1302-01 to TL, by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007–2013 Grant Agreement number 281225 to MD and 260753 to TL). BM is supported by CNRS, the EE-ASI European Collaborative Research Project, the NANOASIT Euronanomed Project and an ERC Advanced Investigator Grant.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* 124: 783 – 801

Aldridge JR Jr, Moseley CE, Boltz DA, Negovetich NJ, Reynolds C, Franks J, Brown SA, Doherty PC, Webster RG, Thomas PG (2009) TNF/iNOS-producing dendritic cells are the necessary evil of lethal influenza virus infection. *Proc Natl Acad Sci USA* 106: 5306 – 5311

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 413: 732 – 738

Andrade WA, Souza Mdo C, Ramos-Martinez E, Nagpal K, Dutra MS, Melo MB, Bartholomeu DC, Ghosh S, Golenbock DT, Gazzinelli RT (2013) Combined action of nucleic acid-sensing Toll-like receptors and TLR11/TLR12 heterodimers imparts resistance to Toxoplasma gondii in mice. *Cell Host Microbe* 13: 42 – 53

Asselin-Paturel C, Boonstra A, Dalod M, Durand I, Yessaad N, Dezutter-Dambuyant C, Vicari A, O'Garra A, Biron C, Briere F, Trinchieri G (2001) Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat Immunol* 2: 1144 – 1150

Bachem A, Guttler S, Hartung E, Ebstein F, Schaefer M, Tannert A, Salama A, Movassaghi K, Opitz C, Mages HW, Henn V, Kloetzel PM, Gurka S, Kroczek

RA (2010) Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. *J Exp Med* 207: 1273 – 1281

Bachem A, Hartung E, Güttler S, Mora A, Zhou X, Hegemann A, Plantinga M, Mazzini E, Stoitzner P, Gurka S, Henn V, Mages HW, Kroczek RA (2012) Expression of XCR1 Characterizes the Batf3-Dependent Lineage of Dendritic Cells Capable of Antigen Cross-Presentation. *Front Immunol* 3: 214

Bain CC, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O, Grip O, Guilliams M, Malissen B, Agace WW, Mowat AM (2013) Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors. *Mucosal Immunol* 6: 498 – 510

Baranek T, Manh TP, Alexandre Y, Maqbool MA, Cabeza JZ, Tomasello E, Crozat K, Bessou G, Zucchini N, Robbins SH, Vivier E, Kalinke U, Ferrier P, Dalod M (2012) Differential responses of immune cells to type I interferon contribute to host resistance to viral infection. *Cell Host Microbe* 12: 571 – 584

Bjorck P (2001) Isolation and characterization of plasmacytoid dendritic cells from Flt3 ligand and granulocyte-macrophage colony-stimulating factor-treated mice. *Blood* 98: 3520 – 3526

Bohuslav J, Kravchenko VV, Parry GC, Erlich JH, Gerondakis S, Mackman N, Ulevitch RJ (1998) Regulation of an essential innate immune response by the p50 subunit of NF-kappaB. *J Clin Invest* 102: 1645 – 1652

Callahan JA, Hammer GE, Agelides A, Duong BH, Oshima S, North J, Advincula R, Shifrin N, Truong HA, Paw J, Barrera J, DeFranco A, Rosenblum MD, Malynn BA, Ma A (2013) Cutting edge: ABIN-1 protects against psoriasis by restricting MyD88 signals in dendritic cells. *J Immunol* 191: 535 – 539

Caminschi I, Proietto AI, Ahmet F, Kitsoulis S, Shin Teh J, Lo JC, Rizzitelli A, Wu L, Vremec D, van Dommelen SL, Campbell IK, Maraskovsky E, Braley H, Davey GM, Mottram P, van de Velde N, Jensen K, Lew AM, Wright MD, Heath WR *et al* (2008) The dendritic cell subtype-restricted C-type lectin Clec9A is a target for vaccine enhancement. *Blood* 112: 3264 – 3273

Cheng CS, Feldman KE, Lee J, Verma S, Huang DB, Huynh K, Chang M, Ponomarenko JV, Sun SC, Benedict CA, Ghosh G, Hoffmann A (2011) The specificity of innate immune responses is enforced by repression of interferon response elements by NF-kappaB p50. *Sci Signal* 4: ra11

Crozat K, Vivier E, Dalod M (2009) Crosstalk between components of the innate immune system: promoting anti-microbial defenses and avoiding immunopathologies. *Immunol Rev* 227: 129 – 149

Crozat K, Guiton R, Contreras V, Feuillet V, Dutertre CA, Ventre E, Vu Manh TP, Baranek T, Storset AK, Marvel J, Boudinot P, Hosmalin A, Schwartz-Cornil I, Dalod M (2010) The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. *J Exp Med* 207: 1283 – 1292

Crozat K, Tamoutounour S, Vu Manh TP, Fossum E, Luche H, Ardouin L, Guilliams M, Azukizawa H, Bogen B, Malissen B, Henri S, Dalod M (2011) Cutting edge: expression of XCR1 defines mouse lymphoid-tissue resident and migratory dendritic cells of the CD8α+ type. *J Immunol* 187: 4411 – 4415

Cusson-Hermance N, Khurana S, Lee TH, Fitzgerald KA, Kelliher MA (2005) Rip1 mediates the Trif-dependent toll-like receptor 3- and 4-induced NF-{kappa}B activation but does not contribute to interferon regulatory factor 3 activation. *J Biol Chem* 280: 36560 – 36566

Dalod M, Salazar-Mather TP, Malmgaard L, Lewis C, Asselin-Paturel C, Briere F, Trinchieri G, Biron CA (2002) Interferon alpha/beta and interleukin 12 responses to viral infections: pathways regulating dendritic cell cytokine expression in vivo. *J Exp Med* 195: 517 – 528

Datta SK, Redecke V, Prilliman KR, Takabayashi K, Corr M, Tallant T, DiDonato J, Dziarski R, Akira S, Schoenberger SP, Raz E (2003) A subset of Toll-like receptor ligands induces cross-presentation by bone marrow-derived dendritic cells. *J Immunol* 170: 4102 – 4110

De Gassart A, Camosseto V, Thibodeau J, Ceppi M, Catalan N, Pierre P, Gatti E (2008) MHC class II stabilization at the surface of human dendritic cells is the result of maturation-dependent MARCH I down-regulation. *Proc Natl Acad Sci USA* 105: 3491 – 3496

Dissanayake D, Hall H, Berg-Brown N, Elford AR, Hamilton SR, Murakami K, Deluca LS, Gommerman JL, Ohashi PS (2011) Nuclear factor-kappaB1 controls the functional maturation of dendritic cells and prevents the activation of autoreactive T cells. *Nat Med* 17: 1663 – 1667

Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, Redmann V, Freitas TC, Blagih J, van der Windt GJ, Artyomov MN, Jones RG, Pearce EL, Pearce EJ (2014) TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKvarepsilon supports the anabolic demands of dendritic cell activation. *Nat Immunol* 15: 323 – 332

Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G, Brint E, Dunne A, Gray P, Harte MT, McMurray D, Smith DE, Sims JE, Bird TA, O'Neill LA (2001) Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. *Nature* 413: 78 – 83

Gatto D, Wood K, Caminschi I, Murphy-Durland D, Schofield P, Christ D, Karupiah G, Brink R (2013) The chemotactic receptor EBI2 regulates the homeostasis, localization and immunological function of splenic dendritic cells. *Nat Immunol* 14: 446 – 453

Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, Helft J, Chow A, Elpek KG, Gordonov S, Mazloom AR, Ma'ayan A, Chua WJ, Hansen TH, Turley SJ, Merad M, Randolph GJ (2012) Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* 13: 1118 – 1128

Gautier G, Humbert M, Deauvieau F, Scuiller M, Hiscott J, Bates EE, Trinchieri G, Caux C, Garrone P (2005) A type I interferon autocrine-paracrine loop is involved in Toll-like receptor-induced interleukin-12p70 secretion by dendritic cells. *J Exp Med* 201: 1435 – 1446

Ginhoux F, Liu K, Helft J, Bogunovic M, Greter M, Hashimoto D, Price J, Yin N, Bromberg J, Lira SA, Stanley ER, Nussenzweig M, Merad M (2009) The origin and development of nonlymphoid tissue CD103+ DCs. *J Exp Med* 206: 3115 – 3130

Guilliams M, Henri S, Tamoutounour S, Ardouin L, Schwartz-Cornil I, Dalod M, Malissen B (2010) From skin dendritic cells to a simplified classification of human and mouse dendritic cell subsets. *Eur J Immunol* 40: 2089 – 2094

Hacker H, Redecke V, Blagoev B, Kratchmarova I, Hsu LC, Wang GG, Kamps MP, Raz E, Wagner H, Hacker G, Mann M, Karin M (2006) Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. *Nature* 439: 204 – 207

Hammer GE, Turer EE, Taylor KE, Fang CJ, Advincula R, Oshima S, Barrera J, Huang EJ, Hou B, Malynn BA, Reizis B, DeFranco A, Criswell LA, Nakamura MC, Ma A (2011) Expression of A20 by dendritic cells preserves immune homeostasis and prevents colitis and spondyloarthritis. *Nat Immunol* 12: 1184 – 1193

Han D, Walsh MC, Cejas PJ, Dang NN, Kim YF, Kim J, Charrier-Hisamuddin L, Chau L, Zhang Q, Bittinger K, Bushman FD, Turka LA, Shen H, Reizis B, Defranco AL, Wu GD, Choi Y (2013) Dendritic cell expression of the signaling molecule TRAF6 is critical for gut microbiota-dependent immune tolerance. *Immunity* 38: 1211 – 1222

Haniffa M, Shin A, Bigley V, McGovern N, Teo P, See P, Wasan PS, Wang XN, Malinarich F, Malleret B, Larbi A, Tan P, Zhao H, Poidinger M, Pagan S,

Cookson S, Dickinson R, Dimmick I, Jarrett RF, Renia L *et al* (2012) Human tissues contain CD141hi cross-presenting dendritic cells with functional homology to mouse CD103+ nonlymphoid dendritic cells. *Immunity* 37: 60 – 73

Hemmi H, Takeuchi O, Sato S, Yamamoto M, Kaisho T, Sanjo H, Kawai T, Hoshino K, Takeda K, Akira S (2004) The roles of two IkappaB kinase-related kinases in lipopolysaccharide and double stranded RNA signaling and viral infection. *J Exp Med* 199: 1641 – 1650

Heng TS, Painter MW, Immunological Genome Project C (2008) The Immunological Genome Project: networks of gene expression in immune cells. *Nat Immunol* 9: 1091 – 1094

Hoebe K, Du X, Georgel P, Janssen E, Tabeta K, Kim SO, Goode J, Lin P, Mann N, Mudd S, Crozat K, Sovath S, Han J, Beutler B (2003) Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. *Nature* 424: 743 – 748

Honda K, Ohba Y, Yanai H, Negishi H, Mizutani T, Takaoka A, Taya C, Taniguchi T (2005a) Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. *Nature* 434: 1035 – 1040

Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T, Shimada N, Ohba Y, Takaoka A, Yoshida N, Taniguchi T (2005b) IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* 434: 772 – 777

Horng T, Barton GM, Flavell RA, Medzhitov R (2002) The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. *Nature* 420: 329 – 333

Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdorfer B, Giese T, Endres S, Hartmann G (2002) Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *Journal of Immunology* 168: 4531 – 4537

Hoshino K, Sugiyama T, Matsumoto M, Tanaka T, Saito M, Hemmi H, Ohara O, Akira S, Kaisho T (2006) IkappaB kinase-alpha is critical for interferon-alpha production induced by Toll-like receptors 7 and 9. *Nature* 440: 949 – 953

Hoshino K, Sasaki I, Sugiyama T, Yano T, Yamazaki C, Yasui T, Kikutani H, Kaisho T (2010) Critical role of IkappaB Kinase alpha in TLR7/9-induced type I IFN production by conventional dendritic cells. *Journal of Immunology* 184: 3341 – 3345

Huang G, Wang Y, Vogel P, Kanneganti TD, Otsu K, Chi H (2012) Signaling via the kinase p38alpha programs dendritic cells to drive TH17 differentiation and autoimmune inflammation. *Nat Immunol* 13: 152 – 161

Huang G, Wang Y, Chi H (2013) Control of T cell fates and immune tolerance by p38alpha signaling in mucosal CD103+ dendritic cells. *Journal of Immunology* 191: 650 – 659

Huysamen C, Willment JA, Dennehy KM, Brown GD (2008) CLEC9A is a novel activation C-type lectin-like receptor expressed on BDCA3+ dendritic cells and a subset of monocytes. *J Biol Chem* 283: 16693 – 16701

Jakubzick C, Gautier EL, Gibbings SL, Sojka DK, Schlitzer A, Johnson TE, Ivanov S, Duan Q, Bala S, Condon T, van Rooijen N, Grainger JR, Belkaid Y, Ma'ayan A, Riches DW, Yokoyama WM, Ginhoux F, Henson PM, Randolph GJ (2013) Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. *Immunity* 39: 599 – 610

Kawashima T, Kosaka A, Yan H, Guo Z, Uchiyama R, Fukui R, Kaneko D, Kumagai Y, You DJ, Carreras J, Uematsu S, Jang MH, Takeuchi O, Kaisho T, Akira S, Miyake K, Tsutsui H, Saito T, Nishimura I, Tsuji NM (2013) Double-stranded RNA of intestinal commensal but not pathogenic bacteria triggers production of protective interferon-beta. *Immunity* 38: 1187 – 1197

Koblansky AA, Jankovic D, Oh H, Hieny S, Sungnak W, Mathur R, Hayden MS, Akira S, Sher A, Ghosh S (2013) Recognition of profilin by Toll-like receptor 12 is critical for host resistance to Toxoplasma gondii. *Immunity* 38: 119 – 130

Kool M, van Loo G, Waelput W, De Prijck S, Muskens F, Sze M, van Praet J, Branco-Madeira F, Janssens S, Reizis B, Elewaut D, Beyaert R, Hammad H, Lambrecht BN (2011) The ubiquitin-editing protein A20 prevents dendritic cell activation, recognition of apoptotic cells, and systemic autoimmunity. *Immunity* 35: 82 – 96

Lawrence T, Bebien M, Liu GY, Nizet V, Karin M (2005) IKKalpha limits macrophage NF-kappaB activation and contributes to the resolution of inflammation. *Nature* 434: 1138 – 1143

Le Bon A, Etchart N, Rossmann C, Ashton M, Hou S, Gewert D, Borrow P, Tough DF (2003) Cross-priming of CD8+ T cells stimulated by virus-induced type I interferon. *Nat Immunol* 4: 1009 – 1015

Lindstedt M, Lundberg K, Borrebaeck CA (2005) Gene family clustering identifies functionally associated subsets of human in vivo blood and tonsillar dendritic cells. *Journal of Immunology* 175: 4839 – 4846

Luber CA, Cox J, Lauterbach H, Fancke B, Selbach M, Tschopp J, Akira S, Wiegand M, Hochrein H, O'Keeffe M, Mann M (2010) Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. *Immunity* 32: 279 – 289

Lutz MB, Schuler G (2002) Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? *Trends Immunol* 23: 445 – 449

Ma A, Malynn BA (2012) A20: linking a complex regulator of ubiquitylation to immunity and human disease. *Nat Rev Immunol* 12: 774 – 785

Mancino A, Habbeddine M, Johnson E, Luron L, Bebien M, Memet S, Fong C, Bajenoff M, Wu X, Karin M, Caamano J, Chi H, Seed M, Lawrence T (2013) I kappa B kinase alpha (IKKalpha) activity is required for functional maturation of dendritic cells and acquired immunity to infection. *EMBO J* 32: 816 – 828

Manicassamy S, Pulendran B (2009) Modulation of adaptive immunity with Toll-like receptors. *Semin Immunol* 21: 185 – 193

Matmati M, Jacques P, Maelfait J, Verheugen E, Kool M, Sze M, Geboes L, Louagie E, Mc Guire C, Vereecke L, Chu Y, Boon L, Staelens S, Matthys P, Lambrecht BN, Schmidt-Supprian M, Pasparakis M, Elewaut D, Beyaert R, van Loo G (2011) A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritis resembling rheumatoid arthritis. *Nat Genet* 43: 908 – 912

Mellman I, Steinman RM (2001) Dendritic cells: specialized and regulated antigen processing machines. *Cell* 106: 255 – 258

Miller JC, Brown BD, Shay T, Gautier EL, Jojic V, Cohain A, Pandey G, Leboeuf M, Elpek KG, Helft J, Hashimoto D, Chow A, Price J, Greter M, Bogunovic M, Bellemare-Pelletier A, Frenette PS, Randolph GJ, Turley SJ, Merad M *et al* (2012) Deciphering the transcriptional network of the dendritic cell lineage. *Nat Immunol* 13: 888 – 899

Nakano H, Yanagita M, Gunn MD (2001) CD11c(+)B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. *J Exp Med* 194: 1171 – 1178

Oganesyan G, Saha SK, Guo B, He JQ, Shahangian A, Zarnegar B, Perry A, Cheng G (2006) Critical role of TRAF3 in the Toll-like receptor-dependent and -independent antiviral response. *Nature* 439: 208 – 211

Ohl L, Mohaupt M, Czeloth N, Hintzen G, Kiafard Z, Zwirner J, Blankenstein T, Henning G, Forster R (2004) CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. *Immunity* 21: 279 – 288

Oldenburg M, Kruger A, Ferstl R, Kaufmann A, Nees G, Sigmund A, Bathke B, Lauterbach H, Suter M, Dreher S, Koedel U, Akira S, Kawai T, Buer J, Wagner H, Bauer S, Hochrein H, Kirschning CJ (2012) TLR13 recognizes bacterial 23S rRNA devoid of erythromycin resistance-forming modification. *Science* 337: 1111 – 1115

Pantel A, Teixeira A, Haddad E, Wood EG, Steinman RM, Longhi MP (2014) Direct Type I IFN but Not MDA5/TLR3 activation of dendritic cells is required for maturation and metabolic shift to glycolysis after poly IC stimulation. *PLoS Biol* 12: e1001759

Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W, Vanhoutte L, Neyt K, Killeen N, Malissen B, Hammad H, Lambrecht BN (2013) Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* 38: 322 – 335

Probst HC, Lagnel J, Kollias G, van den Broek M (2003) Inducible transgenic mice reveal resting dendritic cells as potent inducers of CD8+ T cell tolerance. *Immunity* 18: 713 – 720

Raetz M, Kibardin A, Sturge CR, Pifer R, Li H, Burstein E, Ozato K, Larin S, Yarovinsky F (2013) Cooperation of TLR12 and TLR11 in the IRF8-dependent IL-12 response to Toxoplasma gondii profilin. *Journal of Immunology* 191: 4818 – 4827

Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 118: 229 – 241

Ramirez-Carrozzi VR, Braas D, Bhatt DM, Cheng CS, Hong C, Doty KR, Black JC, Hoffmann A, Carey M, Smale ST (2009) A unifying model for the selective regulation of inducible transcription by CpG islands and nucleosome remodeling. *Cell* 138: 114 – 128

Robbins SH, Walzer T, Dembele D, Thibault C, Defays A, Bessou G, Xu H, Vivier E, Sellars M, Pierre P, Sharp FR, Chan S, Kastner P, Dalod M (2008) Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. *Genome Biol* 9: R17

Rothlin CV, Ghosh S, Zuniga EI, Oldstone MB, Lemke G (2007) TAM receptors are pleiotropic inhibitors of the innate immune response. *Cell* 131: 1124 – 1136

Sancho D, Mourao-Sa D, Joffre OP, Schulz O, Rogers NC, Pennington DJ, Carlyle JR, Reis e Sousa C (2008) Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. *J Clin Investig* 118: 2098 – 2110

Schlitzer A, McGovern N, Teo P, Zelante T, Atarashi K, Low D, Ho AW, See P, Shin A, Wasan PS, Hoeffel G, Malleret B, Heiseke A, Chew S, Jardine L, Purvis HA, Hilkens CM, Tam J, Poidinger M, Stanley ER *et al* (2013) IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* 38: 970 – 983

Schmitz F, Heit A, Guggemoos S, Krug A, Mages J, Schiemann M, Adler H, Drexler I, Haas T, Lang R, Wagner H (2007) Interferon-regulatory-factor 1 controls Toll-like receptor 9-mediated IFN-beta production in myeloid dendritic cells. *Eur J Immunol* 37: 315 – 327

Schraml BU, van Blijswijk J, Zelenay S, Whitney PG, Filby A, Acton SE, Rogers NC, Moncaut N, Carvajal JJ, Reis ESC (2013) Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. *Cell* 154: 843 – 858

Schulz O, Diebold SS, Chen M, Naslund TI, Nolte MA, Alexopoulou L, Azuma YT, Flavell RA, Liljestrom P, Reis e Sousa C (2005) Toll-like receptor 3 promotes cross-priming to virus-infected cells. *Nature* 433: 887 – 892

Schwarz K, Storni T, Manolova V, Didierlaurent A, Sirard JC, Rothlisberger P, Bachmann MF (2003) Role of Toll-like receptors in costimulating cytotoxic T cell responses. *Eur J Immunol* 33: 1465 – 1470

Segura E, Touzot M, Bohineust A, Cappuccio A, Chiocchia G, Hosmalin A, Dalod M, Soumelis V, Amigorena S (2013) Human inflammatory dendritic cells induce Th17 cell differentiation. *Immunity* 38: 336 – 348

Serbina NV, Salazar-Mather TP, Biron CA, Kuziel WA, Pamer EG (2003) TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* 19: 59 – 70

Sporri R, Reis e Sousa C (2005) Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4(+) T cell populations lacking helper function. *Nat Immunol* 6: 163 – 170

Takeda K, Takeuchi O, Tsujimura T, Itami S, Adachi O, Kawai T, Sanjo H, Yoshikawa K, Terada N, Akira S (1999) Limb and skin abnormalities in mice lacking IKKalpha. *Science* 284: 313 – 316

Takeuchi O, Akira S (2010) Pattern recognition receptors and inflammation. *Cell* 140: 805 – 820

Tamoutounour S, Henri S, Lelouard H, de Bovis B, de Haar C, van der Woude CJ, Woltman AM, Reyal Y, Bonnet D, Sichien D, Bain CC, Mowat AM, Reis e Sousa C, Poulin LF, Malissen B, Guilliams M (2012) CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. *Eur J Immunol* 42: 3150 – 3166

Tamoutounour S, Guilliams M, Montanana Sanchis F, Liu H, Terhorst D, Malosse C, Pollet E, Ardouin L, Luche H, Sanchez C, Dalod M, Malissen B, Henri S (2013) Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* 39: 925 – 938

Tze LE, Horikawa K, Domaschenz H, Howard DR, Roots CM, Rigby RJ, Way DA, Ohmura-Hoshino M, Ishido S, Andoniou CE, Degli-Esposti MA, Goodnow CC (2011) CD83 increases MHC II and CD86 on dendritic cells by opposing IL-10-driven MARCH1-mediated ubiquitination and degradation. *J Exp Med* 208: 149 – 165

Vu Manh TP, Alexandre Y, Baranek T, Crozat K, Dalod M (2013) Plasmacytoid, conventional, and monocyte-derived dendritic cells undergo a profound and convergent genetic reprogramming during their maturation. *Eur J Immunol* 43: 1706 – 1715

Wang Y, Huang G, Vogel P, Neale G, Reizis B, Chi H (2012) Transforming growth factor beta-activated kinase 1 (TAK1)-dependent checkpoint in the survival of dendritic cells promotes immune homeostasis and function. *Proc Natl Acad Sci USA* 109: E343 – E352

Yamamoto M, Sato S, Mori K, Hoshino K, Takeuchi O, Takeda K, Akira S (2002) Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling. *J Immunol* 169: 6668 – 6672

Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S (2003a) Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science* 301: 640 – 643

Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T, Takeuchi O, Takeda K, Akira S (2003b) TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat Immunol* 4: 1144 – 1150

Yamazaki C, Miyamoto R, Hoshino K, Fukuda Y, Sasaki I, Saito M, Ishiguchi H, Yano T, Sugiyama T, Hemmi H, Tanaka T, Hamada E, Hirashima T, Amakawa R, Fukuhara S, Nomura S, Ito T, Kaisho T (2010) Conservation of a chemokine system, XCR1 and its ligand, XCL1, between human and mice. *Biochem Biophys Res Commun* 397: 756 – 761

Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, Hieny S, Sutterwala FS, Flavell RA, Ghosh S, Sher A (2005) TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* 308: 1626 – 1629

