Transcriptional Control of Dendritic Cell Differentiation

Izumi Sasaki and Tsuneyasu Kaisho

Abstract Dendritic cells (DCs) are professional antigen presenting cells involved critically not only in provoking innate immune responses but also in establishing adaptive immune responses. Dendritic cells are heterogenous and divided into several subsets, including plasmactyoid DCs (pDCs) and several types of conventional DCs (cDCs), which show subset-specific functions. Plasmactyoid DCs are featured by their ability to produce large amounts of type I interferons (IFNs) in response to nucleic acid sensors, TLR7 and TLR9 and involved in anti-viral immunity and pathogenesis of certain autoimmune disorders such as psoriasis. Conventional DCs include the DC subsets with high crosspresentation activity, which contributes to anti-viral and anti-tumor immunity. These subsets are generated from hematopoietic stem cells (HSCs) via several intermediate progenitors and the development is regulated by the transcriptional mechanisms in which subset-specific transcription factors play major roles. We have recently found that an Ets family transcription factor, SPI-B, which is abundantly expressed in pDCs among DC subsets, plays critical roles in functions and late stage development of pDCs. SPI-B functions in cooperation with other transcription factors, especially, interferon regulatory factor (IRF) family members. Here we review the transcription factor-based molecular mechanisms for generation and functions of DCs, mainly by focusing on the roles of SPI-B and its relatives.

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1 Introduction

Dendritic cells (DCs), in response to signaling through pathogen sensors such as Toll-like receptors (TLRs), produce various cytokines or augment expression of costimulatory molecules, thereby playing critical roles in linking innate and adaptive immunity (Banchereau and Steinman 1998; Akira et al. 2006). Dendritic cells are heterogenous and consist of several subsets, including pDCs and various types of conventional DCs (cDCs) (Liu and Nussenzweig 2010). In addition to their common properties as antigen presenting cells, each subset exhibits a subset-specific function. The DC subsets express a distinct set of pathogen sensors and respond to their signaling in a subset-specific manner. Dendritic cells development is also regulated in a DC-subset-specific manner. The development requires subset-specific transcriptional programs, in which the transcription factors expressed in a subset-specific manner are mainly involved (Belz and Nutt 2012; Moore and Anderson 2013).

2 DC Subsets and Their Development

In murine spleen, DCs are divided into pDCs and cDCs by the expression of several surface molecules. Conventional DCs can be further divided into two major subsets, CD8α⁺CD11b⁻ and CD8α⁻CD11b⁺ cDCs.

DCs are originated from hematopoietic stem cells (HSCs) in the bone marrow (BM) via intermediate progenitors (Fig. 1) (Geissmann et al. 2010). HSCs first differentiate into common myeloid progenitors (CMPs). Common myeloid
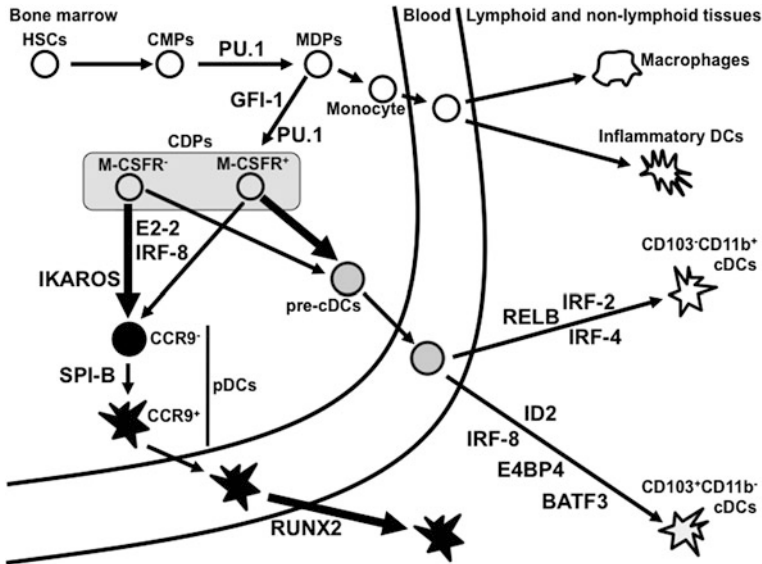
Fig. 1 *DC development in mice* DCs are generated from HSCs through several intermediate progenitors. Transcription factors involved in the developmental steps are indicated

progenitors then give rise to macrophage/DC precursors (MDPs), which retain the ability to differentiate into monocytes and DCs, but not to granulocytes. Monocytes enter the blood and reach peripheral non-lymphoid tissues, where they differentiate to macrophages or inflammatory DCs, although not to cDCs or pDCs. Macrophage/DC precursors develop to common DC precursors (CDPs), which lose the ability to differentiate into monocytes. A zinc finger-containing transcriptional repressor, GFI-1, is required for the transition from MDPs to CDPs, because GFI-1-deficient mice show reduction of all cDCs and pDCs. GFI-1-deficient hematopoietic progenitors are unable to develop into DCs, but instead differentiate into macrophages (Rathinam et al. 2005). Common DC precursors further give rise to pre-classical DCs (pre-cDCs) and pDCs. Pre-cDCs leave the BM, circulate in the blood and reach the lymphoid or non-lymphoid peripheral tissues. In the lymphoid tissues such as spleen or lymph nodes, pre-cDCs give rise to several cDC subsets, including two main subsets, $CD8\alpha^+CD103^+CD11b^-$ and $CD8\alpha^-CD103^-CD11b^+$ cDCs. Conventional DC subsets related to these two cDCs can be seen also in the non-lymphoid tissues such as skin, where CD103 is a more reliable marker to distinguish these two cDCs than CD8α. Therefore, hereafter, we refer them as $CD103^+CD11b^-$ and $CD103^-CD11b^+$ cDCs. Notably, in the intestinal tissues including mesenteric lymph nodes and lamina propria, $CD103^+CD11b^+$ cDCs are also detected as a main cDC population.

A receptor tyrosine kinase, Fms-like tyrosine kinase receptor 3 (FLT3), is expressed in DC progenitors including MDPs and CDPs. Ablation of FLT3 or FLT3 ligand (FLT3L) leads to loss of pDCs and all cDCs (McKenna et al. 2000;

Waskow et al. 2008). Furthermore, mice injected with FLT3L shows increase of all DCs (Maraskovsky et al. 1996). Thus, DC development depends on the interaction of FLT3L with FLT3.

3 Roles of SPI-B in Functions of pDCs

Plasmactyoid DCs can be distinguished from cDCs according to several surface markers including a B cell marker, B220, an I-type lectin, SIGLEC-H, or an integral membrane protein, BM stromal cell antigen 2 (BST-2) (Colonna et al. 2004). Plasmactyoid DCs are present in the BM and lymphoid tissues. Plasmactyoid DCs express nucleic acid sensing TLRs, TLR7 and TLR9, exclusively among pathogen sensors and produce vast amounts of type I IFNs, including IFN-α and IFN-ß, in response to TLR7/9 signaling (Blasius and Beutler 2010). This function of pDCs is a hallmark of pDCs. Plasmactyoid DCs detect viral infection through TLR7/9 and are involved in the anti-viral defense by producing type I IFNs. TLR7/9 can also sense host-derived endogenous nucleic acids and this sensing confers the potential to cause autoimmunity on pDCs. In homeostatic conditions, host-derived nucleic acids are unstable and degraded by tissue-derived nucleases. When nucleic acids are bound by anti-nucleic acid antibodies or anti-microbial peptides, they become stable molecular complexes, which are incorporated by pDCs and lead to pDCs activation. This is supposed to contribute to the pathogenesis of certain autoimmune disorders such as psoriasis or systemic lupus erythematosus (Gilliet et al. 2008). Thus, pDCs have both protective and pathogenic roles in immune responses.

Molecular mechanisms for pDC-specific functions to produce type I IFNs in response to TLR7/9 signaling have been progressively clarified (Fig. 2) (Honda and Taniguchi 2006; Akira et al. 2006; Kaisho and Tanaka 2008). TLR7 and TLR9 are localized in the endoplasmic reticulum in the resting state. Upon activation, they are guided by a chaperone protein, UNC93B1, to the endosome, where they bind their ligands (Beutler et al. 2007; Fukui et al. 2009). TLR7/9 have similar intracytoplasmic portions and can activate indistinguishable signal transduction cascades. The cascade depends on a cytoplasmic TLR adaptor, MyD88, and leads to activation of transcription factors, NF-κB and IRF-7 via MyD88. NF-κB and IRF-7 are critical for induction of proinflammatory cytokine and type I IFN gene expression, respectively (Honda et al. 2005; Honda and Taniguchi 2006). Several molecules are involved in IRF-7 activation (Fig. 2) (Uematsu et al. 2005; Hoshino et al. 2006; Kaisho and Tanaka 2008). IRF-7 activation requires its phosphorylation and phosphorylated IRF-7 translocates to the nucleus, where it binds to the target DNA. Thus, IRF-7 functions as a master regulator for TLR7/9-mediated type I IFN gene induction in pDCs. IRF-7 is expressed at constitutively high levels in pDCs (Izaguirre et al. 2003). This expression is further augmented rapidly and strongly upon TLR7/9 signaling. This expression pattern of IRF-7 drives positive feedback mechanisms and should contribute to the high ability of pDCs to produce
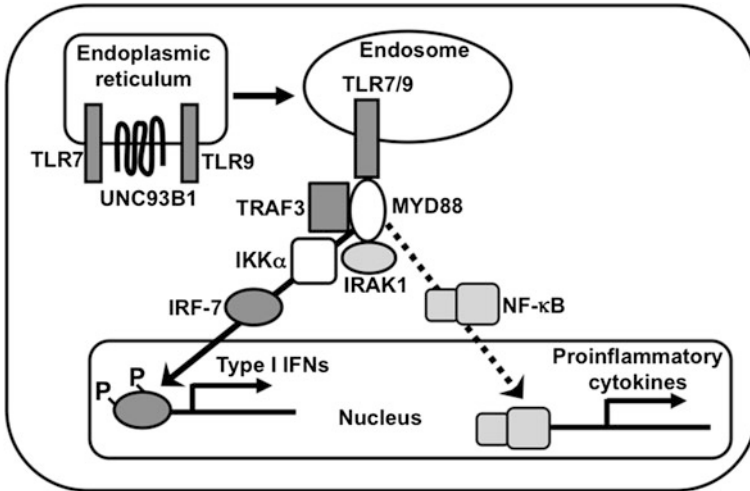
Fig. 2 *Signaling cascades of TLR7/9 in pDCs* TLR7/9 in the endoplasmic reticulum are transported with UNC93B1 to the endosome, where they meet the ligands. TLR7/9 signaling leads to induction of type I IFNs and proinflammatory cytokines via a TLR adaptor, MYD88. An adaptor, TNF receptor-associated factor 3 (TRAF3), a serine threonine kinase, IκB kinase-α (IKKα) and IL-1 receptor-associated kinase 1 (IRAK1) are involved in IRF-7 activation

type I IFNs. But IRF-7 expression is prominently induced also in cDCs stimulated by the stimuli through various TLRs including TLR4, TLR7 or TLR9 and these stimulated cDCs still fail to produce so much amount of type I IFNs as pDCs. Therefore, pDCs should have some other pDC-specific mechanisms to produce high amounts of type I IFNs.

Although a number of molecules have been shown to phosphorylate and activate IRF-7, their expression is not specific to pDCs, but rather ubiquitous. So we have analyzed gene expression profiles of DC subsets and focused on an ETS-family transcription factor, SPI-B, which is highly and constitutively expressed in pDCs (Sasaki et al. 2012).

ETS family consists of around 30 members in mammals (Oikawa and Yamada 2003). The first member, *v-ets,* was identified as a fusion oncogene of the avian transforming retrovirus E26 that can induce leukemia in chickens (Leprince et al. 1983). Then *Ets1* and *Ets2* were identified as the first cellular homologues of *v-ets* (Watson et al. 1985). Thereafter a number of cellular homologues have been revealed. All ETS family members possess an ETS domain which consists of about 85 amino acids and can bind to a purine-rich motif with a GGAA/T core consensus sequence. ETS family members regulate a variety of cellular functions, including proliferation and differentiation. Several members are involved in carcinogenesis by activating the transcription of angiogenesis-inducing genes.

Among ETS family members, SPI-B, PU.1 and SPI-C show closely related overall structures and ETS domains and form an SPI-B-related subfamily (Fig. 3).
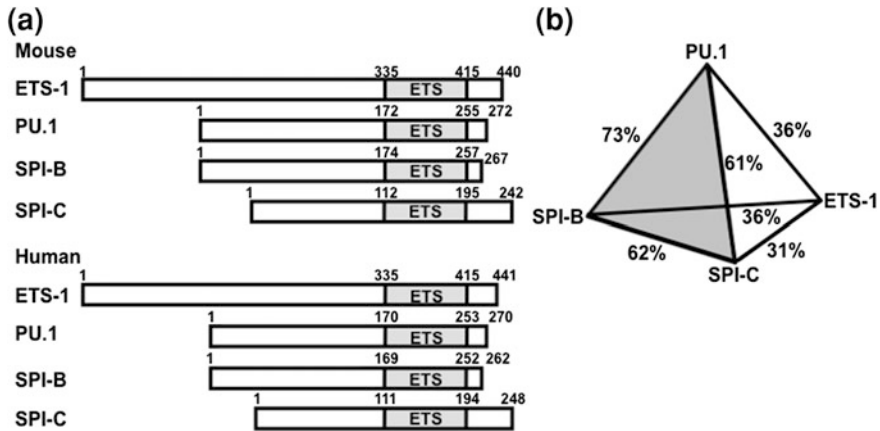
Fig. 3 *SPI-B-related subfamily* **a** Schematic representation of the overall molecular structure of ETS-1, PU.1, SPI-B, and SPI-C. The ETS domain is indicated by shaded boxes. Numbers represent positions of the amino acid residues. **b** Homology of the ETS domains of ETS-1, PU.1, SPI-B and SPI-C. The percentages of conserved amino acids in the ETS domains of indicated murine transcription factors are shown

These SPI-B subfamily members activate the promoters of target genes in cooperation with IRF family members. PU.1 can activate the target genes in cooperation with IRF-4 (Escalante et al. 2002). The target sequence is called as an ETS-IRF composite element (Brass et al. 1996). The elements were initially identified in the immunoglobulin light chain enhancers and found also in various kinds of genes. Crystal structures of the molecular complex consisting of PU.1, IRF-4 and the target DNA show that the DNA bends to juxtapose PU.1 and IRF-4 close together for selective electrostatic and hydrophobic interactions across the central minor groove (Escalante et al. 2002). This should be the basis for the synergistic effects of PU.1 with IRFs. In addition to the high expression in pDCs, this potential of SPI-B subfamily members to synergize with IRF family members highly motivated us to investigate whether or how SPI-B is involved in type I IFN gene induction in pDCs.

The activities of SPI-B on type I IFN promoters with or without IRFs are summarized in Table 1 (Sasaki et al. 2012). The *Ifna* promoter is not activated by SPI-B alone. Meanwhile, IRF-7 can activate the *Ifna* promoter and this IRF-7-induced activation is synergistically augmented when SPI-B is coexpressed. SPI-B does not show such synergistic activity when coexpressed with other IRFs. While IRF-1 can also activate the *Ifna* promoter, coexpression of SPI-B decreases the activity. The *Ifnb* promoter is activated by SPI-B, but not by IRF-7. However, as in the *Ifna* promoter, SPI-B can synergistically augment the transactivating activity with IRF-7. The synergy is also observed with IRF-4 or IRF-8, but at lesser levels than with IRF-7 (Sasaki et al. 2012). IRF-1, which can activate the *Ifnb* promoter, shows additive transactivating ability when coexpressed with SPI-B. Thus, SPI-B can transactivate the type I IFN promoters in the best synergy with IRF-7 among

Table 1 Type I IFN promoter activation 293T cells were transfected with *Ifna* or *Ifnb* promoter-driven luciferase reporter plasmids together with a combination of expression vectors for SPI-B and IRF family members

	IFN-α		IFN-ß	
SPI-B	−	+	−	+
No IRF	−	→	−	↑
IRF-1	↑	→[a]	↑	↑↑[b]
IRF-3	→	→	→	↑
IRF-4	→	→	→	↑↑↑[c]
IRF-5	→	→	→	↑
IRF-7	↑	↑↑↑[c]	→	↑↑↑[c]
IRF-8	→	→	→	↑↑↑[c]

Promoter activation is increased (↑), unchanged (→), or decreased (↓). The numbers of arrows represent the degree of activation

[a] IRF-1-induced activation was decreased by coexpression of SPI-B

[b] IRF-1-induced activation was increased additively by coexpression of SPI-B

[c] SPI-B showed synergistic activation with indicated IRFs

IRF family members, although the underlying molecular mechanisms for the synergy seem different between *Ifna* and *Ifnb* promoters. Although detailed mechanisms are still unclear, this synergy should result from the most intimate association of SPI-B with IRF-7 among IRF family members (Sasaki et al. 2012). Furthermore, analysis on SPI-B-deficient mice clarified that SPI-B is critical for TLR7/9-mediated type I IFN induction (Sasaki et al. 2012). In SPI-B-deficient mice, pDCs show defective production of type I IFNs in response to TLR7 or TLR9 stimuli. SPI-B-deficient mice also show decrease of serum type I IFN levels after injection of TLR7 or TLR9 agonists. Meanwhile, injection of double-stranded RNAs, which can induce type I IFNs through the cytosolic sensors mainly from fibroblasts, but not from pDCs, increase serum type I IFN levels similarly in both wildtype and SPI-B-deficient mice.

SPI-B-deficient pDCs also show defects in induction of TLR7/9-induced IL-12p40 and TNF-α production (Sasaki et al. 2012). This cannot be ascribed to the synergy of SPI-B with IRF-7, because the induction does not require IRF-7 but NF-κB. The NF-κB p65 subunit can activate the promoters of these proinflammatory cytokines. Although SPI-B expression alone fails to activate it, SPI-B can augment promoters of *Il12b* and *Tnf* in synergy with p65. It is unclear at present whether this synergy depends on the association of SPI-B with NF-κB, because the association could not be detected (our unpublished results). The results, however, indicate that SPI-B is also involved in TLR7/9-induced proinflammatory cytokine production in synergy with p65.

In response to TLR7/9 signaling, cDCs fail to produce IFN-α, but some, albeit not so large as in pDCs, amounts of IFN-ß and proinflammatory cytokines. Induction of IFN-ß and proinflammatory cytokines is not impaired in SPI-B-deficient cDCs. Thus SPI-B is dispensable for TLR7/9-induced cytokine production in cDCs in which SPI-B expression is low.
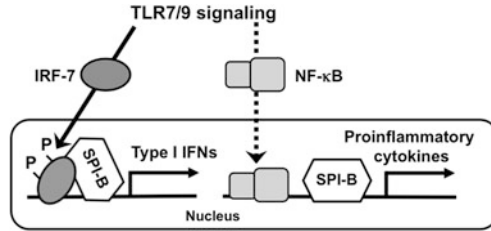
Fig. 4 *The model for the roles of SPI-B in activation of target genes* TLR7/9 signaling leads to activation and nuclear translocation of IRF-7 and NF-κB. In the nucleus, SPI-B functions in synergy with these activated transcription factors

Spib expression is constitutively high in unstimulated pDCs, but is not enhanced, rather suppressed upon TLR7/9 signaling. Although not formally tested, SPI-B is considered to be present in the nucleus. We therefore speculate that SPI-B makes the chromatins or target DNAs accessible for various transcription factors and activates the target genes in synergy with those transcription factors (Fig. 4).

4 Transcriptional Programs for pDC Generation

Knock-down of human SPI-B expression inhibits pDCs generation from CD34$^+$ precursor cells (Schotte et al. 2004). Meanwhile, SPI-B-deficient mice retain pDCs in both BM and spleen (Sasaki et al. 2012). Detailed analysis on SPI-B-deficient mice revealed novel regulatory points for pDC development. Before going into the phenotype of SPI-B-deficient mice, we describe how pDC development is regulated by transcriptional factors.

Generation of pDC depends on a pDC-specific transcription factor, E2-2 (encoded by *Tcf4*) (Fig. 1). E2-2 belongs to an E protein transcription factor family that includes basic helix-loop-helix (bHLH) transcription factors homologous to a drosophila protein, DAUGHTERLESS (Lazorchak et al. 2005). E proteins include E12, E47 (E12 and E47 are encoded by a single gene, *Tcf3*), HEB, and E2-2, which form homodimers or heterodimers with other E protein family members. E protein dimers bind E box sequences (CANNTG) with an apparent preference for C or G in the middle position. The activity of E protein is antagonized by the ID proteins (ID1-4), which sequester them into nonfunctional heterodimers. E2-2-deficient mice die at birth. So BM chimeric mice were generated and analyzed by transferring E2-2-deficient fetal liver cells into irradiated mice (Cisse et al. 2008). In E2-2-deficient chimeric mice, pDCs, defined by expression of CD11c, B220, and BST-2, are completely absent in the BM and all lymphoid tissues. Induced deletion of E2-2 in mature pDCs converts them to cDCs, indicating the roles of E2-2 in keeping the differentiation toward pDCs and against cDCs. ID2, expression of which is high in cDCs, but scarce in pDCs, can inhibit expression of pDC-specific genes and pDC

development (Spits et al. 2000). Upon deletion of E2-2, ID2 expression is induced and it drives differentiation into the cDCs. Thus, differentiation of pDCs and cDCs seems to be determined by the expression balance of E2-2 and its antagonist, ID2. Plasmactyoid DCs are derived from CDPs. Common DC precursors are heterogenous in terms of M-CSFR expression and M-CSFR⁻ CDPs have a more skewed potential to generate pDCs than M-CSFR⁺ CDPs (Fig. 1) (Onai et al. 2013). E2-2 is highly expressed in these M-CSFR⁻ CDPs. The impairment of pDC function and development is also observed in heterozygous E2-2 mutant mice, indicating the haploinsufficiency of E2-2. Furthermore, a human disease, Pitt-Hopkins syndrome, which is caused by monoallelic loss of E2-2, also shows impairment in generation and type I IFN producing ability of pDCs (Amiel et al. 2007). Thus, E2-2 is a master gene required for pDC generation and expression of pDC signature genes in both mice and humans.

Another transcription factor, IRF-8, is highly expressed in CD103⁺CD11b⁻ cDCs and pDCs (Aliberti et al. 2003; Tsujimura et al. 2003). IRF-8-deficient mice show ablation of these two DC subsets, indicating that IRF-8 is critical for generation of them (Fig. 1) (Schiavoni et al. 2002).

Transcription factors expressed more broadly also play important roles in pDC development. IKAROS is a zinc finger type of transcription factors expressed in almost all hematopoietic cells, can associate with itself and the other related members, AIOLOS and HELIOS and essential for multilineage hematopoietic cell development (Georgopoulos et al. 1994). The mice expressing a hypomorphic IKAROS mutant show selective loss of pDCs (Allman et al. 2006). So pDC generation requires higher expression levels of IKAROS than the other hematopoietic cells (Fig. 1).

A transcription factor, RUNX2, is required for pDC-specific homing to the peripheral tissues (Fig. 1). The RUNX family of transcription factors consists of RUNX1, RUNX2, and RUNX3, which are orthologous to the RUNT protein of Drosophila melanogaster (Collins et al. 2009). They form heterodimers with CBFß and regulate the expression of target genes in various cells including T lymphocytes. RUNX2 is highly expressed in pDCs among DC subsets. In RUNX2-deficient mice, peripheral pDCs in the blood and lymphoid tissues are decreased, while BM pDCs are slightly increased (Sawai et al. 2013). This defect is caused by decreased expression of a set of chemokine receptors such as CCR5 in RUNX2-deficient pDCs. Thus, RUNX2 is involved in the migration of pDCs into the peripheral lymphoid tissues. Similar homing defects of pDCs are observed also in DOCK2-deficient mice (Gotoh et al. 2008). DOCK2 is a guanine nucleotide exchange factor that regulates the actin cytoskeleton at the upstream of a small GTPase. In DOCK2-deficient mice, pDCs normally develop in the BM, but peripheral pDCs are severely decreased. DOCK2 is required for the functions of chemokine receptors for pDC homing to the peripheral tissues.

5 Roles of SPI-B in pDC Generation

In SPI-B-deficient mice, pDCs are decreased in the BM, but increased in peripheral lymphoid tissues such as spleen and lymph nodes (Sasaki et al. 2012). Plasmactyoid DCs in the circulating blood are also increased. SPI-B-deficient pDCs show defects in expression of a set of surface molecules, including a type II C-type lectin, LY49Q, BST-2, SIGLEC-H, and B220, which are expressed more abundantly in pDCs than in cDCs. The defective expression of these markers is detected in both BM and splenic pDCs, but the defects are more severe in BM than splenic pDCs. BM chimeric mice analysis indicates that the impairment is caused by intrinsic deficiency of SPI-B in pDCs.

The findings that BM pDCs are decreased, while peripheral pDCs are increased is in contrast to the phenotype of chemokine signaling defective mice and unique for SPI-B-deficient mice (Table 2). In the BM pDC development proceeds from CCR9$^-$ to CCR9$^+$ stage (Fig. 1) (Schlitzer et al. 2011). Plasmactyoid DC-specific genes are enriched in CCR9$^+$ pDCs. In the SPI-B-deficient BM, CCR9$^-$ pDCs are increased, while CCR9$^+$ pDCs are decreased. This indicates the partial developmental block at this stage in SPI-B-deficient BM pDCs. However, this deterred development cannot account for the increase of peripheral pDCs and there should be an additional defect in the SPI-B-deficient mice. In wildtype mice, BM pDCs consist of proliferating and non-proliferating populations, whereas splenic pDCs are mainly non-proliferating (Fig. 5). We can assume that non-proliferating pDCs are more mature than proliferating pDCs. Therefore, the BM contain immature and mature pDCs and the peripheral tissues such as the spleen or lymph nodes mainly contain mature pDCs. In SPI-B-deficient mice, non-proliferating mature BM pDCs are severely decreased, although proliferating immature BM pDCs are comparable with wildtype mice (WT) (Fig. 5) (Sasaki et al. 2012). This should indicate that certain steps for retaining pDCs in the BM are defective in the SPI-B-deficient mice. It can be assumed that SPI-B-deficient BM pDCs cannot be fully retained in the BM and exit from the BM in a precocious state. Such immature pDCs can somehow mature, but the maturation is not completed in the periphery. Thus, precocious exit of the BM pDCs should contribute to the phenotype of the SPI-B-deficient mice. However, the molecular basis for keeping pDCs in the BM until full maturation is unclear at present.

E2-2 directly binds to the promoters of a group of pDC-specific genes including *Irf7, Irf8* and *Spib*. Expression of these genes is decreased in heterozygous E2-2 mutant pDCs. In SPI-B-deficient pDCs, expression of *Tcf4* is not decreased. The results indicate that *Spib* is a target gene of E2-2 and that SPI-B functions downstream of E2-2. However, comparison between E2-2- and SPI-B-dependent genes revealed a group of SPI-B-dependent and E2-2-independent genes (Sasaki et al. 2012). Thus, SPI-B also has its own functions independently of E2-2 and should also contribute to pDC developmental programs in an SPI-B-specific manner.

Table 2 pDC phenotype of gene targeting mice

	RUNX2	DOCK2	SPI-B
BM	↑	→	↓
Spleen	↓	↓	↑
Lymph node	↓	↓	↑
Blood	↓	ND	↑

pDC frequencies are increased (↑), unchanged (→), or decreased (↓) in the deficient mice
ND Not done
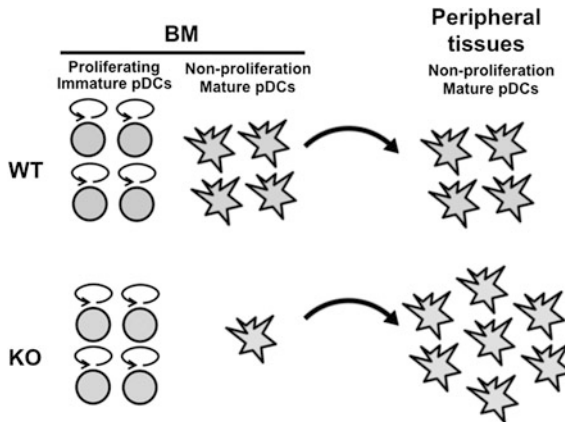
Fig. 5 *Precocious exit of SPI-B-deficient pDCs from the BM* In the WT, BM pDCs consist of both proliferating immature and nonproliferating mature pDCs, while peripheral pDCs consist of mainly nonproliferating mature pDCs. In the SPI-B-deficient mice (KO), immature pDCs are not so affected, but mature pDCs are severely decreased in the BM. Meanwhile, mature pDCs are increased in the peripheral tissues. These mature pDCs still have some functional and phenotypical defects. We assume that SPI-B deficiency should lead to precocious exit of pDCs from the BM and that SPI-B is critical for retaining the pDCs in the BM to complete their differentiation

6 Roles of SPI-B in Non-DCs

SPI-B is also expressed in B cells and the SPI-B-deficient mice were first analyzed for their B cell phenotype (Su et al. 1997). The SPI-B-deficient mice are normal in terms of basal levels of all Ig isotypes and T-independent B cell responses. However, the mutant mice show impairment in T-dependent secondary antigenic responses and produce lower levels of antigen-specific IgG1, IgG2a and IgG2b Abs, although they produce higher levels of antigen-specific IgM Abs after immunization. The SPI-B-deficient mice also show defective generation of germinal centers. SPI-B-deficient splenic B cells show poor proliferative responses against anti-IgM Abs. However, the responses against anti-CD40 Ab, IL-4 and various TLR agonists including TLR4, TLR7 and TLR9 agonists are not impaired (Su et al. 1997; Sasaki et al. 2012). Thus, SPI-B is critical for B cell receptor signaling.

SPI-B is expressed also in a subset of intestinal epithelial cells, called microfold cells (M cells). M cells are found in the follicle-associated epithelium (FAE) which covers the lymphoid follicles of gut-associated lymphoid tissues including Peyer's patches (PPs) (Neutra et al. 1996). M cells have a capacity for phagocytosis and transcytosis, thereby mediating the transport of antigens to underlying lymphoid tissues, initiating mucosal responses and leading to the T and B cell activation and generation of IgA-producing plasma cells. M cells are severely decreased in the mutant mice lacking receptor activator of nuclear factor κB ligand (RANKL), a cytokine of the TNF superfamily (Knoop et al. 2009). RANKL is produced by the mesenchymal cells in the subepithelial dome of PPs and exogenous administration of RANKL into RANKL-deficient mice rescues M cell differentiation, which is detected by sequential expression of various M cell markers. The kinetics and localization of the expression of those markers in M cells induced by RANKL are similar to those in M cells developing naturally around the birth. Shortly (1–2 h) after the treatment with RANKL, *Spib* expression is induced in a subset of FAE cells positive for *Ulex europaeus* agglutinin-1, a M cell-specific lectin. In SPI-B-deficient mice, mature M cells expressing the late stage markers, such as glyco-protein 2 or CCL9, are missing, although early stage M cell markers such as annexin V are normally expressed (Kanaya et al. 2012). SPI-B-deficient mice also show defects in the transcytotic activity across the intestinal epithelial barrier and, in the PPs, lack cells with irregular and sparse microvilli and a pocket-like invagination of the basolateral plasma membrane, which are morphological characteristics of M cells. Furthermore, SPI-B-deficient mice show impaired T cell responses against oral infection of *Salmonella typhimurium*. Thus, SPI-B is critical for generation of morphologically and functionally mature M cells (de Lau et al. 2012) (Sato et al. 2013).

Thus, not only in pDCs but also in B and M cells, SPI-B plays critical roles in the late stage development and acquisition of the functional properties of SPI-B-expressing cells rather than their early development. In the T cell development, SPI-B expression increases at the early stage, but decreases at the stage when pre-TCR signaling functions. SPI-B-deficient mice show no obvious defects in thymocyte differentiation. However, the decrease of SPI-B expression during the T cell development is critical for the cell lineage determination, because enforced expression of SPI-B arrests T cell development and instead accelerates DC development (Lefebvre et al. 2005).

Thus, SPI-B plays important roles in various cell lineages. However, the molecular mechanisms how SPI-B functions in those cells largely remain unclear.

7 Roles of PU.1 in DC and T Cell Generation

PU.1 shows 43 and 73 % identity with SPI-B at the amino acid level in the whole region and the ETS domain, respectively (Fig. 3b). PU.1 is not expressed in pDCs, but in all cDC subsets and their progenitors including MDPs and CDPs.

PU.1-deficient mice show loss of all lymphoid and myeloid progenitors and long-term multilineage repopulating HSC activity. Thus, PU.1 is critical for normal HSC activity and commitment of hematopoietic progenitors (Fig. 1) (Anderson et al. 2000). These severe defects in hematopoiesis made it difficult to clarify the roles of PU.1 in DC lineages. However, inducible deletion of PU.1 in the adult mice clarified the critical involvement of PU.1 in generation of cDCs and pDCs from CDPs (Fig. 1) (Carotta et al. 2010a). This phenotype can be ascribed to reduced expression of FLT3. PU.1 directly regulates FLT3 gene expression in a dose-dependent manner. PU.1 is also necessary for expression of other cytokine receptors including macrophage colony stimulating factor receptor (M-CSFR), granulocyte-macrophage colony stimulating factor receptor (GM-CSFR), and the interleukine 7 receptor (IL-7R) or molecules involved in DC functions. PU.1 is considered to function in synergy with IRFs. For example, PU.1 functionally interacts with IRF-8 to activate the expression of various target genes in myeloid cells (Rehli et al. 2000; Marecki et al. 2001). PU.1 also activates the cytostatin gene expression in synergy with IRF-4 in mature DCs (Xu et al. 2011). The roles of PU.1 in DCs and their progenitors are quite divergent and complex and remain largely unclear.

PU.1 is expressed in the early stage of T cell development and required for generation of T cell precursors (Carotta et al. 2010b). Multiple genes are regulated by PU.1 in early developmental stages of T cells (Zhang et al. 2012). Furthermore, decrease of PU.1 expression in T lineage cells corresponds to the commitment to T cells and loss of differentiating ability to DCs (Anderson et al. 2002). Thus, expression level of PU.1 is critical for determining the development to DCs or T cells.

8 Roles of SPI-C in Macrophages

SPI-C shows 34 and 62 % identity with SPI-B at the amino acid level in the whole region and the ETS domain, respectively, (Fig. 3b). SPI-C expression is scarce in pDCs and cDCs, but abundant in a macrophage subset, splenic red pulp macrophages. In SPI-C-deficient mice, splenic red pulp macrophages are missing (Kohyama et al. 2009). As a result, red blood cells are not efficiently phagocytosed and iron is accumulated in the red pulp, leading to splenomegaly. Vascular cell adhesion molecule (VCAM1) expression is decreased in the spleen of SPI-C-deficient mice and *Vcam1* was found to be a target gene of SPI-C. It, however, remains unclear how SPI-C is involved in generation of splenic red pulp macrophages. SPI-C is also expressed in B cells, albeit at lower levels, than in the red pulp macrophages. However, obvious B cell defect is not observed in SPI-C-deficient mice.

9 Transcriptional Programs for cDC Generation

Development of CD103$^+$CD11b$^-$ and CD103$^-$CD11b$^+$ cDCs is also regulated in a subset-specific transcriptional mechanisms.

9.1 Function and Generation of CD103$^+$CD11b$^-$ cDCs

CD103$^+$CD11b$^-$ cDCs are featured by high ability to incorporate dying cells and crosspresent Ags to generate CD8$^+$ T cell responses (Shortman and Heath 2010). Principally all DC subsets can exhibit crosspresenting activity, but CD103$^+$CD11b$^-$ cDCs highly express a set of molecules required for Ag presentation through MHC Class I. Crosspresentation includes both crosspriming and crosstolerance, which can lead to immune stimulation and immune suppression, respectively. DCs exhibit crosspriming by ingesting tumor or virally infected cells and contribute to anti-viral or anti-tumor defense. Crosstolerance can be achieved by DCs that incorporate host cells or tissues and should function for maintaining the homeostasis. Furthermore, CD103$^+$CD11b$^-$ cDCs are also featured by high ability to produce proinflammatory cytokines. In response to TLR3, TLR4 or TLR9, which recognize double-stranded-RNA, lipopolysaccharide (LPS) or CpG DNA, respectively, CD103$^+$CD11b$^-$ cDCs can produce the highest amounts of the cytokines such as IL-6 or IL-12p40 among all DC subsets.

As described above, IRF-8 is required for generation of pDCs and CD103$^+$CD11b$^-$ cDCs (Fig. 1). BXH2 mice are spontaneous mutant mice harboring a point mutation which leads to the change of arginine into cysteine in the position 294 of IRF-8 within the association domain required for the interaction with partner proteins (Schiavoni et al. 2002; Tailor et al. 2008). The mice show ablation of CD103$^+$CD11b$^-$ cDCs, but not of pDCs. This indicates that IRF-8 is involved in development of pDCs and CD103$^+$CD11b$^-$ cDCs through the differential protein interaction.

Basic leucine zipper transcription factor, ATF-like 3 (BATF3), which is also known as JUN-dimerization protein p21SNFT, belongs to a BATF family and can repress the functions of AP-1 family members, because it lacks a transcription activating domain (Dorsey et al. 1995). Although BATF3 is expressed in both CD103$^+$CD11b$^-$ and CD103$^-$CD11b$^+$ cDCs, BATF3-deficient mice show selective ablation of CD103$^+$CD11b$^-$ cDCs (Hildner et al. 2008). BATF3 is the first transcription factor shown to be critical for generation of CD103$^+$CD11b$^-$ cDCs (Fig. 1), although detailed analysis revealed the presence of CD103$^+$CD11b$^-$ cDCs in the peripheral lymph nodes of C57BL/6 BATF3-deficient mice (Edelson et al. 2011). This is due to the compensatory effects of closely related molecules, BATF and BATF2 (Tussiwand et al. 2012). However, BATF3-deficient mice have been used for a number of experiments, which clearly show the critical roles of CD103$^+$CD11b$^-$ cDCs in the crosspriming immune responses against virus,

bacteria, parasites, and tumors (Murphy et al. 2013). Critical roles of CD103$^+$CD11b$^-$ DCs in crosspriming were also shown by the analysis on the mutant mice in which CD103$^+$CD11b$^-$ DCs can be inducibly ablated upon injection of diphtheria toxin (DT). The mice were generated by knocking the gene for a fusion protein of DT receptor (DTR) and a fluorescence protein into the gene locus of a chemokine receptor, XCR1, which is specifically expressed in CD103$^+$CD11b$^-$ DCs (Yamazaki et al. 2013).

E4 promoter-binding protein 4 (E4BP4) is a basic leucine zipper transcription factor that is required for NK cell development. E4BP4 expression is not restricted to DCs and widely expressed in various tissues including macrophages. E4BP4-deficient mice also show selective loss of CD103$^+$CD11b$^-$ cDCs among DC subsets, indicating the critical involvement of E4BP4 in generation of CD103$^+$CD11b$^-$ cDCs (Fig. 1) (Kashiwada et al. 2011). BATF3 expression is decreased in E4BP4-deficient CDPs and defect in CD103$^+$CD11b$^-$ cDCs generation is rescued by enforced expression of BATF3, indicating that E4BP4 acts upstream of BATF3. As described above, ID2 expression is abundant in all cDC subsets, although low in pDCs. However, ID2 deletion leads to selective deletion of CD103$^+$CD11b$^-$ cDCs, indicating the involvement of ID2 in generation of CD103$^+$CD11b$^-$ cDCs (Fig. 1) (Ginhoux et al. 2009; Jackson et al. 2011). Thus, several transcription factors, although their expression is not specific to CD103$^+$CD11b$^-$ DCs, are required for generation of CD103$^+$CD11b$^-$ DCs.

9.2 Function and Generation of CD103$^-$CD11b$^+$ cDCs

CD103$^-$CD11b$^+$ cDCs are less characterized than the other subsets, but are mainly involved in CD4$^+$ T cell responses (Liu and Nussenzweig 2010). An NF-κB family member, RELB, is expressed highly in CD103$^-$CD11b$^+$ cDCs and is the first transcription factor identified to be critical for generation of CD103$^-$CD11b$^+$ cDCs (Fig. 1) (Burkly et al. 1995; Wu et al. 1998). NF-κB family members include p50, p52, p65, CREL, and RELB. RELB tends to dimerize with p52, while p65 and CREL tend to dimerize with p50. At present, however, the molecular mechanisms by which RELB is involved in generation of CD103$^-$CD11b$^+$ cDCs remain unclear.

IRF-2-deficient mice also show defective generation of CD103$^-$CD11b$^+$ cDCs in the spleen. This phenotype is rescued by type I IFN receptor deficiency. IRF-2 seems to function in preventing CD103$^-$CD11b$^+$ cDCs from the suppressive function of type I IFNs (Fig. 1) (Ichikawa et al. 2004; Honda et al. 2004).

IRF-4 is broadly expressed in a variety of hematopoietic cells and play important roles in B and T cells (De Silva et al. 2012). In B lineage cells, IRF-4 is critical for Ig class switch recombination and plasma cell differentiation. In T lineage cells, IRF-4 is required for Treg cells to regulate Th2 responses and for differentiation of Th2 and Th17 cells. Among splenic DCs, IRF-4 expression is high in CD103$^-$CD11b$^+$ cDCs. IRF-4 deficiency leads to selective loss of

CD103$^-$CD11b$^+$ cDCs and slight reduction of pDCs in the spleen (Suzuki et al. 2004; Tamura et al. 2005). The findings reveal the differential involvement of IRF-4 and IRF-8 in development of DC subsets (Fig. 1).

Further analysis on IRF-4-deficient mice revealed tissue- and subset-specific roles of IRF-4 in DCs. In the skin lymph nodes, one DC subset, which express CD301b, also known as macrophage galactose-type C-type lectin 2 (MGL2), was found (Kumamoto et al. 2013). This DC subset is migratory and also expresses programmed death ligand-2 (PDL2), CD11b, and CCR7, but not CD103. Thus, CD301b$^+$ DC is included in the CD103$^-$CD11b$^+$ fraction of the skin lymph nodes. A DTR gene was knocked into the *Mgl2* locus to generate the mutant mice in which CD301b$^+$ DC can be inducibly depleted. IRF-4 deficiency in CD11c-expressing cells, i.e. DCs, also leads to selective ablation of CD301b$^+$ DCs (Gao et al. 2013). These mutant mice show defective Th2 responses induced by alum, papain, or helminths. Furthermore, CD301b$^+$ DC show high ability to support in vitro Th2 cell differentiation. Thus, IRF-4 is critical for a skin lymph node DC subset, which is required for Th2 responses.

CD103$^+$CD11b$^+$ DCs can be found in the small intestine lamina propria and mesenteric lymph nodes, but not in the spleen. The intestinal CD103$^+$CD11b$^+$ DCs and splenic CD103$^-$CD11b$^+$ DCs are developmentally related, because these two DC subsets show similar expression profiles (Persson et al. 2013; Schlitzer et al. 2013). These DC subsets show high IRF-4 and low IRF-8 expression, which is found also in splenic CD103$^-$CD11b$^+$ DCs. IRF-4 deficiency in DCs leads to severe reduction of CD103$^+$CD11b$^+$ DCs in the lamina propria and mesenteric lymph nodes. IRF-4 is required for survival of CD103$^+$CD11b$^+$ DCs, although it is unclear whether IRF-4 is also involved in the developmental process. Mice with IRF-4-deficient DCs show defective Th17 responses, indicating that the CD103$^+$CD11b$^+$ DCs are critical for Th17 responses. CD103$^+$CD11b$^+$ DCs show high ability to produce IL-6, thereby contributing to Th17 responses. CD103$^+$CD11b$^+$ DCs are also found in the lung and the mutant mice with IRF-4 deficiency in DCs exhibit impaired Th17 responses against *Aspergillus fumigatus* challenge in the lung (Schlitzer et al. 2013). Thus, analysis on the IRF-4-deficient mice clarified that CD103$^+$CD11b$^+$ DCs are critical for Th17 responses in the intestine and lung. Involvement of CD103$^+$CD11b$^+$ DCs in mucosal Th17 responses was also shown by the analysis on the gene-manipulated mice in which CD103$^+$CD11b$^+$ DCs are constitutively absent due to transgenic expression of DT A subunit (Welty et al. 2013).

10 Transcription Programs for Human DC Generation

Much progress has been achieved in the molecular and cellular mechanisms for function and development of murine DCs. It should be demanded how this knowledge can be translated to human systems (Collin et al. 2011). It largely remains unknown whether there exist committed DC progenitors in humans.

However, some mature DC subsets seem preserved. Human peripheral blood contains myeloid DCs and pDCs among the cells which show high expression of MHC Class II and are negative for several cell lineage markers. Plasmactyoid DCs can be defined as blood dendritic cell antigen 4 (BDCA4)$^+$ cells. Human pDCs can produce vast amounts of type I IFNs in response to TLR7/9 signaling. In myeloid DCs, BDCA3$^+$ DCs exist as a small but significant population. The gene expression profile of BDCA3$^+$ DCs closely resembles to that of murine CD103$^+$CD11b$^-$ cells, indicating that they are corresponding subsets. Furthermore, human blood and lung CD1c$^+$ DCs are similar to murine CD103$^+$CD11b$^+$ DCs in terms of expression patterns of genes including IRF-4 and the ability to support Th17 cell responses (Schlitzer et al. 2013). Thus, accumulating evidences show that some, although not all, human DC subsets correspond to murine DC subsets.

The study of primary immunodeficiency should provide us with critical information on the molecular mechanisms for human DC generation. Pitt-Hopkins syndrome is a rare genetic disorder caused by monoallelic loss of functions or deletions of E2-2 (Cisse et al. 2008). The patients show decrease and impaired type I IFN producing capacity of pDCs. Heterozygous mutation, including autosomal dominant or de novo mutation, of a transcription factor, GATA-binding factor 2 (GATA2), is the main cause for DC, monocyte, B and NK lymphoid (DCML) deficiency, which manifests combined mononuclear cell deficiency including complete absence of blood and interstitial tissue DCs (Dickinson et al. 2011). GATA2 is a zinc finger transcription factor involved in the homeostasis of hematopoietic stem cells. Three cases of impaired DC development likely due to mutations of IRF-8 were also reported (Hambleton et al. 2011). Similar to the patients with DCML deficiency, they show susceptibility to poor virulent mycobacteria, including Bacillus Calmette-Guérin. One case carries an autosomal recessive mutation (K108E) and two cases carry an autosomal dominant sporadic mutation (T80A) in the DNA-binding domain of IRF-8. IRF-8 with K108E or T80A mutation shows defective transcriptional activity due to impaired DNA-binding ability. The IRF-8 K108E patient manifests complete loss of peripheral blood myeloid DC, pDCs, and monocytes, while the IRF-8 T80A patients have normal numbers of myeloid DCs including BDCA3$^+$ DCs, pDCs, and monocytes, but lack CD1c$^+$ DCs. The phenotypes are different from the phenotype of IRF-8-deficient mice, which show a specific loss of CD103$^+$CD11b$^-$ DCs, a murine homologue of BDCA3$^+$ DCs. In spite of discordant phenotype, IRF-8 is critical for keeping the DC homeostasis in both human and mice.

11 Conclusions

Accumulating evidences have unveiled subset-specific transcriptional programs of DC development. If the deletion of one transcription factor led to selective ablation of one DC subset, then it should contribute a lot to understanding not only the

molecular mechanisms on the development of the subset but also the critical roles of the subset. However, we should carefully consider about the difference between a transcription factor-deficient mice and DC subset-ablated mice. For example, BATF3 is expressed in CD103$^-$CD11b$^+$ cDCs, which are not deleted in BATF3-deficient mice. Therefore, the possibility that phenotype of the BATF3-deficient mice is caused by the loss of BATF3 in CD103$^-$CD11b$^+$ cDCs should be excluded. It is also possible that certain cells unexpectedly increase due to activation of alternative differentiation pathway following the deletion of one transcription factor. Taking these possibilities into consideration should be necessary in interpreting the phenotype of the transcription factor-deficient mice.

DCs function not only in the host defense but also in the pathogenesis of immune disorders. Activating or depleting specific DC subsets should lead to enhancement of the host defense or amelioration of immune disorders. Therefore, clarifying the molecular mechanisms for DC subsets development should reveal the target mechanisms or molecules for developing novel types of immunoregulatory reagents. Although there are some differences in the human and murine systems, it is still necessary and quite useful to elucidate the molecular mechanisms for DC development in mice and to analyze the human system according to the findings on mice.

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