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Transcription Factor PU.1 Is Necessary for Development of Thymic and Myeloid Progenitor-Derived Dendritic Cells

Karen L. Anderson,* Hugh Perkin,* Charles D. Suh,* Sara Venturini,* Richard A. Maki, † and Bruce E. Torbett²*

Dendritic cells (DCs) are a heterogeneous population of cells that are specialized for Ag processing and presentation. These cells are believed to derive from both myeloid- and lymphoid-committed precursors. Normal human PBMC-derived, human CD14⁺ cell (monocyte)-derived, and mouse hematopoietic progenitor-derived DCs were shown to express the hematopoietic cell-restricted, ets family transcription factor PU.1. These populations represent myeloid progenitor-derived DCs. Hematopoietic progenitor cells from PU.1 gene-disrupted (null) mice were unable to generate MHC class II⁺, CD11c⁺ myeloid-derived DCs in vitro. Mouse thymic DCs are proposed to be derived from a committed lymphoid progenitor cell that can give rise to T cells as well as DCs. Previously, we showed that CD4 and CD8 T cells developed in PU.1 null mice in a delayed manner and in reduced number. We examined the thymus of 10- to 12-day-old PU.1 null mice and found no evidence of DEC-205⁺, MIDC-8⁺ DCs in this tissue. Our findings indicate that PU.1 regulates the development of both thymic and myeloid progenitor-derived populations of DCs, and expand its known role in hematopoietic development.

Dendritic cells (DCs) are specialized APCs that are key in initiating T cell responses (1). This highly effective T cell activation is accomplished by the capacity of DCs to endocytose, process, and present Ags that are bound to cell surface class II molecules and the potent array of cell surface costimulator molecules, such as CD80 (B7-1), CD86 (B7-2), and CD40 (2, 3). Although DCs can be identified in most tissues, they differ considerably in their appearance and surface marker profile depending upon their state of maturation and their tissue residence (reviewed in Refs. 3 and 4). This heterogeneity of the DC population has contributed to the difficulty in ascertaining their origin and differentiation hierarchy. It has been established that DCs can arise from a bone marrow progenitor cell or a circulating version of this cell (5–7). However, phenotypically distinct populations of mature DCs appear to be derived from both myeloid-committed (8, 9) and lymphoid-committed (10) progenitor cells. An example of the latter is believed to reside intrathymically, and to give rise to both T cells and the thymic subset of DCs (2, 10, 11). In contrast to non-thymic tissue DCs that have a predominate role in T cell activation, the thymic DCs have a critical role in negative selection of thymocytes (for a review, see Ref. 2). It has also recently been demonstrated that postmitotic human neutrophil precursors (12) and peripheral blood monocytes of humans and mice (13–15) can be differentiated in vitro into cells displaying DC function and characteristics.

Because DCs are unique in their exceptional ability to stimulate T cells, particularly naive T cells, it is not unreasonable to propose that this function might be orchestrated by a unique set of transcription factors. A few candidate DC-critical factors have been identified. Studies have shown that RelB, a member of the NF-κB family, is expressed specifically in certain populations of DCs (16–18). RelB-deficient mice have very reduced numbers of thymic medullary and splenic DCs, as well as a lack of thymic medullary epithelial cells (17, 19). Similarly, Ikaros transcription factor-null and dominant-negative mutant mice display defects in both myeloid- and lymphoid-derived DC populations (20). The ets family transcription factor PU.1 is expressed in multiple hematopoietic lineages, as well as in human CD34⁺ progenitor cells, and can regulate the expression of many myeloid and lymphoid genes (21, 22). Previously we have shown that mice deficient in PU.1 display specific defects in the development and maturation of multiple hematopoietic lineages (23). In this study we demonstrate that PU.1 is normally expressed in mouse hematopoietic progenitor-derived CD11c⁺ MHC class II⁺ DCs, as well as in human PBMC- and CD14⁺ cell (monocyte)-derived MHC class II⁺CD11a⁺CD14⁻ DCs. In contrast to cells from wild-type mice, when hematopoietic progenitor cells from PU.1 null mice were cultured in a variety of DC-supportive/inductive growth factors, no cells with characteristic DC appearance or cell marker phenotype were produced. Furthermore, although development of a thymic cortex and medulla occurred and T cells were generated in all 8- to 12-day-old PU.1 null mice, DEC-205⁺ and MIDC-8⁺ thymic DCs could not be detected. Thus, PU.1 is required for the differentiation of the distinct myeloid-derived and thymic, lymphoid-derived populations of DCs in mice.

Materials and Methods

Mice

C57BL/6 × 129 PU.1 gene-disrupted mice were produced and identified as previously reported (23).

³Abbreviations used in this paper: DC, dendritic cell; rm, recombinant mouse; rh, recombinant human; cRPMI, complete RPMI.

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Culture medium and cytokines

Culture medium included RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS (HyClone, Logan, UT), with 5 × 10⁻³ M 2-ME (Sigma, St. Louis, MO), 1% t-glutamine (Life Technologies), 1% penicillin/streptomycin (Life Technologies), termed complete RPMI (cRPMI), and IMDM (Life Technologies) supplemented with 10% FCS (HyClone), with 5 × 10⁻³ M 2-ME (Sigma), 1% t-glutamine (Life Technologies), 1% penicillin/streptomycin (Life Technologies), termed complete IMDM (cIMDM). The following human and mouse cytokines were used: recombinant mouse rm-GM-CSF (R&D Systems, Minneapolis, MN), rm-TNF-α (R&D Systems), recombinant rat (rr) stem cell factor (SCF, Amgen, Thousand Oaks, CA), rm-IL-3 (R&D Systems), recombinant human (rh)-GM-CSF (Amgen), rh-IL-4 (R&D Systems), rh-IL-6 (R&D Systems), rh-M-CSF (a kind gift from Dr. David Hume, University of British Columbia, Vancouver, Canada), and rh-TNF-α (R&D Systems).

Multiparameter flow cytometry analysis for cell surface Ags

Three- and four-color flow cytometric analysis was performed as described for mouse and human cells (23) using a Becton Dickinson FACScanBurchell and CellQuest software (Becton Dickinson, San Jose, CA). Aliquot sizes used for flow cytometric analysis were directly conjugated with fluorochromes of choice. Abs used for mouse cell analyses included I-A^d, CD11b, CD11c, Gr-1, CD3, B220, C57, and F4/80 (all from PharMingen, San Diego, CA), and C57, Gr-1, and B220 (Becton Dickinson). All reagents were from Becton Dickinson, and CellQuest (Becton Dickinson, San Diego, CA). CD11c^+ cells were run through two VarioMACS separation columns (Miltenyi Biotec). The purity and efficiency of the CD11c^+ cell separation was evaluated by staining the positively selected and negatively depleted cell fractions with CD145, CD19, CD14, CD3, CD4, CD8, CD83, and CD33 by three- and four-color flow cytometry. CD14^+ purity of cells isolated after positive selection was found to be ≥98%. These cells were cultured identically to whole PBMCs for DC differentiation.

To produce human macrophages, 5 × 10⁶ PBMCs were plated on tissue culture plates in 10 ml of cRPMI, incubated for 5 h at 37°C in a humidified incubator, nonadherent cells were removed, fresh medium containing 5000 U/ml of rh-M-CSF was added, and nonadherent cells were removed the next day. Cultures were fed every 3–4 days by one-half medium changes, and cells were used on days 7–12.

Western blotting

Cell lysates were prepared and SDS-PAGE was performed as described (27). Polyclonal anti-Pu.1 and anti-RelB Abs were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and anti-Actin Ab from Sigma was used at 1:500, 1:500, and 1:200 dilution, respectively. Secondary anti-rabbit Ab was obtained from Santa Cruz Biotechnology. CrossMarker protein standards (Santa Cruz Biotechnology) were included on gels to determine the size of immunoreactive proteins.

Results

Mouse and human DCs express Pu.1

Because Pu.1 has been shown to play a critical role in regulating both the development of multiple lineages and the function of mature hematopoietic cells (23, 27), we examined normal mouse bone marrow-derived and human PBMC-derived DCs for Pu.1 expression. Low density cells were isolated from wild-type mouse bone marrow and cultured in GM-CSF plus TNF-α or M-CSF, cytokines that promote either DC or macrophage generation, respectively (see Materials and Methods). To ensure a relatively pure population of mouse DCs for Pu.1 analysis, CD11c^+ cells were isolated from DC cultures after 11–12 days using immunomagnetic beads. This strategy was utilized because CD11c is present on essentially all mouse myeloid DC populations (3, 4). These isolated cells were recultured for 24 h under DC-promoting conditions to allow recycling of Ab-bound CD11c. As can be seen from flow cytometry analysis, cells obtained from cultures promoting macrophage differentiation were class II^null (I-A^d), and CD11c^-, a cell surface phenotype characteristic of macrophages, whereas cells obtained from cultures promoting DC differentiation were CD11c^+, and class II^high (I-A^d), and CD11c^-, a cell surface phenotype characteristic of DCs (Fig. 1A). Cells from both macrophage and DC cultures were F4/80^+, and Gr-1^-, CD3^−, and B220^− (data not shown). Both populations were also examined for the presence of RelB protein (Fig. 1C), a transcription factor known to be expressed in DCs but not macrophages (16, 17). Expression of RelB was consistent with the flow cytometry analysis demonstrating that DCs were generated under our culture conditions.

Mature PBMCs, specifically monocytes, have been shown to be capable of undergoing DC differentiation following stimulation with appropriate growth factors in vitro (13–15). Furthermore, the existence of circulating dendritic precursor cells in humans and mice has also been established (6, 29). We next examined whether Pu.1 is expressed in human DCs derived from whole PBMCs and isolated CD14^+ cells from PBMCs. Human PBMCs were isolated

isolated from PBMCs using anti-CD14 Ab and a VarioMACS apparatus as specified by the manufacturer. To increase purity, positively selected CD14^+ cells were run through two V5^+ separation columns (Miltenyi Biotec). The purity and efficiency of the CD14^+ cell separation was evaluated by staining the positively selected and negatively depleted cell fractions with CD145, CD19, CD14, CD3, CD4, CD8, CD83, and CD33 by four-color flow cytometry. CD14^+ purity of cells isolated after positive selection was found to be ≥98%. These cells were cultured identically to whole PBMCs for DC differentiation.
FIGURE 1. Murine bone marrow progenitor-derived DCs and human CD14+ cell-derived DCs express PU.1 protein. A, Low-density mononuclear progenitor cells were separated from murine bone marrow (Mouse cells) and cultured to promote macrophage (Macrophage) or DC (Dendritic cell) differentiation as described in Materials and Methods. Cultured cells were analyzed for class II (I-A<sup>+</sup>) and CD11c expression by flow cytometry (n = 3). CD11c<sup>+</sup> cells were purified and used for the protein analysis shown below. B, CD14<sup>+</sup> cells were isolated from human PBMCs (Human cells) and cultured under macrophage- and DC-promoting conditions. HLA-DR and CD14 flow cytometry data are shown for CD14<sup>+</sup> cell-derived DCs (n = 3), and results obtained from PBMC-derived DCs were identical (n = 3, but results not shown). Ig controls consisted of irrelevant species- and isotype-matched Abs. C, A total of 35 μg of total cell protein extract was separated on a 10% SDS polyacrylamide gel, transferred to a membrane, and probed with polyclonal anti-RelB Ab. Immunoreactive protein was seen in murine CD11c<sup>+</sup> bone marrow-derived DC (mBM-DC), human PBMC-derived DC (hPBMC-DC), and CD14<sup>+</sup> cell-derived DC (CD14-DC) samples as well as the murine T cell line BW5147T, but not in murine macrophages (MACS) or human HT1080 cells. D, After probing with a polyclonal anti-PU.1 Ab, band(s) representing the PU.1 protein were seen in extracts from the murine B cell line A20-2J, both murine and human DC samples (described in C) but not in BW5147T cells or HT1080 cells. Blots were stripped and reprobed with anti-actin Ab to check equivalence of sample loading (data not shown). CruzMarker (Santa Cruz Biotechnology) protein size standards were run in the lane marked MW; sizes of standards in kDa is shown to the right of the panel.

and cultured in M-CSF or GM-CSF and IL-4 to induce macrophage and DC differentiation as described in Materials and Methods. To determine the status of PU.1 expression in monocyte-derived DCs, human CD14<sup>+</sup> cells were isolated from peripheral blood (see Materials and Methods) and cultured using the same conditions. Following an 8-day culture period in M-CSF or GM-CSF and IL-4, the surface marker profile of CD14<sup>+</sup> cell-derived macrophages and DCs was examined by flow cytometry analysis. Fig. 1B shows an example of flow cytometry analysis of cells from macrophage and DC cultures. Cells cultured under conditions that promote macrophage differentiation were HLA-DR<sup>+</sup> and CD14<sup>+</sup> (Fig. 1B), CD1a<sup>dull</sup>, and CD86<sup>+</sup> (data not shown), consistent with a macrophage phenotype, whereas cells from DC cultures were HLA-DR<sup>bright</sup>, CD14<sup>+</sup> (Fig. 1B), CD1a<sup>bright</sup>, and CD86<sup>+</sup> (data not shown), consistent with a DC phenotype. Both human PBMC- and CD14-derived DCs demonstrated similar cell surface phenotypes; however, only analysis of CD14-derived DCs is shown (Fig. 1B). Cells from both macrophage and DC cultures were negative for the T cell marker CD3 and the B cell marker CD19 (data not shown).

The human and mouse myeloid DCs that were described above were examined for PU.1 expression. As shown in Fig. 1D, Western blot analysis clearly demonstrated the presence of PU.1 protein in human and mouse DCs. Low-level B cell contamination, a possible contributor of PU.1 protein, was ruled out by RT-PCR analysis for CD19 (data not shown). Thus, we have demonstrated that myeloid DCs generated from either mouse bone marrow progenitors or human peripheral blood cells express the transcription factor PU.1.

**PU.1-deficient mouse hematopoietic progenitor cells fail to generate DCs in culture**

Hematopoietic progenitor cells derived from normal mice have been shown to be capable of differentiating into mature, functional DCs under appropriate culture conditions (Refs. 25, 30, and 31, and above). To determine whether PU.1-deficient hematopoietic progenitors were able to generate such cells in vitro, we isolated and pooled low density mononuclear cells from the liver and bone marrow of PU.1 null neonates. This step was required to obtain sufficient hematopoietic progenitors because the bone marrow of PU.1 null mice is vastly reduced in all hematopoietic cells, due at least in part to osteopetrosis (24, 32). These progenitors were cultured using the method of Lutz et al. (25) with the addition of IL-3 to the culture medium in some cases, because in its absence all PU.1-deficient cells died (Table I and Ref. 24). Our previous work has shown that PU.1 null cells require IL-3 for survival and growth, and fail to express detectable cell surface receptors for and proliferate to GM-CSF (24). Following the culture period, the cells were analyzed by flow cytometry for their expression of DC and myeloid markers, the results of which are summarized in Table I. Only PU.1 null cells maintained in IL-3 and GM-CSF survived, and cells compatible with a DC phenotype were not detectable in any cultures established from PU.1 null mice. Lastly, PU.1 null
cells cultured under these conditions expressed Gr-1 (Table I) and morphologically were similar to immature neutrophils, as reported previously (24, 27).

Macrophages and thymic DCs cannot be detected in the thymus of PU.1 null mice

Our in vitro studies demonstrated the inability of PU.1 null hematopoietic progenitors to produce myeloid DCs under cytokine conditions appropriate for wild-type cells. Recently, it has been shown that populations of lymphoid lineage-derived DCs, including those present in the thymus, can develop in vivo in GM-CSF-cytokine and -receptor null mice (33, 34). Given the absence of GM-CSF receptor expression in PU.1 null mice, we next addressed whether the loss of PU.1 expression affected the development of thymic DCs. Rudimentary thymus from fetal (35) and neonatal (23) PU.1 null mice is devoid of T cells. However, if mice are kept alive using intensive antibiotic therapy, thymic development is observed to begin between 5 and 8 days after birth (23). CD4 and CD8 single- and double-positive cells are found in approximately normal proportions, although their number is 5- to 10-fold reduced compared with normal litters (23). Histologic examination of the thymus of a 10-day-old PU.1 null mouse clearly demonstrated cortical and medullary areas (Fig. 2, A, D, F, and H; PU.1 null) and the presence of double positive and single positive CD4 and CD8 cells by flow cytometry (data not shown). Previously, we demonstrated that PU.1 was required for development of monocytes/macrophages in the blood, spleen, and liver (23). Consistent with these results, F4/80+ cells were not detected in the thymus of a 10-day-old PU.1 null mouse (Fig. 2, compare A and B). Immature thymocytes have a rapid turnover, due to the majority of thymocytes not undergoing positive selection and subsequently undergoing apoptosis (28). In the cortex of a normal thymus these apoptotic cells are rapidly engulfed by F4/80+ macrophages (28). The presence of a few apoptotic cells, identified by the TUNEL method, could be seen in the thymic cortex of a 10-day-old wild-type mouse (Fig. 2C). In contrast, the thymic cortex and medulla of a PU.1 null littermate contained tremendously elevated numbers of apoptotic (dark red-purple staining) cells (Fig. 2D), presumably the result of the absence of clearance by macrophages. Also, visible in the cortical and medullary regions was intensely blue-purple counterstained (hematoxylin) cellular debris. Therefore, it appeared that phagocytic cells normally responsible for clearance of dead and dying cells in the thymus were highly reduced, absent, and/or not functional in PU.1 null mice.

To determine whether thymic DCs were affected by the absence of PU.1, serial sections from the same null thymi used for F4/80 immunohistology and TUNEL analysis were stained with two Abs known to identify thymic DCs: DEC-205 and the DC subset marker MIDC-8. DEC-205, the multilectin receptor (36) related to the macrophage mannose receptor, is expressed in a number of DC populations, including most thymic DCs that are predominantly localized in the medulla (2, 37). In contrast to the wild-type littermate controls (Fig. 2E), we were unable to detect median staining for DEC-205 in thymus sections from a PU.1 null mouse (Fig. 2F). As reported (38–40), DEC-205 was expressed on thymic cortical epithelial cells in both wild-type and PU.1 null mice (Fig. 2, E and F). MIDC-8, which recognizes intracellular granules in thymic medullary DCs (41), was also used for immunohistology. As was seen for DEC-205, MIDC-8+ cells were readily apparent in normal thymus but not the PU.1 null thymus (Fig. 2, G and H). Thus, our combined data from PU.1 null mice demonstrate ongoing T cell differentiation and the presence of a thymic cortex and medulla. However, PU.1 null thymi lack F4/80+ macrophages, phagocytic clearance of dead and dying cells, and detectable DEC-205+, MIDC-8+ DCs.

Discussion

The transcription factor PU.1 has previously been shown to be expressed in human CD34+ progenitors and in neutrophils, monocytes/macrophages, mast cells, early erythroid cells, and B cells (21, 42, 43), but not T cells (23, 43). In this study we have demonstrated that human and mouse myeloid-derived DCs also express PU.1. A vital role for PU.1 in myeloid DC development is suggested by the absence of development of DCs from primary PU.1 null hematopoietic progenitors under appropriate DC-supportive culture conditions. Furthermore, DCs could not be readily detected in the thymus of neonatal to 10- to 12-day-old PU.1 null mice, providing evidence that PU.1 also must play an important role in the development of the distinct thymic DC population.

DCs have been shown to derive from a bone marrow progenitor by both allogeneic bone marrow transplantation studies and in vitro culture techniques. These types of experiments have identified a MHC class II-negative mouse progenitor shared by DCs, neutrophils, and monocytes/macrophages (8), although more recently a distinct DC-CFU cell has been demonstrated in human bone marrow (44). Despite some lack of certainty in their lineage relationship, it is clear that DCs share many similarities with myeloid cells, although key differences also exist. The demonstration that peripheral blood monocytes and postmitotic neutrophil precursors can both be induced to differentiate to a DC phenotype is further evidence for this close relationship (12–14). Because PU.1 is expressed in bone marrow-derived myeloid lineages, it was not unexpected to find PU.1 expression in DCs derived from mouse bone marrow progenitor cells as well as in human peripheral blood cell/monocyte-derived DCs. These DC populations are considered to be of myeloid origin, unlike the thymic DCs and some splenic DC populations, which are believed to originate from a lymphoid-committed precursor cell (10, 11, 34, 45). To our knowledge, this study represents the first direct demonstration of expression of PU.1 in human and mouse myeloid DCs.

Populations of DCs are heterogeneous in their phenotype, but are broadly grouped into those derived from a myeloid (bone-marrow) progenitor and those derived from a lymphoid progenitor. The latter have been identified in the mouse thymus by repopulation studies using purified thymic progenitor cells, and indicate the existence of a precursor cell capable of developing into T cells or DCs (10, 11). Although we have not demonstrated directly that PU.1 is expressed in thymic DCs, the results of our studies would

Table I. Cells expressing a DC phenotype cannot be cultured from PU.1 null hematopoietic progenitors

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<th>Ab</th>
<th>Percentage of Marker Positive Cells After Culture</th>
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† Low density mononuclear cells were prepared from normal bone marrow and PU.1 null bone marrow/liver, and then cultured for DCs for 7-12 days as described in Materials and Methods. Following the culture period, cells were stained with a panel of mAbs, and percentage of positive staining cells are shown above. Results are shown from a representative experiment; three experiments were performed with similar results.

4 Sample was pooled bone marrow and liver cells cultured in GM-CSF.

3 Sample was pooled bone marrow and liver cells cultured in GM-CSF and IL-3.

2 Sample was bone marrow in GM-CSF.

1 Few cells survived in GM-CSF after 1 wk.
indicate that it plays a vital role in the development of this population. We found no DEC-205⁺, MIDC-8⁺ cells in the thymus of 10- to 12-day-old PU.1 null mice, although CD4⁺, CD8⁺ single- and double-positive T cells were present. Similarly, T cells but not DEC-205⁺ cells were found when spleens from 12-day-old PU.1 null mice were analyzed by flow cytometry and immunohistology (B. E. Torbett and C. D. Surh, unpublished results). Although PU.1 expression is high in B cells, it has not been found at any stage of definitive T cell development yet examined. If PU.1 is expressed in thymic DCs, perhaps its down-regulation represents a critical decision point in the determination of thymic T cell vs thymic DC differentiation. This question must remain open at present. Because the markers we examined represent markers for mature DCs, we cannot exclude the possibility that committed DC precursors have

FIGURE 2. Phagocytes and DCs are absent from the thymus of PU.1 null mice. Frozen sections of thymus from 10-day-old wild-type and PU.1 null mice were prepared and subjected to TUNEL assay or stained with a series of Abs. Note the presence of a distinct cortex and medulla (labeled cor and med in E, F, G, and H) in the PU.1 null as well as the normal tissue. Also note presence of intensely but nonspecifically blue/purple-stained cell debris at the cortico-medullary boundary and in the medulla in PU.1 null sections. A and B, Staining for the mature macrophage marker F4/80 was abundant in the normal thymus but absent from the PU.1 null thymus. C and D, TUNEL assay (TdT) revealed few detectable apoptotic cells in normal thymus; in contrast, high numbers of apoptotic thymocytes were seen in PU.1 null thymus. E and F, The multilectin receptor DEC-205 was expressed on thymic cortical epithelial cells in normal and at a reduced level in PU.1 null mice. However, whereas cells in the medulla of the normal thymus reacted with DEC-205, consistent with thymic medullary DCs, no positive-staining cells were seen in the medulla of PU.1 null thymus. G and H, Another marker of DCs, MIDC-8, was expressed abundantly in the medulla of normal thymus but was not detectable in the PU.1 null thymus. Magnification, ×165.
been generated in PU.1 null mice but are unable to fully differentiate in the absence of PU.1 due to other PU.1-related defects.

We observed failure of both lymphoid-derived and myeloid-derived DC development in the absence of PU.1. This suggests either that PU.1 may be required for specification of the DC developmental program in a very early shared progenitor (such as the human CD34+ cell), or that PU.1 is required for expression of multiple phenotype-defining genes that are similarly expressed in both the lymphoid and myeloid-derived DCs. One possibility is that the absence of the PU.1 regulated GM-CSF receptor expression (24, 46) in PU.1 null myeloid cells contributes to the loss of myeloid DC development in vitro (34). However, both myeloid and lymphoid DCs were found in GM-CSF and GM-CSF receptor null mice (34). A similar loss of both myeloid and lymphoid DCs has been observed in mice that express a nonfunctional or a dominant-negative form of the Ikaros zinc-finger transcription factor (20). Another transcription factor, the NF-kB family member RelB, was found to be expressed in inducting DCs (16) in which it is expressed at high levels (18). RelB gene-disrupted mice failed to develop myeloid DCs, but also had very low numbers of thymic, lymphoid DCs as a result of abnormal thymic architecture (17, 18). Although we have directly demonstrated PU.1 expression in myeloid DCs, its expression in lymphoid DCs is unresolved and we can only speculate as to whether the absence of PU.1 has a direct or an indirect effect on thymic DC development. Unlike RelB-deficient mice (17), however, the thymic architecture of PU.1 null mice appears intact and medullary epithelial cells were present based on UEA-1 immunostaining (C. D. Surh, unpublished results).

In summary, we have shown that PU.1 is expressed in and is crucial for development of myeloid DCs. Lymphoid DC development in the mouse thymus is also disrupted in the absence of PU.1, although the extent and nature of the disruption will require further investigation. Nevertheless, it is clear that PU.1 is required for normal development of both major mouse DC populations. Our data from the PU.1 null mouse model suggest that the formation of a thymic cortex and medulla, and the homing and expansion of T cell progenitors, can occur in the absence of accessory cells such as macrophages and DCs.

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