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Article

The Transcription Factor PU.1 Controls Dendritic Cell Development and Flt3 Cytokine Receptor Expression in a Dose-Dependent Manner

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SUMMARY

The transcription factor PU.1 plays multiple context and concentration dependent roles in lymphoid and myeloid cell development. Here we showed that PU.1 (encoded by *Sfpi1*) was essential for dendritic cell (DC) development in vivo and that conditional ablation of PU.1 in defined precursors, including the common DC progenitor, blocked Flt3 ligand-induced DC generation in vitro. PU.1 was also required for the parallel granulocyte-macrophage colony stimulating factor-induced DC pathway from early hematopoietic progenitors. Molecular studies demonstrated that PU.1 directly regulated *Flt3* in a concentration-dependent manner, as *Sfpi1*$^{+/-}$ cells displayed reduced expression of *Flt3* and impaired DC formation. These studies identify PU.1 as a critical regulator of both conventional and plasmacytoid DC development and provide one mechanism how altered PU.1 concentration can have profound functional consequences for hematopoietic cell development.

INTRODUCTION

The ETS family transcription factor PU.1 plays multiple roles in hematopoiesis. PU.1 (encoded by the gene *Sfpi1*) regulates numerous genes within the myeloid and lymphoid lineages, including those encoding the developmentally important cytokine receptors, macrophage colony stimulating factor receptor (M-CSFR), granulocyte-macrophage colony stimulating factor receptor (GM-CSFR), and the interleukin 7 receptor (IL-7R, reviewed in Dakic et al., 2007). Mice with *Sfpi1* germline mutations have profound impairment in hematopoiesis and die either in late gestation or shortly after birth (McKercher et al., 1996; Scott et al., 1994). In contrast, enforced expression of *Sfpi1* in hematopoietic progenitors suggests an instructive and concentration-dependent role of PU.1 in promoting macrophage and dendritic cell (DC) development (Bakri et al., 2005; DeKoter and Singh, 2000; Laiosa et al., 2006; Nerlov and Graf, 1998; Spooner et al., 2009). Studies using mice with either a conditional mutation that allows inactivation of *Sfpi1* in adult hematopoietic cells or with reduced *Sfpi1* expression throughout adult hematopoiesis demonstrated that PU.1 deficiency resulted in dramatically perturbed hematopoiesis and greatly enhanced granulopoiesis (Dakic et al., 2005; Houston et al., 2007; Iwasaki et al., 2005; Rosenbauer et al., 2004). Analysis of the multipotent progenitors revealed that PU.1 ablation led to the loss of all identifiable lymphoid and myeloid progenitor populations and long-term multilineage repopulating hematopoietic stem cell (HSC) activity (Dakic et al., 2005; Iwasaki et al., 2005). These findings demonstrate that PU.1 is required for the maintenance of normal HSC function and the regulation of commitment of adult hematopoietic progenitors.

The concentration-dependent functions of PU.1 in cell-fate decisions are mirrored by the dynamic nature of *Sfpi1* expression during hematopoiesis. Using PU.1-GFP reporter mice, we and others have demonstrated that within adult hematopoietic progenitors, *Sfpi1* is highly expressed in the HSCs and the earliest myeloid and lymphoid progenitors but is downregulated in megakaryocyte-erythrocyte lineage precursors (Back et al., 2005; Nutt et al., 2005). Upon lineage commitment, *Sfpi1* is expressed highly by myeloid cells, at low amounts by B cells, and is silenced in the erythrocytic lineage, in mature NK cells and in developing thymocytes. Within the DC lineage, *Sfpi1* shows a bimodal expression pattern, being highly expressed by conventional DCs (cDCs) but present in low amounts in plasmacytoid DCs (pDCs) (Back et al., 2005; Nutt et al., 2005).

DCs are bone marrow (BM)-derived antigen-presenting cells of the immune system that play important roles in regulating immune responses. Studies on the developmental origin of DCs showed that both cDCs and pDCs could be generated by *Flt3*-expressing myeloid or lymphoid progenitors, as well as by the common DC progenitors (CDPs) (D'Amico and Wu, 2003; Karsunky et al., 2003; Naik et al., 2007; Onai et al., 2007). Flt3 is a receptor tyrosine kinase that is expressed on a subset of multipotent progenitors. Mice with a deficiency in Flt3 or Flt3 ligand (Flt3L) exhibited reduced DC numbers (McKenna et al., 2000; Waskow et al., 2008), whereas mice treated with Flt3L showed a dramatic increase in DC numbers (Maraskovsky et al., 1996). These observations indicate an essential role of Flt3 in steady-state DC development; however, the regulation of *Flt3* expression remains poorly understood.

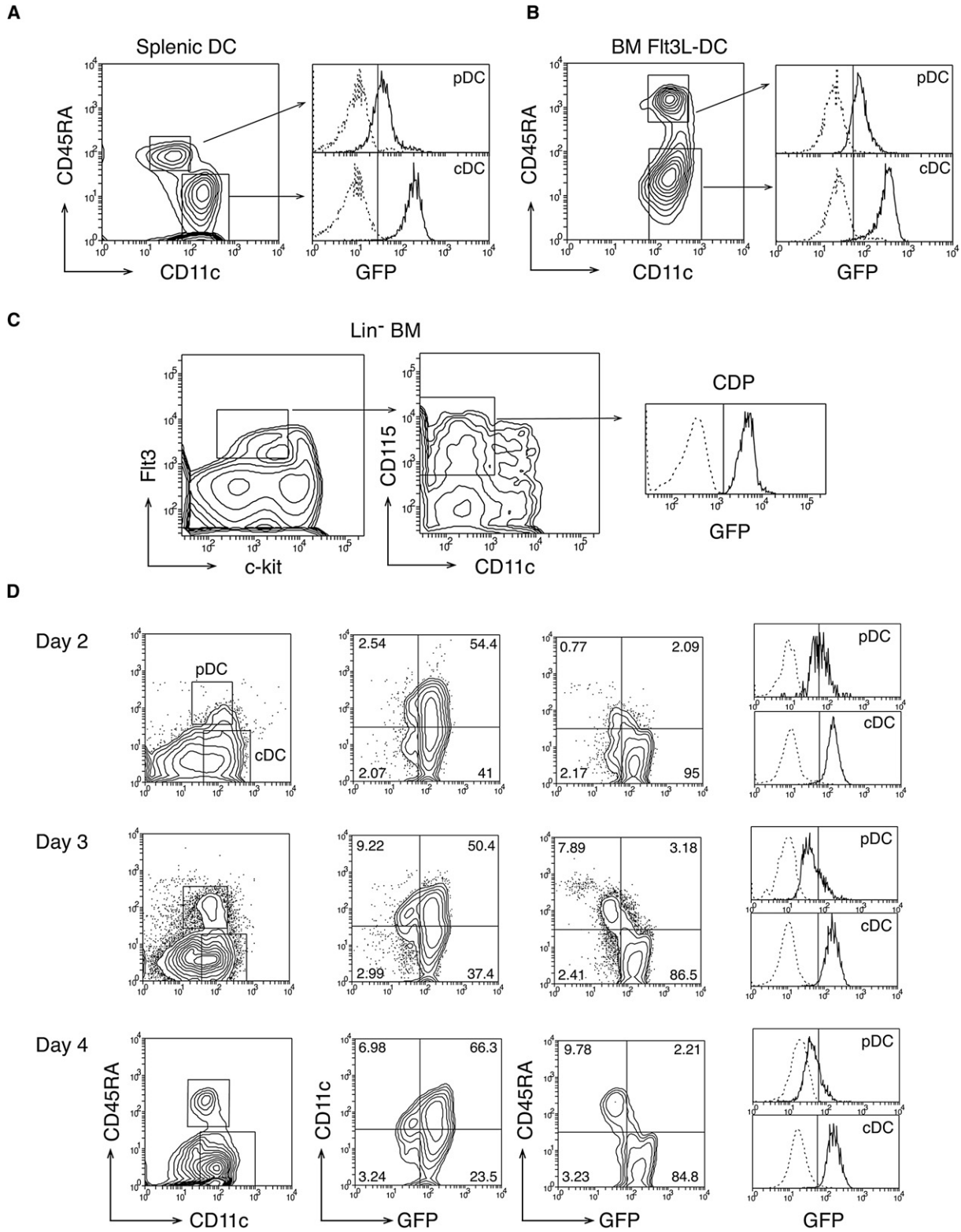

Figure 1. Expression of PU.1 by Mouse DC Populations

(A and B) Splenic DCs (A) and Flt3L BM cell cultures (B) were analyzed by flow cytometry for the expression of PU.1-GFP in gated cDC ($CD11c^{hi}CD45RA^{-}$) and pDC ($CD11c^{int}CD45RA^{+}$) populations.

(C) CDPs were identified from Lin^{-} BM as $Flt3^{+}c\text{-}kit^{lo}$ (left panel) and $CD115^{+}CD11c^{-}$ (middle panel) and analyzed for PU.1-GFP.