Published in final edited form as:

Eur J Immunol. 2008 September ; 38(9): 2386–2388. doi:10.1002/eji.200838763.

E-box protein E2-2 is a crucial regulator of plasmacytoid DC development

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Abstract

DC play central roles in priming both innate and adaptive immune responses. Multiple DC subsets have been identified on the basis of their phenotype and function. Plasmacytoid DC (pDC) are professional IFN-producing cells that play an essential role in anti-viral immunity. A series of recent studies demonstrates that the regulation of pDC development is different from other types of DC. In this issue of the *European Journal of Immunology*, new insight is provided into how human pDC development is regulated by various transcription factors, in particular by the Ets family protein Spi-B and E-box protein E2-2.

Keywords

DC; Developmental immunology; Transcription factors

DC serve a critical role in the immune system as pathogen sensors and activators of adaptive immunity. Several DC subsets exist, which are distinguished by differences in their surface phenotype, tissue localization, antigen presentation function and cytokine secretion. Plasmacytoid DC (pDC) and conventional DC (cDC) are two principal DC subsets in the peripheral blood and tissue [1]. Murine cDC can be further categorized into three subsets, based on their surface expression of CD4 and CD8, *i.e.* CD4⁺/CD8[−], CD4[−]/CD8⁺ and double negative [1]. pDC are professional type I IFN-producing cells of the immune system, capable of secreting 100–1000-fold higher levels of IFN than other blood cell types following infection [1]. Subsequent to activation and IFN secretion, pDC undergo maturation and adopt antigen-presentation function [1, 2]. pDC have an important role in establishing anti-viral status and shaping the adaptive immune response, while cDC are potent stimulators of T-cell responses by virtue of their inherent capacity for antigen-presentation and cytokine secretion.

It has taken many years to understand how DC development is regulated, *i.e.* which cell population/s is/are the progenitor/s of DC? What kinds of factors control the development of DC? And what is/are the mechanism/s through which these factors regulate DC development? It is well established that DC are derived from bone marrow HSC. The Flt3⁺ bone marrow subset, which is developmentally restricted compared with HSC [3], contains the majority of progenitors for pDC as well as cDC subsets [4]. Compelling evidence points to an essential function for Flt3 and its ligand (Flt3L) in DC development [5–8]. Flt3L

stimulates the growth of cDC and pDC *in vitro* from hematopoietic progenitors isolated from the bone marrow and the fetal liver [5, 6]. Administration of Flt3L *in vivo* induces a dramatic rise in the number of DC in the bone marrow, spleen and other peripheral tissues, as well as mobilization of DC into circulation [7, 8]. Recent evidence suggests that Flt3L signaling induces certain hematopoietic progenitor subsets to develop preferentially along the pDC and cDC lineages *via* a mechanism that involves several transcription factors [9–11]; for example, deletion of STAT3 in the hematopoietic system greatly reduces the number of CD11c⁺ DC (both cDC and pDC) and suppresses responses to Flt3L *in vivo* [9], while deletion of the Ets family transcription factor PU.1 causes severe deficiency in cDC development [12, 13].

It has been demonstrated that pDC and cDC are also induced from human CD34⁺ hematopoietic progenitors in Flt3L-supplemented cultures. By using this culture system, Spits *et al.* showed that the basic helix–loop–helix transcription factor Id2 blocks the development of pDC but not cDC [14]. Furthermore, gene knockdown of Spi-B, an Ets family transcription factor, strongly inhibited pDC development from CD34⁺ progenitors [15]. Although these studies demonstrated that these transcription factors play crucial roles in pDC development, further investigation is required for a detailed understanding of the molecular machinery involved.

In this issue of the *European Journal of Immunology*, Nagasawa *et al.* [16] show that the E-box protein E2-2 is a crucial regulator of human pDC development. The investigators isolated pDC and pDC progenitors from the thymus and evaluated E-box protein (HEB, E2-2 and E2A) expression levels. Interestingly, E2-2 was highly expressed in pDC while only low-level expression was detected in progenitors. Nagasawa *et al.* [16] then went on to demonstrate that over-expression of E2-2 enhanced pDC development from CD34⁺ hematopoietic progenitors. In contrast, inhibition of E2-2 expression by RNA interference impaired the induction of pDC. Moreover, impaired pDC development by enforced expression of the E-box protein inhibitor Id2 could be rescued by E2-2 over-expression, indicating the reciprocal functions of Id2 and E2-2. Furthermore, Nagasawa *et al.* [16] showed that the Ets family transcription factor Spi-B suppressed Id2 expression. Over-expression of Spi-B, however, failed to rescue impaired pDC development caused by enforced Id2. These data suggest that Spi-B controls pDC development by downregulating endogenous Id2 and acts by promoting E2-2 activity, which results in the suppression of Id2 expression.

Interferon regulatory factors (IRF) not only play a critical role in controlling type 1 IFN responses, but also serve as important transcriptional regulators of DC development. While IRF4-deficient mice showed defects in the development of CD4⁺ cDC and pDC, IRF8-deficient mice exhibited impaired development of CD8α⁺ cDC and pDC [17, 18]. These data indicate that both IRF4 and IRF8 are necessary for the development of pDC. Further studies on IRF4 and IRF8 double-gene-deficient mice show defects in the development of all DC populations, suggesting a nonredundant role of each factor for DC development [19]. Although these studies demonstrate the importance of IRF4 and IRF8 in DC development, the molecular mechanisms by which they regulate DC maturation are still unclear.

As E2-2 positively controls pDC development, it will be interesting to investigate whether E2-2 links to IRF4 and/or IRF8. There are at least two possibilities for cross-talk between these critical regulators. First, E2-2 may directly control the expression of IRF4 and/or IRF8. E-proteins (like E2-2) bind to E-box DNA elements either as homodimers or as heterodimers with other basic helix–loop–helix proteins. Interestingly, the consensus E-box sequence (CANNTG) is found in the IRF4 and IRF8 promoter regions (E. E and Y. J. L., unpublished observations). In addition, we have recently demonstrated that STAT5

activation by GM-CSF is essential for the inhibition of pDC development induced by Flt3L. Furthermore, we showed that STAT5 binds to the *Irf8* promoter region and IRF8 expression was directly suppressed by STAT5 [11, 20]; however, the detailed molecular mechanisms by which STAT5 negatively regulates IRF8 expression remain to be determined. Therefore, it would be of interest and important to see whether STAT5 controls IRF8 expression through interaction with E2-2 and/or inhibition of E2-2 binding to the *Irf8* promoter.

Another possibility is that E2-2 and Spi-B control the transcriptional activity of IRF4 and/or IRF8, but not their expression. It is known that Ets factors (like Spi-B) and IRF can interact and bind to ETS-IRF composite DNA elements (EICE). Additionally, it has been reported that a ternary complex of PU.1, IRF4 and E47 bind to an E-box and EICE element and regulate B-cell gene expression [21]. Taken together with previous data, the current findings of Nagasawa *et al.* [16] suggest that Spi-B might form a complex with E2-2 and IRF4/IRF8 and bind to E-box and EICE element in target genes that specify pDC development.

Nagasawa *et al.* [16] demonstrate the crucial regulatory role of E2-2 in human pDC development using the human CD34⁺ progenitor culture system. Furthermore, they suggest that the positive role for E2-2 in pDC development may be coupled with interaction and/or regulation of IRF family proteins; however, Nagasawa *et al.* [16] employed gene over-expression and RNA interference experiments to address E2-2 function in the development of pDC *ex vivo*. Direct analysis of E2-2 mice is hampered by their postnatal lethality [22]. Thus, the generation of cell-typespecific E2-2-deficient mice (*e.g.* bone marrow or DC-deficient) or analysis of bone marrow chimeric mice transplanted with E2-2-deficient HSCs will be required for *in vivo* confirmation of Nagasawa *et al.*'s *ex vivo* results. By analyzing IRF expression and function in E2-2-deficient progenitors and pDC, the molecular pathway by which E2-2 controls the development of pDC can be clearly elucidated.

Acknowledgments

We thank S. S. Watowich for her critical review of the paper.

Abbreviations

cDC	conventional DC
pDC	plasmacytoid DC
IRF	interferon regulatory factor
EICE	ETS-IRF composite DNA elements

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