Notch1 Deficiency Dissociates the Intrathymic Development of Dendritic Cells and T Cells

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Abstract

Thymic dendritic cells (DCs) form a discrete subset of bone marrow (BM)-derived cells, the function of which is to mediate negative selection of autoreactive thymocytes. The developmental origin of thymic DCs remains controversial. Although cell transfer studies support a model in which T cells and thymic DCs develop from the same intrathymic pluripotential precursor, it remains possible that these two types of cells develop from independent intrathymic precursors. Notch proteins are cell surface receptors involved in the regulation of cell fate specification. We have recently reported that T cell development in inducible Notch1-deficient mice is severely impaired at an early stage, before the expression of T cell lineage markers. To investigate whether development of thymic DCs also depends on Notch1, we have constructed mixed BM chimeric mice. We report here that thymic DC development from Notch1−/− BM precursors is absolutely normal (in terms of absolute number and phenotype) in this competitive situation, despite the absence of Notch1−/− T cells. Furthermore, we find that peripheral DCs and Langerhans cells are also not affected by Notch1 deficiency. Our results demonstrate that the development of DCs is totally independent of Notch1 function, and strongly suggest a dissociation between intrathymic T cell and DC precursors.

Key words: Notch1 • dendritic cell • cell fate • T cell • development

Introduction

Dendritic cells (DCs)1 play a key role in T cell immune responses by endocytosing, processing, and presenting foreign antigens to specific T cells (1, 2). All DCs originate from bone marrow (BM) precursors, but it is not clear whether these precursors differentiate and mature to DCs in the BM itself, or whether they migrate to and differentiate within other organs.

Different lines of evidence point to the existence of two types of DCs in the mouse. Although all DCs express CD11c and MHC class II, the two subsets can be distinguished according to their differential expression of cell surface markers such as CD8α−, M−ac−1, and DEC−205. The CD8α+M+ac+1−DEC−205+ subset of DCs is thought to be of lymphoid origin, whereas the CD8α−M−ac−1−DEC−205− DCs are believed to be myeloid derived. In the spleen, where the function of DCs is to present foreign antigens to specific T cells, both subpopulations of DCs are present. However, thymic DCs whose function is to mediate negative selection of autoreactive thymocytes (3) constitute only the lymphoid-related subset (4–6).

The development of thymic DCs and T cells has been reported to be closely linked via an intrathymic common precursor (6, 7). In support of this hypothesis, intrathymic transfer of either early T cell precursors (CD4−CD44+CD25−CD117+) or progenitor T cells (CD4−CD44+CD25−CD117+) into sublethally irradiated mice results in the production of both thymic DCs and mature T cells. However, the next downstream population (defined as CD4−, CD44−CD25+, CD117+) has lost DC potential and generates only T cells (8). These data are consistent with a lymphoid-related origin for thymic DCs and support the existence of a common intrathymic T/DC pre-
cursor (7, 8). Nevertheless, this hypothesis has not been proven at the clonal level, and it remains possible that the populations transferred contain distinct DC and T cell precursors that cannot be distinguished phenotypically.

Further evidence that DC and T cell development might be closely linked is derived from gene-targeted mice. Mice homozygous for an Ikaros dominant-negative mutation (deletion of the DNA-binding domain) lack all cells of lymphoid origin, such as T, B, and NK cells, as well as lymphoid- and myeloid-derived DCs (9). More interestingly, mice homozygous for an Ikaros null allele (deletion of the C/EBP homodomain of Ikaros) lack B and NK cells, as well as lymph nodes, but maintain a certain T cell differentiation potential (10). Although no myeloid DCs were found in such mice, lymphoid-related DCs were present in the thymus, suggesting a correlation between T cell and thymic DC development (11).

Notch gene family members have been shown to play crucial roles in binary cell fate decisions in many developmental systems (12). Notch proteins are conserved transmembrane receptors containing EGF repeats in their ectodomain that are implicated in ligand binding. The cytoplasmic domain harbors six ankyrin repeats and is involved in intracellular signaling (13). To date, four mammalian Notch homologues (Notch1–4) that interact with transmembrane-bound ligands such as Jagged1, Jagged2, Delta1, and Delta-like3 have been identified (14–24).

Several reports suggest a role for Notch family members in T cell development. Notch1, 2, and 3 as well as the Delta and Delta-like ligands have been shown to be involved in T cell development. Notch signaling in T cells is thought to be mediated by Delta-like ligands and is involved in the development of CD4 versus CD8 T cells (30, 31).

The putative ability of Notch1 to regulate the B/T lineage decision (32), and Notch signaling in T cell development is supported by previous reports showing that Notch1 signaling is involved in the development of CD4 versus CD8 T cells (30, 31).

Materials and Methods

Generation of Mice with a loxP-flanked Notch1 Allele. The activation of the Cre recombinase was performed as described previously (32). In brief, adult mice received five intraperitoneal injections of 250 μg poly I:C (Sigma Chemical Co.) at 2-d intervals. 2 d after the last injection, mice were killed, and the BM was prepared for BM transplantation by T cell depletion. Genomic DNA was prepared from a portion of the BM-depleted BM cells for assessing Notch1 deletion efficiency by Southern blot, and was quantified using a Phosphorimager (Molecular Dynamics).

Flow Cytometry and Antibodies. Four-color FACSC® staining was performed as described elsewhere (36) using the following mAbs: anti-CD45.1–FITC or –biotin, anti-CD45.2–FITC or –PE, anti-CD3–PE, anti-CD44–FITC or –allophycocyanin (APC), anti-Gr1–APC, anti-CD11b–PE, anti-CD44–CyChrome, anti-CD8α–CyChrome or –APC, anti-CD4–CyChrome or –APC, anti-NK1.1–PE (PharMingen). Anti-F4/80–biotin was purified and conjugated in this laboratory. Biotinylated antibodies were revealed with either streptavidin–CyChrome (PharMingen) or streptavidin–APC (Molecular Probes). FACSC® analysis was performed using four colors on a FACSCalibur™ flow cytometer (Becton Dickenson), and was analyzed using CELLQuest™ software (Becton Dickenson). Dead cells were excluded by live gating of forward scatter and side scatter, and 100,000–200,000 cells were analyzed in each file.

Isolation of DCs from the Thymus and Spleen. DC-enriched cell suspensions were prepared as described previously (37). In brief, organs were cut into small pieces and digested with collagenase A (0.5 mg/ml Boehringer Mannheim) and DNAase I (40 μg/ml Boehringer Mannheim) in RPMI 1640 medium supplemented with 5% FCS for 10 min at 37°C with continuous agitation. Digested fragments were filtered through a sieve, and the cell suspension was washed twice in PBS supplemented with 5% FCS and 5 mmol/liter EDTA containing 5 μg/ml DNase I. The cells were then resuspended in cold isosmotic Optiprep™ solution (Nyegaard Diagnostics), pH 7.2, 1.061 g/cm³, containing 5 mmol/liter EDTA to dissociate DC–thymocyte complexes, and the low density fraction was obtained by centrifugation at 1,700 g.
for 10 min. This low density fraction was washed twice in PBS-EDTA-FCS. DCs were identified in the low density cell fraction as MHC class II~CD11c~B220~F4/80~ cells.

Isolation of Langerhans Cells from the Skin. Langerhans cell (LC) preparation was performed as described previously (37, 38). In brief, ears were rinsed with 70% ethanol, split using forceps into dorsal and ventral halves, and incubated with 0.5% trypsin (Sigma Chemical Co.) in PBS containing 5% FCS for 30 min at 37°C to allow the separation of the epidermal sheets from dermis. Subsequently, epidermal sheets were cultured for 24 h in 24-well tissue culture plates in the presence of 100 ng/ml GM-CSF (provided by Immunex Corp., Seattle, WA). LCs, together with keratinocytes, were released into the culture medium. Epidermal cell suspensions were obtained by filtering the culture medium and epidermal sheets through a sieve, and were washed in PBS with 5% FCS. LCs cells were identified in the epidermal cell suspension as MHC class II~CD11c~ and CD11c~ cells.

Analysis of DCs and Other Hematopoietic Lineages in the Mixed BM Chimeras. Two groups of mixed BM chimeras were constructed, CD45.1~w/CD45.2~ Notch1lox/lox (n = 8) and CD45.1~w/CD45.2~ Notch1~ (n = 15). Cells bearing DC markers were analyzed on DC-enriched preparations from pooled thymi and spleens derived from the BM chimeras. Analyses of other hematopoietic lineages such as granulocytes, macrophages, B cells, and NK cells were performed on BM and spleens from individual mice.

Results

Experimental Strategy. As the block in thymocyte development after induced inactivation of Notch1 is at a very early stage (32), Notch1 may also be involved in thymic DC differentiation, affecting a putative intrathymic common T/DC precursor. Since the deletion efficiency of Notch1 (mediated by the IFN-α-inducible MxCre retransgene) in the thymus is only ~40%, but is close to 100% in the BM, we decided to investigate this question using BM chimeras. To this end, CD45.2~ Notch1lox/lox MxCre/− (Notch1~), CD45.2~ Notch1lox/lox (control), and CD45.1~ wt mice were treated with the IFN-α inducer polyI-polyC five times at 2-d intervals to delete an essential portion of the N otch1 gene (Fig. 1 A). 2 d after the last injection, BM was harvested and prepared for transfer. To assess the Notch1 deletion efficiency, genomic DNA was prepared from a sample of induced Notch1~ or control BM and analyzed by Southern blot. The deletion efficiency in Notch1~ BM was close to 100%, as expected (Fig. 1 B). Lethally irradiated wt hosts (CD45.1~) were reconstituted with either CD45.2~ Notch1~ BM or CD45.2~ control BM, each mixed in a 2:1 ratio with CD45.1~ wt BM (hereafter described as N otch1~ or control chimeras, respectively), and analyzed 3 mo later for the different myeloid and lymphoid lineages derived from each donor population.

Notch1~ BM Precursors Fail to Generate Thymic or Peripheral T Cells. The distribution of the two allelic markers CD45.1 and CD45.2, which denote the donor origin of the cells, showed an equivalent contribution of leukocytes from each donor population in the blood of both control and Notch1-deleted chimeras (Fig. 2 A). In the control chimeras, B220~ (B) and CD3~ (T) cells derived from both CD45.1~ and CD45.2~ BM were present, as expected. However, in the Notch1-deleted chimeras, although B220~ B cells were derived from both donor populations, >99% of CD3~ T cells were derived from the CD45.1~ wt BM, and <0.3% from the CD45.2~ Notch1-deficient BM (Fig. 2 A).

In the thymus of control chimeras, reconstitution was similar to that observed in blood and other peripheral organs with an equal contribution from both CD45.1~ and CD45.2~ BM donor populations. In contrast, in the Notch1-deleted chimeric thymus, 98% of cells were derived from CD45.1~ wt BM (Fig. 2 B), in spite of the fact that absolute thymocyte numbers were similar in both control and Notch1-deleted chimeras (~90 × 106). Furthermore, most of the residual CD45.2~ cells of Notch1~ donor origin were CD4−CD8−B220− B cells (Fig. 2 B), confirming that Notch1-deficient precursors are unable to generate T cells in a competitive situation (32).

Figure 1. Inducible targeting of the Notch1 gene. (A) Schematic representation of the murine Notch1 gene. The Notch1 protein contains a signal peptide, 36 EGF repeats followed by a cysteine-rich domain (LN) in the extracellular domain, a transmembrane domain (TM), cytoplasmic ankyrin repeats (Cdc10), and a PEST sequence. The genomic organization of the Notch1 locus derived from control mice is partially shown (1); the exon coding for the leader peptide (filled square) is flanked by two loxP sequences (gray triangles), followed by the exon coding for the first EGF repeat (gray box). After induction of the Cre recombinase, the genomic portion harboring the exon coding for the leader peptide is deleted (2). Arrows indicate EcoRI fragments that differ in size between the genomic locus derived from control mice and the locus after deleting the loxP-flanked gene segment (induced Notch1~). (B) Southern blot analysis of EcoRI-digested genomic DNA derived from the BM used for setting up the mixed chimeric mice. The probe indicated in A reveals a 5.8-kb fragment for the allele derived from control animals (1), whereas deletion of the flanked loxP segment gives rise to a 2.3-kb fragment (2). Deletion efficiency was calculated after PhosphorImager® analysis to be >98%.

A

Notch1

EGF

LN

TM

Cdc10

PEST

RI

RI

Control

induced Notch1~

1.58 kb

2.3 kb

After Cre recombination

B

Control

induced Notch1~

Figure 1.
Notch1\(^{-/-}\) BM Precursors Have the Full Potential for Generating Thymic DCs. To determine whether induced Notch1\(^{-/-}\) BM precursors are able to generate thymic DCs, the low density thymocyte fraction was prepared from control and Notch1\(^{-/-}\) BM precursors, and was stained with a combination of cell surface markers specific for DCs. As expected, a characteristic thymic DC population with the phenotype MHC class II\(^{+}\)CD11c\(^{+}\)CD8\(^{+}\) derived from both CD45.1\(^{+}\) and CD45.2\(^{+}\) BM origin was detected in control chimeras (Fig. 3 A). Surprisingly, in the competitive situation where T cells could not be generated, a phenotypically identical population of CD45.2\(^{+}\) DCs of Notch1\(^{-/-}\) donor origin was detected in addition to the wt CD45.1\(^{+}\) DCs (Fig. 3 A). Even the absolute numbers of thymic DCs derived from CD45.2\(^{+}\) control or CD45.2\(^{+}\) Notch1\(^{-/-}\) BM were similar compared with DCs derived from wt BM (Fig. 3 B). This result clearly demonstrates that thymic DC development is Notch1 independent, and furthermore, strongly suggests a developmental dissociation between thymic DC and T cell precursors.

Notch1\(^{-/-}\) BM Precursors Are Capable of Generating Peripheral DCs and LCs. To investigate whether Notch1 deficiency could also affect the generation of myeloid-derived DCs, we analyzed splenic DCs in the same chimeric mice. As shown in Fig. 4, both lymphoid-related DCs (characterized by the expression of CD8\(^{+}\)a) and myeloid-related DCs (which are CD8\(^{+}\)a\(^{-}\)) of both CD45.1\(^{+}\) and CD45.2\(^{+}\) origin were present in similar numbers in the spleens of control and Notch1\(^{-/-}\) BM chimeras. This result indicates that Notch1 deficiency does not affect the generation of either myeloid- or lymphoid-related splenic DCs.

Skin DCs, known as LCs, are immature DCs that differentiate into mature DCs after antigenic stimulation and migrate to the T cell areas of the draining lymph nodes. To investigate if the generation of LCs was affected by the loss of Notch1, LCs were isolated from the epidermis of
Figure 3. Induced Notch1−/− BM generates thymic DCs in mixed BM chimeras. (A) Mixed chimeric mice were analyzed 3 mo after reconstitution with a 1:2 mixture of wt (CD45.1) and control (Notch1lox/lox, CD45.2) or induced Notch1−/− (CD45.2) BM-derived populations. Thymi of these chimeric mice were pooled, and the DC fraction was enriched as described in Materials and Methods. The DC-enriched fraction was stained using anti-F4/80, anti-CD11c, anti-MHC class II, and anti-CD8α antibodies. The graph shows a FACS® analysis of CD11c versus MHC class II+ cells, as well as histograms for the expression of CD8α gated on CD11c+ MHC class II+ cells. (B) Absolute cell numbers of thymic DCs derived from control and Notch1−/− chimeric mice. The bars represent average values per thymus of two independent experiments, and the triangles represent the average number of DCs per thymus from pooled thymi. n = 4 for wt (CD45.1) plus control (CD45.2), and n = 8 and 7 for wt (CD45.1) plus Notch1−/− (CD45.2) chimeras.

Figure 4. Induced Notch1−/− BM generates splenic DCs in mixed BM chimeras. The BM chimeric mice described in Fig. 3 were also analyzed for splenic DC development. Spleens of chimeric mice were pooled, and the DC fraction was enriched as described in Materials and Methods. The DC-enriched fraction was stained using anti-CD45.1 or anti-CD45.2, together with anti-F4/80, anti-CD11c, and anti-CD8α antibodies. The graph shows a FACS® analysis of CD11c versus CD8α gated on F4/80+ cells for the two different groups of chimeric mice. Absolute numbers of the myeloid-related (CD11c−CD8α+) and lymphoid-related (CD11c+CD8α+) DCs per spleen are indicated next to the quadrants.

Figure 5. The generation of LCs is Notch1 independent. The same chimeric mice as described in the legend to Fig. 4 were analyzed for the presence of LCs, which were prepared from epidermal sheets of mouse ears as described in Materials and Methods. The cell populations were stained for the expression of CD45.1, CD45.2, and MHC class II. Percentages of LCs are indicated in the upper right quadrant.
Notch1 is expressed on BM CD34

Notch1 in T cell fate determination.

Collectively, affected by Notch1 deficiency, even in a situation of commingling both thymic and splenic DCs, as well as LCs) is uniform throughout BM. The finding that Notch1 is dispensable for the development of myeloid and/or erythroid differentiation (40, 41). Further evidence for Notch function in myelopoiesis comes from studies using CD32 cells, which are progenitor cells that can differentiate into granulocytes in the presence of a cytokine cocktail. Expression of a dominant active form of Notch1 by induction (a ligand for the Notch receptor) was found to inhibit differentiation of 32D cells in response to the cytokine cocktail (40, 42). Our data indicating that Notch1 is dispensable for the development of DCs, granulocytes, and macrophages in vivo appear to conflict with previous results suggesting that constitutive Notch1 activity perturbs myeloid differentiation (40, 43). This discrepancy may be due either to the experimental protocol used for studying myeloid differentiation, or simply to the fact that we cannot exclude the possibility that redundant Notch signaling from other Notch gene family members may rescue the development of myeloid lineages. Nevertheless, our results are in agreement with a recent report in which retroviral infection of BM cells with a dominant active form of Notch1 did not affect myeloid differentiation, although it had profound effects on lymphoid differentiation (33).

Implications for Myeloid Differentiation. Two independent lines of evidence implicate Notch1 in a binary lineage decision of a CLP to develop into a B cell or a T cell. First, as shown here and elsewhere (32), Notch1 expression in the absence of Notch1 is dispensable for the development of myeloid lineages. Nevertheless, our results are in agreement with a recent report in which retroviral infection of BM cells with a dominant active form of Notch1 did not affect myeloid differentiation. Second, the finding that Notch1 is dispensable for the development of DCs, granulocytes, and macrophages in vivo appears to conflict with previous results suggesting that constitutive Notch1 activity perturbs myeloid differentiation (40, 43). This discrepancy may be due either to the experimental protocol used for studying myeloid differentiation, or simply to the fact that we cannot exclude the possibility that redundant Notch signaling from other Notch gene family members may rescue the development of myeloid lineages. Nevertheless, our results are in agreement with a recent report in which retroviral infection of BM cells with a dominant active form of Notch1 did not affect myeloid differentiation, although it had profound effects on lymphoid differentiation (33).

Discussion

The data presented in this report demonstrate that Notch1 deficiency does not affect the development of T cells from all other known myeloid and lymphoid lineages. Thus, although T cell development from Notch1 negative precursors is arrested at a very early stage, the development of B cells, NK cells, macrophages, granulocytes, and DCs (including both thymic and splenic DCs, as well as LCs) is unaffected by Notch1 deficiency, even in a situation of competitive repopulation in mixed BM chimeras. Collectively, these results reveal an obligate and selective role of Notch1 in T cell fate determination.

Implications for Myeloid Differentiation. The finding that Notch1 is expressed on BM CD34+ progenitors (39), together with the observation that Notch ligands are expressed on BM stromal cells, initially led to the suggestion of a role for the Notch pathway in myeloid and/ or erythroid differentiation (40, 41). Further evidence for Notch function in myelopoiesis comes from studies using 32D cells, which are progenitor cells that can differentiate into granulocytes in the presence of a cytokine cocktail. Expression of a dominant active form of Notch1 by induction (a ligand for the Notch receptor) was found to inhibit differentiation of 32D cells in response to the cytokine cocktail (40, 42). Our data indicating that Notch1 is dispensable for the development of DCs, granulocytes, and macrophages in vivo appear to conflict with previous results suggesting that constitutive Notch1 activity perturbs myeloid differentiation (40, 43). This discrepancy may be due either to the experimental protocol used for studying myeloid differentiation, or simply to the fact that we cannot exclude the possibility that redundant Notch signaling from other Notch gene family members may rescue the development of myeloid lineages. Nevertheless, our results are in agreement with a recent report in which retroviral infection of BM cells with a dominant active form of Notch1 did not affect myeloid differentiation. Additionally, our results are in agreement with a recent report in which retroviral infection of BM cells with a dominant active form of Notch1 did not affect myeloid differentiation. Furthermore, our results are in agreement with a recent report in which retroviral infection of BM cells with a dominant active form of Notch1 did not affect myeloid differentiation.

Figure 6. The development of granulocytes, macrophages, B cells, and NK cells is Notch1 independent. (A) Chimeric BM were analyzed for the presence of granulocytes, macrophages, and B cells by staining with mAbs specific for Gr1, M ac-1, and B220. Chimeric spleens were analyzed for the presence of NK cells by staining N K1.1 gated on CD3+ cells. The histograms show the expression pattern of these markers in the CD45.2+ cells derived from either control (thin line) or induced Notch1−/− (bold line) BM cells. (B) Absolute cell numbers (×106) for Mac-1+, Gr1+, B220+, and NK1.1+(CD3+) cells in the CD45.2− fraction derived from either control or induced Notch1−/− BM cells are calculated and shown as bar diagrams. The bars represent average values, and the triangles represent values from individual mice (n = 8 for both control and induced Notch1−/− chimeric mice).

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widely held hypothesis (Fig. 7 A) that thymic DCs are a lymphoid lineage derived from a common intrathymic precursor that also gives rise to T cells: (a) intrathymic transfer of sorted populations of CD44\(^+\)CD4\(^-\) precursors or CD44\(^+\)CD25\(^+\) progenitor T cells gives rise to both thymic DCs and T cells in sublethally irradiated recipients (7, 8); and (b) the development of thymic DCs and T cells could not be clearly dissociated in gene-targeted mice deficient for either Ikaros or RelB transcription factors (10, 11, 44). However, both of these lines of evidence are ultimately based on correlations, and it remains possible that thymic DCs and T cells are derived from independent precursors that cannot be phenotypically distinguished.

The clear-cut dissociation of intrathymic T cell and DC development in chimeric mice reconstituted with Notch1\(^+/+\) BM cells provides a serious challenge to the hypothesis that thymic DCs represent a lymphoid lineage derived from a common T/DC precursor. Indeed, as shown here and elsewhere (32), T cell development from Notch1\(^+/+\) BM precursors is blocked at a very early stage (before expression of CD25), and preliminary results suggest that the few remaining CD44\(^+\) intrathymic cells of Notch1\(^+/+\) origin do not express typical markers of CLPs such as Sca-1 and CD117 (data not shown). Nevertheless, thymic DC development is totally unaffected (in terms of both phenotype and absolute cell numbers) by Notch1 deficiency. Taken together with the evidence that Notch1 probably controls T/B fate specification in a binary fashion (described above), the simplest interpretation of our data would be to postulate that in the absence of Notch1 signaling, CLPs are deviated to the B cell lineage either before or shortly after their entry into the thymus. According to this scenario (Fig. 7 B), Notch1-deficient thymic DCs could not be derived from a CLP, and hence would presumably originate from an independent thymic DC precursor population that has yet to be precisely identified.

Although we favor the hypothesis that T cells and thymic DCs are derived from distinct intrathymic precursors that differ in their developmental dependence on Notch1, other interpretations of our data cannot be excluded. For example, it is possible that thymic DCs arising from Notch1\(^+/+\) precursors represent an aberrant developmental pathway in which CLPs are redirected to the DC fate in the absence of Notch1 signaling. According to this model, differentiation into thymic DCs could be considered as an alternative default pathway (or primary fate) of intrathymic CLPs. Nevertheless, the fact that thymic DC development from Notch1\(^+/+\) precursors is not noticeably perturbed (with respect to either phenotype or absolute cell numbers) is difficult to reconcile with an altered T/DC cell fate specification, particularly when the effect of Notch1 deficiency on the T/B lineage decision is so dramatic.

Finally, the possibility cannot be formally excluded that other developmental pathways may lead to the production of thymic DCs in normal mice. For instance, thymic DCs may develop from a lymphoid precursor that is more closely related to B cells than to T cells. Such a putative B/DC precursor would presumably retain its bipotentiality in the absence of Notch1. Alternatively, thymic DC development may be a highly flexible process, with both lymphoid and nonlymphoid precursors having the potential to give rise to DCs depending on the availability of microenvironmental cues.

In conclusion, our data demonstrate that Notch1 is indispensable for the development of all myeloid and lymphoid lineages with the exception of T cells. Moreover, taken together with a recent report by Rodewald et al. (45), they seriously challenge the widely held hypothesis that thymic DCs and T cells share a common intrathymic precursor.

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