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Organ-Specific Cellular Requirements for In Vivo Dendritic Cell Generation

Tewfik Miloud,* Nathalie Fiegler,* Janine Suffner,* Günter J. Hämmerling,*1 and Natalio Garbi**,*1

Bone marrow-derived dendritic cell (DC) precursors seed peripheral organs, where they encounter diverse cellular environments during their final differentiation into DCs. Flt3 ligand (Flt3-L) is critical for instructing DC generation throughout different organs. However, it remains unknown which cells produce Flt3-L and, importantly, which cellular source drives DC development in such a variety of organs. Using a novel BAC transgenic Flt3-L reporter mouse strain coexpressing enhanced GFP and luciferase, we show ubiquitous Flt3-L expression in organs and cell types. These results were further confirmed at the protein level. Although Flt3-L was produced by immune and nonimmune cells, the source required for development of the DC compartment clearly differed among organs. In lymphoid organs such as the spleen and bone marrow, Flt3-L production by hematopoietic cells was critical for generation of normal DC numbers. This was unexpected for the spleen because both immune and nonimmune cells equally contributed to the Flt3-L content in that organ. Thus, localized production rather than the total tissue content of Flt3-L in spleen dictated normal splenic DC development. No differences were observed in the number of DC precursors, suggesting that the immune source of Flt3-L promoted pre-cDC differentiation in spleen. In contrast, DC generation in the lung, kidney, and pancreas was mostly driven by nonhematopoietic cells producing Flt3-L, with little contribution by immune cells. These findings demonstrate a high degree of flexibility in Flt3-L–dependent DC generation to adapt this process to organ-specific cellular environments encountered by DC precursors during their final differentiation. The Journal of Immunology, 2012, 188: 1125–1135.

Homoeostasis of immune cells involves regulated differentiation of hematopoietic precursors for generating normal numbers of mature cells. To achieve this, hematopoiesis is mostly restricted to primary lymphoid organs such as bone marrow (BM) and the thymus, where organized cellular networks provide factors required for differentiation into mature immune cells (1). Dendritic cells (DCs) represent an exception to this because they are derived from BM precursors that enter the systemic circulation and terminally differentiate in lymphoid and nonlymphoid organs throughout the body (2–4). During their differentiation process, therefore, DC precursors encounter diverse cellular environments specific to each particular organ.

In the steady state, DCs are broadly divided into migratory and lymphoid-organ-resident DCs (5, 6). The latter include CD8α and CD8β subpopulations of CD11c+ MHC class II+ (MHC-II+) conventional DCs (cDCs) with different functional characteristics (7–9). Migratory DCs are initially present in nonlymphoid organs such as skin, gut, and lung, also constituting a mixed cellular group in terms of development, phenotype, and function (10, 11). Resident cDCs in secondary lymphoid organs have been reported to display a short half-life of ~2 d (12, 13) to ~2 wk (14), depending on the methodology used. In addition, migratory DCs continuously egress from peripheral tissues into draining lymph nodes in steady state (15). Therefore, the pool of migratory and resident DCs is constantly being replaced to maintain a constant pool size. Considering the half-life of cDCs and a mouse life span of ~1.5 yr, the complete cDC pool is renewed ~40–150 times.

Different DC precursors seed lymphoid and nonlymphoid organs. Of these, CD11c– (16–18) and immediate CD11c+ pre-cDC (14, 19–21) precursors differentiate into DCs in lymphoid organs. In addition, CD103+ and CD11b+ DCs in nonlymphoid organs originate from circulating pre-cDCs and Ly6C+ monocytes, respectively (10, 22–25). Although much has been recently learned on DC precursors and the cytokines driving their differentiation, it is largely unknown how different tissues instruct DC development and homeostasis. The cellular composition of disparate organs, such as the spleen and lung, varies considerably and it is unknown whether there are organ-specific characteristics that promote DC development.

Different key growth factors for DC development have been identified (26). DC differentiation in lymphoid organs is mostly driven by Flt3 ligand (Flt3-L) (16, 27–29) with little contribution by other cytokines (30). DC development in nonlymphoid organs depends on Flt3-L, M-CSF, or GM-CSF (10, 24, 25, 31). For example, most CD103+CD11b+ DCs in peripheral organs are
developmentally related to CD8+ cDCs in lymphoid organs in that they also arise from pre-cDCs and require Flt3-L and similar transcription factors for their generation (10, 11). Flt3-L induces mTOR activation (32) and is required both during the differentiation of early hematopoietic precursors in the bone marrow (33) and during the final steps of DC differentiation in peripheral organs (16, 29). Earlier studies have shown that Flt3-L is produced by a variety of different organs and cell lines (34–39), but little is known about its in vivo distribution.

In this study, we report on the cellular requirements for Flt3-L–dependent generation of DCs in lymphoid and nonlymphoid organs. Using a novel bacterial artificial chromosome (BAC) transgenic reporter mouse to characterize Flt3-L production, we find widespread expression in a large variety of organs and cell types. However, there are specific cellular requirements for relevant Flt3-L provision in different organs, which reflect their particular characteristics. For example, Flt3-L production by hematopoietic cells is necessary for DC development in the spleen, whereas production by nonhematopoietic cells is required in nonlymphoid organs. We also demonstrate that the cellular source rather than the organ content of Flt3-L is dominant for DC generation in spleen and bone marrow, suggesting that DC development in those organs proceeds within specific cellular environments where close interactions between Flt3-L–producing cells and DC precursors take place.

Materials and Methods

Mice and cell depletion in vivo

C57BL/6N mice (B6), congenic B6.SJL-Ptprca Pep3b/BoyJ (CD45.1) mice (Charles River), flt3l−/− mice (Taiconic), and enhanced GFP (eGFP)–expressing mice (16) were bred at the German Cancer Research Center in specific pathogen–free conditions. Experiments were conducted according to institutional guidelines and regulations (Zentrales Tierlabor, German Cancer Research Center). Flt3-L reporter BAC transgenic mice were generated as previously reported (16, 40). eGFP and CBGr99 luciferase cDNA were separated by a 2A linearized construct was injected into the pronuclei of fertilized C57BL/6N mouse eggs. The resulting transgenic mouse lines were designated FL-2

Organ-specific development of DCs

By i.p. injection of 1 mg anti-NK1.1 depleting Ab (PK136) (16) at the time of birth, NK cells were depleted in vivo (44). Adherent cells were removed from the spleen and bone marrow. Flt3-L provision in different organs, which reflect their particular characteristics, was measured by bioluminescence imaging as described previously (16). The number of cells of interest per lymphoid organ was calculated based on their percentage and the total viable count obtained by trypan blue exclusion. For nonlymphoid organs, cells were enumerated by flow cytometry by adding a known amount of Calibrite FITC beads (BD Biosciences).

Flt3-L ELISA

Flt3-L ELISA was performed as described previously (41). Organ samples were homogenized in 1 ml buffer containing 1% Nonidet P-40 (Fluka) as previously described (16, 43). The Flt3-L amount in the postnuclear fraction was measured using the Quantikine mouse Flt3-L immunoassay (R&D Systems) following the manufacturer’s instructions.

Real-time qPCR

Real-time quantitative PCR was performed as described previously (16). RNA from sample homogenates was prepared using the RNAasy Minikit (Qiagen), and DNA was translated into single-stranded cDNA using the Superscript cDNA Synthesis Kit (Invitrogen) and random hexamers (Amersham Biosciences). Gene expression levels were determined using real-time quantitative PCR TaqMan technology and SYBR green incorporation (Applied Biosystems). The murine Flt3-L 5′-hprt primer and 5′-hprt probe used were: 5′-GGGATGTTTACACGAGGAGATG-3′, 5′-GTTTGGTGGAAGTCATACTGCA-3′, 5′-CTTCCAGAAAGGTTTGTCTC-3′. The egfp primers used were: 5′-TATATATCATGGCCGCAAAAGC-3′, 5′-TTGGTGCGGCTTCTGGAGG-3′. The hprt primers used were: 5′-ACACCTGTAATTATTTACTGCGCA-3′, 5′-TGAAAAAGCCAAAATACAGGCTA-3′. PCR was performed on 43 ng cDNA template per 25 μl reaction volume for all samples following instructions provided by the manufacturer.

Statistical analysis

Comparisons between two samples were performed with Student t test. Comparisons between three or more samples were performed with one-way ANOVA using the Tukey or Dunnett test for multiple comparisons. Data were analyzed using Prism 5 (GraphPad) and statistical significance was set at p < 0.05. Data are expressed as mean values ± SEM.

Results

Expression analysis of Flt3-L in different organs and cell types

Flt3-L is critical for the differentiation of DC populations in lymphoid and nonlymphoid organs (10, 16, 27, 29). However, little...
is known about the source of Flt3-L in tissues and primary cells. To investigate its distribution in vivo, we have generated a BAC transgenic Flt3-L reporter mouse line, termed FL-GL, that expresses eGFP and luciferase as individual proteins under the control of the flt3l promoter and cis regulatory elements present in the BAC (Fig. 1A). Thus, expression analysis of eGFP or luciferase activity reports on flt3l transcription. Noninvasive bioluminescence imaging of FL-GL mice revealed strong light emission throughout the body, suggesting ubiquitous activity of the flt3l promoter (Fig. 1B), which was confirmed by ex vivo analysis of individual organs (Fig. 1C). In agreement with ubiquitous promoter activity, Flt3-L protein was detected in all organs albeit with different amounts. The lung, kidney, and liver contained the highest amount of Flt3-L (>700 pg/organ), whereas all other organs investigated contained lower amounts (≤200 pg/organ; Fig. 1D, 1E). Although soluble Flt3-L was present in serum (Fig. 1F), we observed similar organ Flt3-L content following intracardial perfusion of mice, indicating that the amount of blood-borne Flt3-L had no influence (data not shown). Although the spleen contains relatively high numbers of DCs and their precursors, we observed only an intermediate to low amount of Flt3-L in that organ (Fig. 1D, 1E). Surprisingly, Flt3-L expression in BM, the ultimate source of Flt3-L-dependent hematopoietic precursors, was even lower than in spleen (Fig. 1G).

In order to investigate Flt3-L expression at the single-cell level, we quantified eGFP expression in FL-GL mice by flow cytometry (referred to in this article as FL-eGFP). Since DC differentiation is initiated in BM and finalised in peripheral organs such as spleen (2, 16, 44), we focused our analysis on these two organs. In addition we also investigated expression in the lung because of its high Flt3-L protein content (Fig. 1D, 1E). Independently of the organ investigated, all immune and non-immune cells analyzed in FL-GL mice expressed FL-eGFP, indicating ubiquitous flt3l transcription across different cell types (Fig. 2 and Supplemental Figs. 1 and 2). Similar results were obtained by RT-PCR (data not shown). There were nevertheless quantitative differences between cell types. NK cells showed the highest FL-eGFP level, followed by mast cells, basophils, NK cells, T cells, B cells, and myeloid cells (Fig. 2A–D, Supplemental Fig. 2). Because the frequency of mast cells in the spleen is extremely low, we analyzed this population in the peritoneal exudates of naive mice. FL-eGFP expression within each cell population was homogenous, indicating that there were no differences depending on the subpopulation analyzed or their activation status (Fig. 2A, Supplemental Fig. 1C, and data not shown). Nonimmune cells such as endothelial cells, epithelial cells, and fibroblasts showed intermediate FL-eGFP levels comparable to those of B cells and myeloid cells (Fig. 2A–D and Supplemental Fig. 1).

The difference in Flt3-L mRNA expression between NK cells and B cells was similar to the difference in FL-eGFP mean fluorescence intensity (2.2-fold; Fig. 2E), indicating that flow cytometric quantification of FL-eGFP in FL-GL mice indeed parallels flt3l mRNA expression. Next, the hierarchy of flt3l promoter activity in lymphoid cells was confirmed at the protein level using NK cells, B cells, and T cells sorted from wild type (wt) spleen. NK cells expressed the highest amount of Flt3-L (∼1.5 pg/10⁶ cells), whereas B cells expressed the lowest (∼0.05 pg/10⁶ cells; Fig. 2F). Thus, FL-eGFP intensity highly correlated with Flt3-L protein content among NK, B, and T cells (r² = 0.98219). Because of the low yield of stromal and rare immune cells obtained, we were not able to quantify Flt3-L protein in these cell populations. These results show that the flt3l promoter is ubiquitously active in different organs and cells. Unexpectedly, expression of Flt3-L in NK cells was the highest, whereas expression in nonimmune cells was in general intermediate.

**DC development in lymphoid organs is differentially regulated by the source of Flt3-L**

It is known that hematopoietic stem cells and DC precursors are in close association with both the immune and nonimmune cell compartments (21, 45). Therefore we next investigated whether DC development relies on a specific source of Flt3-L expression. For this investigation, we produced BM chimeric mice restricting Flt3-L production either to the hematopoietic compartment (wt...
FIGURE 2. Widespread Flt3-L expression by immune and nonimmune cells. A, Representative FACS histogram overlays for the expression pattern of flt3L-eGFP by different spleen cell populations in FL-GL reporter mice. Live cell gates were set for NK cells (NK1.1+CD3−), basophils (Ter119+CD11c−CD11b+), NK cells (NK1.1+CD3−), T cells (CD3+), B cells (CD19+), granulocytes (Gr1+CD11b+SSC−), cDCs (CD11c+MHC-II+), pDCs (CD11c+PDCA-1+), Monocytes (CD11c−CD11b+SSC−), CD31+ endothelial cells (EC, CD31+CD45.2−), and CD31+ stromal cells (CD31+CD45.2−). The gating strategy for stromal cells is shown in Supplemental Fig. 1. The gating strategy for basophils is shown in Supplemental Fig. 2. All analysis were performed on live cells. B–D, Quantification of Flt3-L protein expression in NP-40 lysates of sorted splenic B cells and NK cells from B6 mice in the top panel, flt3L-eGFP expression in splenic B cells and NK cells from FL-GL mice is shown in the bottom panel. flt3L-eGFP fold increase was calculated as in B–D. E, Quantification of Flt3-L protein expression in NP-40 lysates of sorted splenic NK cells, T cells, and B cells of B6 mice. Data are expressed as mean ± SEM (n = 3). One representative of at least three independent experiments is shown. EC, endothelial cells with phenotype as in A; EpC, epithelial cells (CD19+CD3−NK1.1−ter119+CD45.2−CD31−EpcAM−).

 Redistribution of Flt3-L expression to stromal, nonhematopoietic cells is required for normal DC development. Flt3-L produced by immune cells was mandatory and sufficient for cDC and pDC generation in the spleen (Fig. 3 A–C). In contrast, restricted expression of Flt3-L to the stromal, nonhematopoietic compartment (flt3L−→wt) was only able to induce partial generation of splenic cDCs and pDCs reaching ∼50% of normal numbers (Fig. 3A–C). The chimerism in the NK cell, B cell, and myeloid compartments was ∼97% of cells derived from the donor BM, whereas that of NKT and T cells was ∼75%. Nevertheless, similar results in DC reconstitution were obtained in BM chimeras using rag1−→ recipient mice, in which all NKT and T cells were of donor origin (Supplemental Fig. 3B). The phenotype of cDCs in the BM chimera was similar in terms of CD11c, MHC-II, CD8, and CD11b expression (Supplemental Fig. 3C), indicating that there was no bias in the development of certain cDC subpopulations. Thus, Flt3-L production by hematopoietic cells is sufficient and necessary to generate a normal DC compartment in the spleen, whereas the contribution of Flt3-L from stromal origin is limited.

In BM, most DCs are pDCs (∼0.8%), whereas cDCs represent only ∼0.1% of leukocytes (Fig. 3D). Using the same set of BM chimeras, we also observed that Flt3-L production by immune cells was mandatory and sufficient for pDC development in BM (Fig. 3D, 3F). However, both the hematopoietic and nonhematopoietic sources of Flt3-L were each sufficient and thus redundant for development of the less abundant cDCs (Fig. 3D, 3E).

These results show that although both the immune and nonimmune compartments in BM and spleen are capable of Flt3-L production, there are important differences regarding the requirement for normal DC development. Flt3-L production by immune cells was mandatory and sufficient for cDC and pDC generation in the spleen (Fig. 3B, 3C) and for BM pDCs (Fig. 3F). In contrast, there was a high degree of redundancy in Flt3-L production for cDC generation in the BM (Fig. 3E).

Flt3-L provided by nonhematopoietic cells is required for CD103+CD11b+ DC homeostasis in nonlymphoid organs

CD11c+MHC-II+ DCs in nonlymphoid organs are heterogeneous in terms of function and development (10, 11). Approximately 70% of lung DCs are CD103+CD11b+ and are largely dependent on Flt3-L for their development, whereas CD11c−CD11b− DCs represent ∼20% of total lung DCs and do not require Flt3-L for their development (10). We thus investigated the source of Flt3-L required for generating lung CD103+ DCs using the BM chimera approach described in this study. Total leukocyte numbers in the lung were not significantly altered in the different BM chimeras (Supplemental Fig. 3A). However, there was a tendency toward
numbers in WT DC populations, we observed that this tendency of decreased DC generating normal numbers of CD103+CD11b nonhematopoietic compartment is sufficient and dominant for normal numbers of DCs (Fig. 4A). A normal numbers of different chimeras (Fig. 4B) not require Flt3-L for development, was not decreased in the flt3l by the non-hematopoietic compartment (Fig. 4C). Interestingly, the requirement for a hematopoietic or nonhematopoietic origin of Flt3-L expression, but not in the lung (Fig. 4). Nonlymphoid organs was more complex. Development of this subpopulation in the kidney, pancreas, and liver was dependent on the hematopoietic and nonhematopoietic compartments to total organ amount of Flt3-L production to the immune (WT→WT) compartments resulted in intermediate levels of Flt3-L protein in the spleen compared with control chimeras (WT→WT; Fig. 5A). No differences were found between WT→flt3−/− and flt3−/−→WT chimeras, indicating that both sources equally contributed to the total Flt3-L amount in the spleen. This finding was in clear contrast with the requirements for DC generation because the hematopoietic source of Flt3-L was sufficient and necessary, but the nonhematopoietic source was not (Fig. 3B, 3C). In BM, however, most of the Flt3-L was derived from immune cells, with nonhematopoietic cells providing only ~5% of the amount (Fig. 5B). This finding again did not correlate with cDC generation in BM, because both sources were sufficient and redundant to obtain a normal cDC compartment (Fig. 3E). Finally, most of the Flt3-L in serum and nonlymphoid organs originated from nonhematopoietic cells (Fig. 5C–G), the latter reflecting the requirement of this source for normal CD103+ DC homeostasis in the lung, kidney, and pancreas.

These results show that the contribution of the hematopoietic and nonhematopoietic compartments to total organ amount of Flt3-L greatly differs in spleen, BM, and nonlymphoid organs. Unexpectedly, Flt3-L content in the spleen and BM did not correlate with the number of splenic cDCs and pDCs, and BM cDCs, respectively. Thus the source rather than the amount of Flt3-L in the spleen and BM was dominant for generating normal numbers of DCs in those organs, strongly suggesting that DC precursors come into close interaction with certain Flt3-L-producing cells during their differentiation.

The number of pre-cDCs in spleen is independent of the source of Flt3-L

Generation and differentiation of early myeloid progenitors in BM is promoted by Flt3/Flt3-L expression (27, 46). Therefore, we in-
vestigated whether the number of downstream DC precursors in the spleen was modulated depending on the source of Flt3-L. Although the number of splenic pre-cDCs in mice lacking Flt3-L was halved, we found a normal pre-cDC compartment independently of whether Flt3-L was of hematopoietic or nonhematopoietic origin (Fig. 6A). These data indicate that a hematopoietic source of Flt3-L, which is required for normal cDC generation in the spleen, is not critical for maintenance of splenic pre-cDCs but promotes their final differentiation.

Pre-cDCs are initially generated in the BM from MDPs and CDPs (21). Like in the spleen, the number of pre-cDCs in BM did not depend on the source of Flt3-L (Fig. 6A). Similar findings were obtained for MDPs and CDPs in BM (Fig. 6B, 6C). These findings are in agreement with a redundancy in the source of Flt3-L for normal BM cDC generation (Fig. 3E) and underline the fact that although nonhematopoietic cells produce only a minor fraction of Flt3-L present in the BM, they are able to support normal numbers of DC precursors and their progeny in that organ.

Flt3-L production necessary for DC development in the spleen does not hinge on a specific immune cell population

Having established that Flt3-L expression by immune cells, rather than the total Flt3-L amount in spleen, is mandatory for generating normal numbers of splenic DCs (Fig. 3B, 3C), we next investigated...
whether there was a specific leukocyte population required to provide Flt3-L for DC homeostasis in that organ. To investigate this question, we performed cell depletion and DC repopulation experiments.

NK and NKT cells produced the highest Flt3-L amount on a per-cell basis in the spleen (Fig. 2A, 2B). However, deficiency of NK cells in IL15−/− mice (47) did not result in a reduced pool size of cDCs in the spleen in the steady state (Supplemental Fig. 4A). In addition, we followed an independent approach by depleting NK and NKT cells with anti-NK1.1 Ab in CD11c.DOG mice whose DC compartment was being repopulated following a single dose of DC-depleting DT (Fig. 7A, left panel). Because of de novo DC generation in the spleen, the splenic DC compartment 4–5 d after DT administration in CD11c.DOG mice is mostly repopulated, comprising ∼75% of the normal number of DCs (Supplemental Fig. 4B) (16). Therefore, we hypothesized that if NK or NKT cells are essential at providing Flt3-L for DC development, the number of cDCs at day 5 after DT treatment would be reduced in CD11c.DOG mice treated with anti-NK1.1 Ab compared with CD11c.DOG mice not treated with anti-NK1.1 Ab. Injection of Ab against NK1.1 resulted in more than 95% depletion of NK and NKT cells in the spleen (Supplemental Fig. 4C). As expected, the cDC pool in CD11c.DOG mice was in expansion 5 d after DT administration, reaching ∼75% of that in B6 mice (Fig. 7A, right panel, and Supplemental Fig. 4D). Similar results were observed in NK cell-depleted CD11c.DOG mice (Fig. 7A, right panel, and Supplemental Fig. 4E) or pDC (data not shown) differentiation in spleen.

We next followed a similar approach to elucidate the role of monocytes and granulocytes for splenic cDC development (Fig. 7B, left panel). In this case, we used anti–Gr-1 Ab to deplete granulocytes (Gr-1hi) and monocytes (Gr-1−; Supplemental Fig. 4E). Again, we did not observe a role of these cells for cDC (Fig. 7B, right panel, and Supplemental Fig. 4F) or pDC (data not shown) differentiation in spleen.

We then investigated whether Flt3-L production by T and B lymphocytes was necessary to support splenic DC generation. T and B cell-deficient rag1−/− mice contain significantly fewer cDCs in the spleen than do wt mice (Fig. 7C, 7D). However, comparing splenic cDC number in B6 and rag1−/− mice lacking lymphocytes may be misleading, because the spleen anatomy and cellular composition in rag1−/− mice are altered, resulting in a drastically reduced size. We thus compared rag1−/− and flt3l−/−rag1−/− mice, which have similar spleen cellularity (Supplemental Fig. 4G) and can thus be directly compared for cDC numbers. flt3l−/− mice contain more than 5-fold fewer splenic cDCs than B6 wt mice, whereas splenic cDC numbers are reduced only by half in flt3l−/−rag1−/− mice compared with rag1−/− mice (Fig. 7D). The number of cDCs in flt3l−/− reflects the general role of Flt3-L expression on cDC generation in comparison with B6 mice, whereas the number of cDCs in flt3l−/−rag1−/− mice shows the role of Flt3-L production by cells other than T and B lymphocytes in comparison with rag1−/− mice. Thus, expression of Flt3-L in mice lacking T and B cells leads to only a minimal increase in splenic cDCs, suggesting that lymphocyte-expressed Flt3-L contributes to DC generation. We attempted to discriminate between the role of B and T cells in normalizing DC numbers by transferring the corresponding cells into rag1−/− mice. Although we observed avid lymphopenia-induced proliferation, there was only a residual reconstitution of the T and B cell compartments (∼1–3 × 106 of each cell type per spleen from day 7 after transfer); consequently, we did not observe a significant increase in the number of splenic cDCs (data not shown). In addition, we quantified DC numbers in the spleen of B cell-deficient jht−/− mice or T cell-deficient lck−/− mice. However, both jht−/− and lck−/− mice showed a strongly disturbed spleen homeostasis with a generalized alteration in the number of specific immune cells, thus making a conclusion on DC homeostasis not possible (data not shown).

These experiments show that Flt3-L production by NK, NKT, granulocytes, and monocytes is redundant and not critical for DC

FIGURE 5. Relative contribution of hematopoietic and nonhematopoietic compartments to Flt3-L amount. Flt3-L protein levels in the spleen (A), BM (B), serum (C), one lung (D), one kidney (E), pancreas (F), and liver (G) of the indicated BM chimera mice quantified by ELISA 11 wk after reconstitution. Data are expressed as mean value ± SEM (n = 3–6). Similar results were obtained in three independent experiments. ***p < 0.001 (ANOVA). n.d., Not detected; n.s., nonstatistically significant.
homeostasis in the spleen, and they suggest that Flt3-L production by T or B cells influences cDC generation in the spleen.

Discussion
BM-derived DC precursors constitutively seed peripheral organs, where they finally differentiate into DCs (2-4, 21, 44). Generation of DCs in lymphoid organs and CD103+ DCs in nonlymphoid organs depends on Flt3-L for differentiation of immediate pre-cDC precursors expressing the Flt3-L receptor Flt3 (10, 16, 20, 29). In this study, we investigated the source of Flt3-L that is required for generating a normal DC pool focusing on the spleen, BM, lung, pancreas, kidney, and liver. Our results show that Flt3-L expression is ubiquitous regarding organs and cell types but, interestingly, specific cellular sources are required for DC development in different organs. In accordance with this, we find that the cell type producing Flt3-L and not the total organ amount governs the extent of DC generation in the lymphoid organs we investigated.

Using FL-GL mice, a novel Flt3-L reporter strain expressing eGFP and luciferase under the endogenous Flt3l promoter, we show that Flt3-L is constitutively expressed in all organs (Fig. 1 B, C). Quantification of Flt3-L mRNA expression (34, 48). Ubiquitous promoter activity resulted in Flt3-L protein expression in all organs in a hierarchical manner. Lungs contained the highest Flt3-L amount, whereas lymphoid organs with extensive DC generation such as the spleen and BM expressed rather low amounts (Fig. 1D, 1E, 1G). In terms of DC homeostasis, we believe that ubiquitous Flt3-L expression outside the BM is necessary to maintain constitutive DC generation in different organs because Flt3+ pre-cDC precursors, which seed peripheral organs, require Flt3-L for their final differentiation (10, 16, 29).

Although comprehensive studies on primary cells are lacking, T cells, fibroblasts and several stromal cell lines are believed to be major producers of Flt3-L on a per-cell basis (34, 35, 38, 39, 48). Contrary to this, we found that all cells investigated in the BM, thymus, spleen, and lung produced Flt3-L, but NK cells were the highest producers, whereas T cells, stromal cells, and other non-immune cells expressed intermediate levels comparable to other immune cells (Fig. 2 and data not shown). It is uncommon that hematopoietic factors are produced by most cells throughout the body. For example IL-7, a cytokine driving T cell differentiation, is mostly produced by BM stromal cells and the thymus (49, 50), whereas T cell progenitors differentiate. Flt3-L-dependent DC development is, however, not confined to a specific organ but proceeds in most locations of the body, which is in agreement with ubiquitous Flt3-L expression.

Because the cellular composition varies considerably in different organs, DC precursors may obtain the necessary Flt3-L from
interact. cDCs and CD103+ DCs derive from pre-DCs, whereas the source for DC development may be a result of the different progenitor cells involved in lymphoid and nonlymphoid organs regarding the Flt3-L content dictated splenic DC development. The differences observed in Flt3-L production between CD103+ DCs in a Flt3-L–dependent fashion. In this study, we hypothesized that production by certain immune cell types was redundant because of the ubiquitous Flt3-L expression (Fig. 2A). Our findings are strongly suggestive of DC development in the spleen being promoted either within confined cellular niches or by dynamic interactions with specific cell populations. Recent data using two-photon imaging showed that pre-DCs are highly motile during their conversion into DCs (21), arguing for dynamic interactions during DC differentiation. Further investigation is needed to clarify this issue.

Although Flt3-L production by immune cells was required to establish a normal cDC compartment in the spleen (Fig. 3B), we hypothesized that production by certain immune cell types was redundant because of the ubiquitous Flt3-L expression (Fig. 2A). Indeed, absence or depletion of NK cells, NKT cells, granulocytes, or monocytes did not interfere with the number of splenic cDCs or with reconstitution of the DC compartment following partial elimination of DCs in CD11c.DOG mice (Fig. 7A, 7B, and Supplemental Fig. 4). However, we found that T and B cells contributed to splenic cDC homeostasis. We obtained this evidence by comparing the effect of Flt3-L expression in mice containing or not containing T and B cells. The spleen of flt3−/− mice contained more than 5-fold fewer cDCs than its wild type counterpart, whereas lymphocyte-deficient rag1−/−/flt3−/− spleens contained half the number of cDCs than lymphocyte-deficient spleens with otherwise normal Flt3-L expression (rag1−/−/flt3−/−; Fig. 7C, 7D). These findings demonstrate that there exists a high degree of redundancy within innate cell-derived Flt3-L for DC generation, and they suggest that T and B cells specifically contribute to splenic cDC homeostasis.

DC development is unique, being a continuum initiated in the BM and finalized in peripheral organs where DC precursors terminally differentiate into subpopulations such as pDCs, cDCs, and CD103+ DCs in a Flt3-L–dependent fashion. In this study, we
provide a basis for understanding how DC generation occurs in such a diverse range of organs throughout the body. Although Flt3-L is widely expressed by immune and nonimmune cells, a high degree of flexibility in the cellular source is required for DC generation depending on the organ in question. This study highlights the adaptation of DC development to specific cellular environments encountered in different organs. Future investigations on the regulation of Flt3-L expression and DC precursors during inflammation will help to understand DC homeostasis in pathologies such as cancer and autoimmunity.

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Disclosures

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References