Langerhans cells (LCs) are antigen-presenting cells (APCs) residing in the epidermis that play a major role in skin immunity. Our earlier studies showed that when skin is inflamed LCs are replaced by bone marrow–derived progenitor cells, while during steady-state conditions LCs are able to self-renew in the skin. Identification of the LC progenitors in bone marrow would represent a critical step toward identifying the factors that regulate LC generation as well as their trafficking to the skin. To determine LC lineage origin, we reconstituted lethally irradiated CD45.2 mice with rigorously purified lymphoid and myeloid progenitors from CD45.1 congenic mice. Twenty-four hours later, we exposed the mice to UV light to deplete resident LCs and induce their replacement by progenitors. Reconstitution with common myeloid progenitors (CMPs), common lymphoid progenitors (CLPs), granulocyte-macrophage progenitors (GMPs), or early thymic progenitors led to LC generation within 2 to 3 weeks. CMPs were at least 20 times more efficient at generating LCs than CLPs. LCs from both lineages were derived almost entirely from fetal liver kinase-2+ (Flk-2+) progenitors, displayed typical dendritic-cell (DC) morphology, and showed long-term persistence in the skin. These results indicate that LCs are derived mainly from myeloid progenitors and are dependent on Flt3-ligand for their development. (Blood. 2006;107:1383-1390)

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Materials and methods

Animals

Four- to 8-week-old C57BL/6 mice (CD45.1) or congenic C57BL/6 mice (CD45.2) were obtained from Jackson Laboratories (Bar Harbor, ME) or the Stanford Animal Facility (Stanford, CA). All animals were maintained according to the PHS Policy for Humane Care and Use of Laboratory Animals.

The online version of the article contains a data supplement.

Reprints: Ines Mende, Stanford Blood Center, 3373 Hillview Ave, Palo Alto, CA 94304; e-mail: imende@stanford.edu.

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Cytokines and media

Epidermal cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Gibco/Invitrogen, Gaithersburg, MD), 1-glutamine (5 mM; Gibco/Invitrogen), and penicillin G (100 U/mL) and streptomycin (100 μg/mL; Bio-Whittaker, Walkersville, MD). Recombinant murine GM-CSF and murine TNF-α were purchased from Peprotech (Rocky Hill, NJ) and reconstituted in sterile PBS. Cell staining and sorting were done in PBS with 2% FBS and 0.05% sodium azide.

Antibodies

The following fluorochrome-conjugated antibodies were produced in our laboratories: M1/70 (anti–Mac-1/CD11b), 8C5 (anti–Gr-1), 6B2 (anti-B220), KT-31 (anti–CD3), GK1.5 (anti–CD4), 53-6.7 (anti–CD8), A20.1.7 (anti-CD45.1), and AL1-4-A2 (anti–CD45.2). Goat anti–rat IgG (PE or Cy5-PE conjugated) was purchased from Caltag (Burlingame, CA). Monoclonal antibodies to I-Ab-FITC, CD45.1-PE and CD11c-APC, B220-TXR, and TCR (anti–I-Ab-FITC, CD45.1-PE and CD11c-APC, B220-TXR, and TCR) were produced in our laboratories: M1/70 (anti–Mac-1/CD11b), 8C5 (anti–Gr-1), 6B2 (anti-B220), KT-31 (anti–CD3), GK1.5 (anti–CD4), 53-6.7 (anti–CD8), A20.1.7 (anti-CD45.1), and AL1-4-A2 (anti–CD45.2). Goat anti–rat IgG (PE or Cy5-PE conjugated) was purchased from Caltag (Burlingame, CA). Monoclonal antibodies to I-Ab-FITC/CD45.1-PE overnight followed by extensive washing and immersion in mounting media (Molecular Probes, Eugene, OR). Stainings were analyzed on a Leica microscope (Leica, Wetzlar, Germany) at a 10 × 40 magnification and pictures were processed using Openlab software (Improvision, Lexington, MA) and Adobe Photoshop software (Adobe Systems, San Jose, CA).

Isolation of hematopoietic progenitor cells from BM

Progenitor populations were isolated from BM or thymus based on phenotype as previously described: hematopoietic stem cells (HSCs; Lin-/CD127-/c-Kit+/Sca+/Thy1.1+); common myeloid progenitors (CMPs; Lin-/CD34+/CD16/32–/c-Kit+/Sca+); granulocyte/macrophage progenitors (GMPs; Lin-/CD34+/CD16/32–/c-Kit+/Sca+); common lymphoid progenitors (CLPs; Lin-/CD127-/c-Kit+/Sca+Thy1.1+/c-Kit+); and progenitors pro-T1 (Lin-/CD44+/CD11b+/c-Kit+/Thy1.1+/CD25−) and pro-T2 (Lin-/CD44+/c-Kit+/Thy1.1+/CD25−).23 All populations were double sorted on a FACS Vantage SE (BD, San Jose, CA) to high purity (> 99%). Lineage specificity of progenitor populations was confirmed by flow cytometric analysis of spleen and thymus of mice that underwent transplantation.

Transplantation of congenic hematopoietic progenitor cells and recruitment to the skin

Mice received 9.5 Gy x-irradiation prior to intravenous injection of progenitors in 150 μL sterile PBS. In order to ensure survival, mice received 3 × 104 autologous BM cells together with the progenitors. Twenty-four hours after progenitor transfer, mice were exposed to UV light (wavelength: 254 nm; voltage: 8 W; source: 38 cm from target) for 20 minutes.

LC isolation

LCs were isolated from epidermal sheets of mouse ears as described previously20 to determine progenitor-derived LCs at 2 to 3 weeks after transplantation. Briefly, ears were incubated in 0.5% trypsin/5 mM EDTA, which allows separation of epidermal sheets from the dermis. The epidermal sheets were cultivated in RPMI10% FBS supplemented with GM-CSF and TNF-α for 24 hours, and migratory cells were stained with I-Aβ-FITC, CD45.1-PE and CD11c-APC, B220-TXR, and TCRγδ-biotin antibodies for flow cytometric analysis.

In situ immunofluorescence

Epidermal sheets for in situ immunofluorescence staining were obtained by shaving mouse ears and culturing the dorsal and ventral halves of the ears in 0.5 M ammonium thiocyanate to separate the epidermis from the dermis. Epidermal sheets were then fixed with acetone and double-labeled with I-Aβ-FITC/CD45.1-PE overnight followed by extensive washing and immersion in mounting media (Molecular Probes, Eugene, OR). Stainings were analyzed

Results

Myeloid progenitors give rise to epidermal LCs in vivo

To study the origin of LCs, lethally irradiated mice received transplants of defined BM progenitor populations obtained from congenic donors (Figure 1A). Twenty-four hours after transplantation, the mice were exposed to UV light to induce the recruitment of LC progenitors to the skin. As shown in Figure 1B, 2 weeks after CD45.2 recipients of 1 × 104 CD45.1+ CMPs or 4 × 104 CD45.1+ GMPs were exposed to UV light, donor-derived I-Aβ+CD11c+ LCs were easily detected by flow cytometric analysis of epidermal-cell suspensions. On average, between 5% and 10% of the LCs were replaced by donor-derived cells following transplantation of 1 × 104 cells, giving rise to its expected lineages and not to other lineages.

Epidermal LCs can be generated by lymphoid progenitors at low efficiency

To determine if lymphoid progenitor cells are also capable of giving rise to LCs after UV light–induced skin inflammation, mice received transplants of either CLPs or early thymic progenitors, such as pro-T1 or pro-T2 cells. At 2 weeks after transfer of 1 × 104 CLPs, few CLP-derived LCs could be detected (Figure 2A). As the maximum peak of thymic and splenic DCs generated by CLPs is reached at about 3 weeks after intravenous transplantation of 1 × 104 CLPs,12 we decided to evaluate the capacity of CLPs to give rise to LCs after 3 weeks. Indeed, a small but significant number of CLP-derived LCs were present in the epidermis at this
LCs DEVELOP FROM MYELOID PROGENITORS

LCs are defined as I-Ab+ cells representative of at least 5 mice that underwent transplantation per progenitor population.

Table 1. LC reconstitution by various progenitor populations at different time points after adoptive transfer

<table>
<thead>
<tr>
<th>Progenitor population</th>
<th>Progenitor no. (\times 10^3)</th>
<th>dEC/tEC, % average (range)</th>
<th>dLC/tLC, % average (range)</th>
<th>dEC/tEC, % average (range)</th>
<th>dLC/tLC, % average (range)</th>
<th>dEC/tEC, % average (range)</th>
<th>dLC/tLC, % average (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSCs</td>
<td>3</td>
<td>7.14 (0.7-27.7)</td>
<td>17.68 (7.1-35.2)</td>
<td>2.58 (0.9-8.4)</td>
<td>22.40 (11.5-40.2)</td>
<td>2.55 (1.1-4.0)</td>
<td>16.50 (3.5-23.1)</td>
</tr>
<tr>
<td>CMPs</td>
<td>10</td>
<td>0.43 (0.7-1.1)</td>
<td>8.72 (1.8-14.0)</td>
<td>ND</td>
<td>ND</td>
<td>0.5 (0.24-1.0)</td>
<td>5.15 (3.1-6.7)</td>
</tr>
<tr>
<td>CMPs</td>
<td>30</td>
<td>0.58 (0.2-0.9)</td>
<td>14.86 (4.3-29.2)</td>
<td>ND</td>
<td>ND</td>
<td>0.12 (0.04-0.29)</td>
<td>1.59 (1-3.2)</td>
</tr>
<tr>
<td>GMPs</td>
<td>40</td>
<td>0.09 (0.01-0.17)</td>
<td>1.60 (0.1-4.6)</td>
<td>ND</td>
<td>ND</td>
<td>0.12 (0.04-0.29)</td>
<td>1.59 (1-3.2)</td>
</tr>
<tr>
<td>CLPs</td>
<td>10</td>
<td>0.04 (0.01-0.1)</td>
<td>0.13 (0.06)</td>
<td>0.07 (0.03-0.12)</td>
<td>0.46 (0.1-1.2)</td>
<td>0.48 (0.15-0.65)</td>
<td>1.55 (0.6-2.4)</td>
</tr>
<tr>
<td>CLPs</td>
<td>30</td>
<td>0.02 (0.01-0.04)</td>
<td>0.39 (0.23-0.55)</td>
<td>0.11 (0.09-0.13)</td>
<td>0.45 (0.38-0.52)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pro-T1</td>
<td>50</td>
<td>0.09 (0.03-0.2)</td>
<td>0.55 (0.1-1)</td>
<td>0.35 (0.06-0.81)</td>
<td>3.44 (1.4-9)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pro-T2</td>
<td>60</td>
<td>0.01 (0-0.02)</td>
<td>0.09 (0-0.3)</td>
<td>0.09 (0.03-0.25)</td>
<td>0.28 (0.1-1)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.02 (0.0-0.07)</td>
<td>0.00 (0-0.2)</td>
<td>0.02 (0.0-0.06)</td>
<td>0.04 (0-0.2)</td>
<td>0.08 (0-0.18)</td>
<td>0.12 (0-0.44)</td>
</tr>
</tbody>
</table>

Results are based on multicolor flow cytometric analysis of epidermal cells isolated from mice at indicated time points after progenitor transfer. Data were obtained from at least 3 mice (3 mo) or 5 mice (2 wk and 3 wk) that underwent transplantation per progenitor population, with the exception of 30 \(\times 10^6\) CLPs (2 mice each).

dEC/tEC indicates ratio of donor-derived (CD45.1+) epidermal cells per total (CD45.1+) epidermal cells (%); dLC/tLC, ratio of donor-derived LCs per total LCs (%); LCs are defined as I-Ab+/CD11c+ cells, and donor-derived LCs, as CD45.1+/I-Ab+/CD11c+ population; and ND, not determined.

Both myeloid- and lymphoid-derived LCs persist in the skin

In contrast to other DCs, in the absence of inflammation, LCs are capable of maintaining themselves in the skin for more than one year. To determine if the LCs derived from progenitor cells during inflammation can provide a durable source of LCs in the skin, we analyzed epidermal-cell suspensions from mice 3 months after transplantation and UV light treatment. At this time point, we still detected both CMP- and CLP-derived LCs in the skin, and the ratio of CMP progenitor-derived LCs was in the same range as obtained after short-term analysis (Figure 3A; Table 1). In contrast, the LC reconstitution efficiency of CLPs increased over time, but remained substantially lower than that of CMPs (Figure 3B; Table 1). These results indicate that the optimum time point to detect LC reconstitution after CLP transfer is later than for other CLP-derived DC populations. Even after transplantation of the further differentiated GMP population, it was still possible to find GMP-derived LCs at 3 months after UV light–induced inflammation (Figure 3A). To exclude false-positive results due to contamination of transplanted progenitors with HSCs, skin and thymus were analyzed for the presence of donor-derived CLPs at 3 months after transplantation and UV light treatment.

### Table 1. LC reconstitution by various progenitor populations at different time points after adoptive transfer

<table>
<thead>
<tr>
<th>Progenitor population</th>
<th>Progenitor no. (\times 10^3)</th>
<th>2 wk after transfer</th>
<th>3 wk after transfer</th>
<th>3 mo after transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>dEC/tEC, %</td>
<td>dLC/tLC, %</td>
<td>dEC/tEC, %</td>
</tr>
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<td>HSCs</td>
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<td>0.09 (0.01-0.17)</td>
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<td>ND</td>
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<tr>
<td>CLPs</td>
<td>10</td>
<td>0.04 (0.01-0.1)</td>
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<td>30</td>
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</tr>
<tr>
<td>Pro-T1</td>
<td>50</td>
<td>0.09 (0.03-0.2)</td>
<td>0.55 (0.1-1)</td>
<td>0.35 (0.06-0.81)</td>
</tr>
<tr>
<td>Pro-T2</td>
<td>60</td>
<td>0.01 (0-0.02)</td>
<td>0.09 (0-0.3)</td>
<td>0.09 (0.03-0.25)</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.02 (0-0.07)</td>
<td>0.00 (0-0.2)</td>
<td>0.02 (0-0.06)</td>
</tr>
</tbody>
</table>
Progenitor-derived epidermal LCs display the same morphology as host-derived epidermal LCs

A distinguishing feature of LCs and other DCs is their characteristic morphology. To assess the morphology and distribution of donor-derived LCs, we double stained epidermal sheets obtained from mice that received transplants of progenitors with antibodies to I-Ab and CD45.1. The results confirmed the presence of CD45.1/H11001/I-Ab/H11001/LC-like cells located in the epidermis (Figure 4A). The cells always appeared in clusters, typically in areas devoid of host LCs. The morphology of progenitor-derived LCs was further assessed in cytospin preparations of sorted CD45.1/H11001/CD11c/H11001/I-Ab/H11001/B220/H9252/TCR/H9253/H9254/H11002 cells. Both CMP- and CLP-derived LCs displayed typical DC morphology (Figure 4B). To further characterize myeloid- and lymphoid-derived LCs, we analyzed sorted CD45.1+/CD11c+/I-Ab+/B220−/TCRγδ− cells for expression of Cd207 mRNA, as Langerin is a reliable LC marker in both humans and mice. Langerin expression could be detected at similar levels in all sorted LC populations irrespective of their developmental origin (Figure 4C). Sorted epidermal γδT cells served as a negative control.

The capacity to generate LCs lies mainly in the Flk2+ fractions of CMPs and CLPs

Flt3-ligand (FL) represents an important growth factor for the generation of DC subsets in the spleen. To study the role of FL in LC development, we took advantage of the fact that both CMPs and CLPs are heterogeneous with respect to their expression of the FL receptor, fetal liver kinase-2 (Flk-2). When mice were reconstituted with Flk2+/H11001 and Flk2+/H11002 fractions of CMPs and CLPs, only the Flk2+/H11001 progenitors from CLPs gave rise to LCs as determined by flow cytometry (Figure 5A) and immunofluorescence staining of epidermal sheets (Figure 5B). On average, 1.0% of total LCs was reconstituted with 1/104 Flk2+/H11001 CLPs at 3 weeks after adoptive transfer, which is about twice the number obtained after transfer of 1/104 unseparated CLPs (Figure 5C). This corresponds to the percentage of Flk2+ cells within the CLP population (50%-70%). For CMPs, the LC reconstitution by donor-derived cells averaged 11.4% 2 weeks after transfer of 1/104 Flk2+/H11001 CMPs, compared with 8.7% after transfer of 1/104 CMPs or 2.6% after transfer of 1/104 Flk2− CMPs (Figure 5C).

Figure 2. LC reconstitution by lymphoid progenitor cells. (A) Histogram plots represent CD45.1 expression profile of epidermal-cell suspensions 2 and 3 weeks after adoptive transfer of either 10⁴ CLPs or 5 × 10⁴ pro-T1 or control cells (autologous BM only). Contour plots show corresponding I-Ab/CD11c expression profile of gated CD45.1+ and CD45.1− epidermal cells from mice that underwent transplantation. (B) Contour plot shows B220/TCRγδ profile of CLP-derived LCs (CD45.1+; I-Ab+, CD11c+ population) isolated from the epidermis of mice that received transplants of CLPs at 3 weeks after adoptive transfer. (C) Top contour plot shows CD45.1/CD45.2 expression profile of total spleen cells at 3 weeks after adoptive transfer of 10⁴ CLPs. Bottom contour plot represents TCR/CD19 expression profile of gated CLP-derived (CD45.1+) spleen cells. (D) Chart shows average LC reconstitution (% of total LCs) obtained per 10⁴ progenitor cells at 2 weeks (both myeloid and lymphoid progenitors) and 3 weeks (only CLPs, pro-T1, pro-T2) after adoptive transfer. ND indicates not determined. Data are representative of at least 5 transplantations per progenitor population with similar results.
results (Figure 6B). Therefore, the expression of these receptors (Figure 6) does not correlate completely with LC generation potential of the progenitors (Figures 1-3; Table 1).

Discussion

Recently our laboratory has shown that LCs self-renew in the skin during steady-state conditions and are replaced by bone marrow–derived progenitors only during inflammatory conditions.\textsuperscript{20} Using an inflammatory model system, we could demonstrate for the first time that both CMPs and CLPs have the capacity to generate LCs in vivo. This has been shown both quantitatively by flow cytometry as well as qualitatively by microscopic analysis of epidermal sheets. Our data indicate that CMPs have a superior capacity to generate LCs, as we could detect a several-fold-higher reconstitution rate of LCs by CMPs in the skin than after transplantation of similar numbers of CLPs. In combination with our observation that LC reconstitution by myeloid progenitors is faster than by all tested lymphoid progenitors, we can conclude that mainly myeloid progenitors are recruited to the epidermis upon inflammation and replace the depleted resident LC population, especially as CMPs are about 10-fold more abundant in the BM than CLPs. Previously, the assumption that LCs are of myeloid origin was based exclusively on data from in vitro cultures, which showed that human blood monocytes and even a CD14\textsuperscript{+} dermal progenitor have the capacity to differentiate into LC-like cells that show the typical Birbeck granules and show expression of Langerin and CD1a.

To date, few studies have been performed to delineate the origin of LCs in vivo. One previously published report indicates a lymphoid origin of LCs.\textsuperscript{8} In this study, early thymic progenitors have been shown to give rise to LCs after 2 weeks. Although we confirmed that a small percentage of LCs can indeed be generated from early thymic progenitors, the percentages of pro-T1– or pro-T2–derived LCs were lower than those detected in the previous study and significantly lower than after transfer of CMPs. Unfortunately, the previous study did not evaluate the capacity of any myeloid progenitors or CLPs to give rise to LCs in comparison, so that the results are difficult to compare with our data. One reason for the higher LC generation capacity of thymic progenitors in the previous study might be due to the longer exposure to UV light, as LC depletion and inflammatory conditions are crucial to induce LC reconstitution by BM-derived progenitors. Furthermore, our study provides a detailed morphologic and phenotypic analysis of CLP- and CMP-derived LCs, as well as giving more insight into the mechanism of LC generation with regard to time course of LC generation and correlation to Fik2 and chemokine receptor expression on progenitor cells.

In a recent attempt to identify the LC precursors resident in the skin, dendritic epidermal leukocytes (DELs) isolated from fetal skin were shown to differentiate into LCs in vitro.\textsuperscript{30} Unfortunately, the capacity of DELs to generate LCs in vivo was not evaluated in this study.\textsuperscript{30} Whether such skin-resident LC precursors originate from CLPs or CMPs during inflammation, or represent an independent source of LCs, remains unanswered. Our observation that progenitor-derived LCs typically appeared in clusters suggests that CMP- and CLP-derived progenitors may continue to proliferate after they are recruited to the skin. Previously, CCR2 has been shown to play an important role in the recruitment of LC precursors to the skin,\textsuperscript{20} but as CCR2 expression on the progenitor populations does not correlate with the LC generation potential observed in our

Chemokine receptor expression on hematopoietic progenitors does not correlate with LC generation potential

As the recruitment of LC progenitors to the skin requires the presence of inflammatory chemokines, such as CCL20 and, specifically, the chemokine receptors CCR2 and CCR6 have been reported to be involved in this process.\textsuperscript{3,20} We determined the expression profile of those receptors in the progenitor populations. RT-PCR analysis for CCR2 and CCR6 demonstrates that only GMPs, and at lower levels CMPs, express CCR2, while none of the progenitors shows expression of CCR6 (Figure 6). Flow cytometric analysis of the progenitor populations after staining with either CCR2 or CCR6 antibodies confirms that CMPs and GMPs are the only progenitor populations that express CCR2. In contrast to the RT-PCR results, CMPs show slightly higher expression of CCR2 than GMPs (Figure 6B), pointing to posttranslational regulation of CCR2 surface expression. As expected, all progenitor populations were negative for CCR6 expression, confirming the RT-PCR
experiments, this further argues that mainly downstream progenitors, and not the transplanted BM progenitors, migrate to the skin. However, CCR2 expression might partially account for the superior LC potential of myeloid progenitors compared with lymphoid progenitors, as GMPs and CMPs but neither HSCs, CLPs, nor any of thymic progenitors show expression of CCR2. Both CMPs and GMPs can therefore potentially migrate directly to the skin, which might explain the faster LC reconstitution by myeloid progenitors.

The identification of murine hematopoietic progenitor populations has had a major impact on the ability to study the development of discrete leukocyte lineages, including DCs. Both “lymphoid” CD8α+ and “myeloid” CD11b+/CD8α– splenic DC subsets have been shown to be derived mainly from CPMs, while thymic DCs originate preferentially from lymphoid progenitors.11,12 Recently, even splenic CD11c+ CD45RA+ Ly6C+ CD11b– interferon-alpha–producing plasmacytoid dendritic cells (IPCs) have been identified as primarily myeloid derived, while the IPC population present in the thymus develops preferentially from CLPs.31,32 The different homing patterns of the various progenitor populations likely influence the developmental origins of DCs in particular.
tissues. Conversely, differences in tissue microenvironment might favor the development of DC precursors from either lymphoid or myeloid progenitors. Thus it is likely that the thymic environment may favor the development of lymphoid DCs, while splenic and peripheral tissues favor the development of myeloid-derived DCs and LCs, even though we cannot exclude that the inflammatory environment induced by UV light exposure might skew LC development toward the myeloid lineage.

Although UV light is the most effective inflammatory stimulus to induce LC chimerism, a variety of other inflammatory stimuli, such as the contact sensitizer DNFB, croton oil, or induction of skin irritation by tape stripping, are also capable of recruiting BM-derived LC progenitors to the skin (data not shown). Unfortunately, the levels of LC chimerism induced by these stimuli after reconstitution of C57Bl/6 mice with CD45.1 congenic BM were significantly lower than after UV light exposure. This did not allow us to analyze LC reconstitution after adoptive transfer of committed progenitors, which have a limited life span. Nevertheless, it is likely that the same progenitors that are the source of LCs in our model system (mainly Flk2\(^+\) CMPs) give rise to LCs during all inflammatory conditions that induce LC reconstitution by BM-derived progenitors. Replacement of LCs by BM-derived progenitors also occurs during the course of graft-versus-host disease (GVHD) after allogeneic BM transplantation. As induction of LC chimerism before allogeneic BM transplantation can also prevent GVHD,\(^3\) understanding the mechanisms responsible for LC development and recruitment during inflammatory conditions might prove useful in the development of new prophylactic therapeutic approaches to GVHD or other diseases involving skin inflammation.

While recent studies indicate that DC subsets in addition to LCs can be of both myeloid or lymphoid origin,\(^11,12,31,32\) it is still not clear if the developmental origins of these cells has an impact on their functional properties, although no major differences have been shown between CLP- or CMP-derived cells of the same DC subset. Unfortunately, the low numbers of progenitor-derived LCs prevented us from studying the capacity of these cells to ingest and present antigen or migrate to lymphoid organs. However, we could not detect any phenotypic or morphologic differences between LCs derived from CMPs or CLPs. Furthermore, all LC populations showed similar expression of Cd207 mRNA. Langerin, a typical LC marker, is a c-type lectin receptor that is involved in presentation of nonpeptide antigens to T cells\(^33\) and is also a potent inducer of Birbeck granules.\(^34\) Recent studies have confirmed that although other murine DC populations express Langerin, LCs are the only Langerin-expressing cells in the skin.\(^35,36\) Thus, despite their numeric differences, the LCs derived from myeloid and lymphoid progenitors appear to be indistinguishable from one another and from conventional LCs.

Flt3-ligand (FL) is an important growth factor that not only expands early hematopoietic progenitors but also promotes the development of both conventional DCs as well as plasmacytoid DCs (PDCs). Flk2 (fetal liver kinase), the cellular receptor for FL, is expressed on most early hematopoietic progenitors and the fact that both CMPs and CLPs contain Flk2\(^+\) as well as Flk2\(^-\) populations allowed us to study the effect of FL expression on LC generation.\(^28\) The much greater capacity of Flk2\(^+\) progenitors to generate LCs indicates that FL plays an important role not only in the development of conventional DCs and PDCs but also during LC development, confirming the promoting effect of FL on LC development observed in human in vitro cultures.\(^37\) Our finding that the Flk2\(^+\) fraction of hematopoietic progenitors is the main source of LCs is consistent with results observed previously for conventional DCs in the spleen\(^28,29\) and further supports the DC potential of Flk2\(^+\) progenitors.

In contrast to splenic and thymic DC populations, a direct effect of FL administration on LC development is difficult to determine in vivo, as FL injections do not increase total LC numbers in vivo,\(^38\) which is most likely due to limited numbers of available LC niches in the epidermis. In addition, FL expands but does not accelerate splenic DC generation from progenitors, therefore FL administration should have no direct effect on LC recovery after UV light–induced inflammation, which is also in accordance with previous data reporting no effect on LC recovery after LPS-induced depletion.\(^38\) We have shown before that splenic DC numbers derived from either CMPs or CLPs increase relative to the percentage of Flk2\(^+\) cells within the progenitor population, but independent of their lineage origin.\(^28\) It is therefore likely that in vivo FL administration has the same promoting effects on CMPs and CLPs during LC development in vivo as observed for conventional splenic DCs, with the exception that total LC numbers in the epidermis are not affected by FL.

Taken together, our results demonstrate for the first time that both CMPs and CLPs have the capacity to generate LCs in vivo. On a per-cell basis, CMP reconstituted LCs in the skin more efficiently and more rapidly than CLPs or early thymic progenitors. With the additional knowledge that CMPs are at least 10-fold more abundant than CLPs in the BM, we can conclude that mainly myeloid progenitors are recruited to the epidermis upon inflammation and replace the depleted resident LC population. Given the much greater capacity of Flk2\(^+\) progenitors to generate LCs, we also conclude that FL plays an important role in the development of LCs.

**Acknowledgments**

We wish to thank Dr Pia Björck and Claudia Benike for critical reading of the paper.
References

Flk2⁺ myeloid progenitors are the main source of Langerhans cells

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