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The process of lymphopoiesis begins in the bone marrow (BM) and requires multiple cellular intermediates. For T cell production, lymphoid progenitors exit the BM and home to the thymus where maturation and selection ensue. These processes are dependent on a number of factors, including chemokines and adhesion molecules. Although the β2 integrin CD11a plays an important role in the migration of lymphocytes to lymph nodes, the role of CD11a in T cell development is largely undefined. Our studies now show that, in CD11a−/− mice, thymic cellularity was decreased and early T cell development was partially impaired. Remarkably, CD11a was critical for generation of common lymphoid progenitors (CLPs) and lymphoid-primed multipotent progenitors. However, in intact CD11a−/− mice, peripheral B and T cell subsets were only modestly altered, suggesting that compensatory mechanisms were operating. In contrast, competitive BM-reconstitution assays revealed an essential role for CD11a in the generation of thymocytes and mature T and B cells. This defect was linked to the requirement for CD11a in the development of CLPs. Furthermore, our results identified CLPs, and not lymphoid-primed multipotent progenitors, as the requisite CD11a-dependent precursor for lymphocyte development. Thus, these findings established a key role for CD11a in lymphopoiesis. The Journal of Immunology, 2014, 193: 000–000.

Hematopoietic stem cells (HSCs) are responsible for the continuous maintenance of immune cells during adult life. These rare self-renewing cells first appear within the fetal liver of embryonic mice at embryonic day 11.5 (1). HSCs begin to traffic into the bone marrow (BM) shortly prior to birth (2). Within the adult BM, murine HSCs lack expression of all lineage markers (Lin−) and express the markers Sca-1 and c-kit (CD117) (3). This Lin−Sca-1+c-kit+ (LSK) subset is heterogeneous in nature. LSKs that express the cytokine receptor Flt3 (CD135) have lost self-renewing capacity and are called multipotent progenitors (MPPs), because they still possess multilineage potential (4, 5). MPPs can give rise to early lymphoid progenitors and lymphoid-primed multipotent progenitors (LMPPs), which can express RAG genes, as well as IL-7Rα, indicating the earliest point of commitment toward the lymphoid lineages (6–9). Further downstream in LSKs are the common lymphoid progenitors (CLPs) (10). Although initially thought to only give rise to cells within the lymphoid lineages, recent data suggest that CLPs are also heterogeneous, and some fraction of this population still possesses myeloid potential (11, 12). From the BM, T cell progenitors enter the circulation and home to the thymus (13). Although the identity of the thymic settling progenitor (TSP) is still open to debate, studies show that either LMPPs or CLPs can give rise to T cells in the thymus (14, 15).

In some ways, the homing and traversing of progenitors through thymic endothelium parallels the sequence of events required for entry of lymphocytes into lymph nodes. Numerous studies suggested that entry into the thymus is tightly regulated, and homing and adhesion molecules, including PSGL-1 and the chemokine receptors CCR7 and CCR9, have been implicated in thymic settling and T cell development in vivo (16–20). In addition, in short-term adoptive-transfer experiments, Scimone et al. (21) showed that Ab blockade of CD18 (the β2 integrin chain) results in reduced migration of progenitors into the thymus. Because the β2 integrin family consists of four members defined by distinct α-chains pairing with β2 (CD11a–d), the contribution of each α integrin to T cell development remains unknown.

CD11a is the α-chain of the α2β2 integrin (also known as leukocyte function associated Ag 1, LFA-1). Although CD11a plays a critical role in the homing of lymphocytes across high endothelial venules to enter lymph nodes (22), the role of CD11a in T lymphopoiesis is not clearly defined. A number of earlier studies examined whether expression of CD11a affected thymocyte binding to thymic epithelium to influence thymocyte maturation; a consensus function for CD11a was not identified. Affinity modulation of CD11a is also important for its function, and it is possible that this may be regulated during T cell development (23). In one study, blocking CD11a in fetal thymic organ cultures resulted in the inhibition of double-positive (DP; CD4+CD8+) thymocyte development (24), whereas in another study, anti-CD11a inhibited T cell development from fetal liver progenitors but not from fetal thymus progenitors (25). Treating mice from birth with anti-CD11a, but not anti-β2, mAb resulted in a selective loss of mature CD8 thymocytes (26), whereas blocking CD11a, or...
its ligand ICAM-1, inhibited intrathymic CD4 T cell migration and Ag recognition (27). In the past, two groups used different lines of CD11a-deficient mice to examine the role of this molecule in productive immune responses (28, 29). Although one of these studies reported normal numbers of thymocytes and T cells (28), detailed analyses of individual subsets were not performed. In the second study, the investigators concluded that T cell selection was intact based on their analysis of TCR-transgenic T cells deficient in CD11a (29). Thus, although some of the evidence points to a role for CD11a in T cell development, additional investigation is required to define the developmental stage(s) at which CD11a might operate in vivo.

In the current study, we used CD11a-deficient mice to define the role of this β2 integrin during lymphocyte development. We examined the generation of various BM progenitor subsets and analyzed intrathymic T cell differentiation in CD11a−/− mice and in mixed chimeric mice generated from wild-type (WT) and CD11a−/− BM cells. Intact CD11a−/− mice exhibited noticeable, but relatively minor, defects in T cell development, despite major defects in lymphoid progenitor generation in the BM. In mixed BM chimeras, CD11a−/− cells exhibited a severe defect in their potential to generate thymocytes and peripheral T and B cells. This defect was likely due to the nearly complete block in CLP development, even in a setting in which upstream LSK development was normal. Overall, our results demonstrate a novel role for CD11a in the generation of lymphoid progenitors in the BM that is essential to normal lymphocyte development.

**Materials and Methods**

**Mice**

CD11a−/− mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME) and further maintained in the Center for Comparative Medicine at the University of Connecticut Health Center. C57BL/6 mice (both CD45.2 and CD45.1) were purchased from the National Cancer Institute–Charles River Laboratories (Frederick, MD). All animal protocols were carried out in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee.

**Tissue preparation**

Spleen, thymus, and BM were isolated from WT, CD11a−/−, or mixed chimeric mice. Single-cell suspensions of spleen and thymus were prepared by crushing each of the organs through 40-μm cell strainers (BD Biosciences). RBCs in the spleen were lysed before preparing samples for staining. BM was isolated from the femur and tibia of one leg by rapid centrifugation at ~6000 rpm and suspended in RPMI 1640.

**Abs and flow cytometry**

mAbs specific for the following Ags were purchased from BioLegend, eBioscience, and BD Biosciences: CD4, CD8, CD3, GL3 (TCRγδ), CD19, CD44, CD25, NK1.1, CD11a, CD11b, CD11c, Gr-1, Ter119, Flt3, Sca-1, CD27, c-kit, CD127, and Ly6G. Viability was analyzed using LIVE/DEAD cell stain (Invitrogen). Cells were stained with Abs for 20–30 min at 4°C, washed with FACS buffer (PBS/0.2% BSA/0.01% NaN3), and fixed using 2% paraformaldehyde/PBS (Electron Microscopy Sciences). Fluorescence was measured using an LSR II (BD Biosciences) and analyzed using FlowJo software (TreeStar, Ashland, OR). Whole BM was analyzed for progenitor populations that were negative for lineage markers (Lin−; CD11b, CD11c, Ter119, NK1.1, CD3, CD19, Gr-1). Fluorescence minus one (FMO) controls were used as indicated in the figure legends, either as the negative controls to detect expression of various cell surface markers or to assist in gating of certain populations.

**Progenitor isolation and in vitro culture**

BM cells isolated from WT and CD11a−/− mice were depleted of Lin+ cells (CD4, CD8, CD19, CD11b, CD11c, NK1.1, Gr-1, Ter119) using MACS beads (Miltenyi Biotec) before sorting on a FACSAria II (BD Biosciences). Progenitors were defined as Lin− Sca-1− c-kit+. Sorted cells were then seeded on either OP9 or OP9-DL1 cells (generously provided by Dr. J.C. Zúñiga-Pflücker, University of Toronto, Toronto, ON, Canada) in vitro for the indicated times in the presence of 5 ng/ml Flt-3L and 1 ng/ml IL-7 (30).

**Mixed bone marrow chimeras**

Whole BM was isolated from WT (CD45.2+/CD45.1−) and CD11a−/− (CD45.2−/CD45.1+) donor mice. Cells were then mixed in either a 1:1 or 1:3 (WT/CD11a−/−) ratio, and a total of 2–3 million BM cells was injected i.v. via the tail vein into WT (CD45.1+) hosts that had been lethally irradiated (1000 rad). Lymphoid tissues were harvested after 13–24 wk to allow for full reconstitution of the immune compartment.

**Statistical analysis**

Statistical significance was analyzed by the Student t test or ANOVA using Prism 5 (GraphPad), with $p < 0.05$ considered significant.

**Results**

**Alterations in peripheral cellularity in CD11a-deficient mice**

In the course of our previous studies examining the role of CD11a in the T cell response to infection (31), we observed several interesting effects of CD11a deficiency on the lymphoid compartment of unfected controls. In naïve CD11a−/− mice, there was no...
significant decrease in the overall cellularity of the spleen compared with WT controls, despite some variation in the total size (Fig. 1A). However, the percentage and total numbers of CD3\(^+\) T cells and CD19\(^+\) B cells were reduced in the spleens of CD11a\(^{-/-}\) mice (Fig. 1B, Table I). The T cell defect was largely accounted for by a loss of CD4 T cells (Table I). We also noted a significant increase in the percentages of NK cells and TCR \(\gamma\delta\) T cells in CD11a\(^{-/-}\) spleens, although only the latter exhibited increased total cell numbers (Fig. 1B, Table I). These changes suggested a potential requirement for CD11a in lymphocyte development and prompted us to examine the primary lymphoid tissues responsible for B and T cell generation, the BM and thymus, respectively. No difference in the total number of BM cells in CD11a\(^{-/-}\) versus WT mice was noted (Fig. 1C), but there was a consistent 2-fold decrease in the total cellularity of the thymus in CD11a\(^{-/-}\) mice (Fig. 1D). Therefore, considering the reduction in peripheral T cells and the reduction in overall thymus size, we hypothesized that CD11a was required for the optimal generation of T cells.

**Expression of CD11a on thymic progenitors**

To examine the role of CD11a in the step-wise process of T cell development in primary lymphoid organs, we first determined CD11a expression levels on progenitor populations in the adult thymus. Individual subsets of double-negative (DN; CD3\(^{-}\)CD11a) thymocytes expressed varying levels of CD11a. Interestingly, early thymic progenitors (ETPs; c-kit\(^+\) DN1 cells) (32) expressed high levels of CD11a, followed by an apparent downregulation of CD11a expression at the DN2 and DN3 stages. CD11a was up-regulated between the DN3 and DN4 stages and again within the DP stage, corresponding with the acquisition of CD3 expression (Fig. 2). Ultimately, there were only slight differences in the mean fluorescence intensity of CD11a expression among CD3 DP, CD4 SP, CD8 SP, and TCR \(\gamma\delta\) T cells in the thymus (Fig. 2B). Overall, our analysis indicated that CD11a expression was regulated during thymocyte maturation, suggesting a potential functional relationship between CD11a expression and T cell development.

**Differentiation of thymocytes is not dependent on CD11a expression**

Because all T cell progenitors expressed CD11a, we determined whether the 2–3-fold decrease in overall thymus size in CD11a-deficient mice was the result of a block at a particular checkpoint during thymocyte maturation. In the CD11a\(^{-/-}\) thymus, a minimal, but statistically significant, reduction in the percentage of DP cells was observed \((p = 0.0037)\). Analysis of single-positive (SP) thymocytes indicated a modest decrease in the frequency of CD4 SP cells \((p = 0.0053)\) that was accompanied by an increased frequency of TCR \(\gamma\delta\) T cells in the CD11a\(^{-/-}\) thymus (Fig. 3A). These relative trends observed in thymic CD4 SP and TCR \(\gamma\delta\) T cell populations were maintained in the periphery of CD11a\(^{-/-}\) mice (data not shown; Table I). Examination of the DN subsets revealed that the frequency of DN1 thymocytes, ETPs, and the small subset of c-kit\(^+\) DN2 cells was significantly reduced in CD11a\(^{-/-}\) mice (Fig. 3B, 3C). The total number of cells in the DN1 and ETP subsets also was significantly reduced (~3-fold) in the CD11a-deficient thymus (Table II). Despite these reductions, the increased frequency of the DN3 subset and equivalent numbers of the DN2 and DN3 cells in the absence of CD11a suggested that compensatory mechanisms might have been operating at these early stages of thymocyte development.

![FIGURE 2. Expression of CD11a on T cell precursors within the thymus. Thymocytes from WT B6 mice were examined for CD11a expression on the indicated populations using flow cytometry. DN (CD3\(^{+}\) TCR\(\gamma\delta\) CD4\(^{-}\) CD8\(^{-}\)) stages were separated based on CD44 and CD25 expression (DN3–4). ETPs were defined as the c-kit\(^+\)CD25\(^{-}\) DN subset, whereas DN2 were identified as the c-kit\(^+\)CD25\(^{+}\) DN subset. Comparison of CD11a on CD3 DP (CD3\(^{+}\) TCR\(\gamma\delta\) CD8\(^{-}\)), CD3\(^+\) DP (CD3\(^{+}\)CD4\(^{-}\)CD8\(^{-}\)), and total DP (CD4\(^{+}\)CD8\(^{-}\)) are shown in comparison with the following mature SP T cells: CD4 SP (CD3\(^{+}\) TCR\(\gamma\delta\) CD4\(^{-}\)), CD8 SP (CD3\(^{+}\) TCR\(\gamma\delta\) CD8\(^{-}\)), and TCR \(\gamma\delta\) (CD3\(^{-}\) TCR\(\gamma\delta\)). (A) Data are representative graphs comparing CD11a expression of developing thymocytes with the FMO control of all thymocytes (lowest graph). (B) Mean fluorescence intensity of CD11a expression in the indicated thymocyte subsets analyzed in three mice. Error bars represent SEM.](http://www.jimmunol.org/)

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Table I. Absolute numbers (\(\times 10^{6}\)) of lymphocytes in the spleen of WT and CD11a\(^{-/-}\) mice

<table>
<thead>
<tr>
<th>Population</th>
<th>WT</th>
<th>CD11a(^{-/-})</th>
<th>(p) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>22.70 ± 0.8</td>
<td>16.82 ± 1.9</td>
<td>0.03</td>
</tr>
<tr>
<td>CD3(^{+})TCR(\gamma\delta) CD4(^{-})</td>
<td>13.45 ± 0.6</td>
<td>6.31 ± 0.9</td>
<td>0.006</td>
</tr>
<tr>
<td>CD3(^{+})TCR(\gamma\delta) CD8(^{-})</td>
<td>8.39 ± 0.7</td>
<td>7.91 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>TCR(\gamma\delta)</td>
<td>0.56 ± 0.0</td>
<td>2.72 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NK</td>
<td>3.37 ± 0.2</td>
<td>3.19 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>CD19</td>
<td>45.40 ± 3.7</td>
<td>22.09 ± 1.2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are inclusive of five mice/group. Mice ranged in age from 8 to 12 wk. Two individual experiments were performed.
Developmental stages. Nevertheless, the most noteworthy outcome of CD11a deficiency was an overall decrease in thymus cellularity.

**CD11a is critical for lymphoid progenitor development in the BM**

Given the defects observed in the ETP population, we compared lymphoid progenitor populations in the BM of WT and CD11a−/− mice. First, we examined CD11a expression on WT progenitors and observed that, on average, only ∼40% of total Lin− cells expressed CD11a (Fig. 4A, left panel). However, the earliest BM progenitors (LSKs, Flt3L-LSKs, and CLPs) were found within the CD11a+ population, and their CD11a expression levels were comparable (Fig. 4A, right panel). CD11a−/− mice had normal frequencies of total Lin− BM cells (data not shown). There was a trend toward increased percentages of LSKs in CD11a−/− mice compared with WT mice, although this was not statistically significant (Fig. 4B, 4F, p = 0.06). Interestingly, further analysis revealed a severe defect in the progression of LSKs to subsequent hematopoietic intermediates in the absence of CD11a. Within the LSK subset, expression of Flt3 identifies MPPs and LMPPs (4, 5, 9), and Flt3+LSKs were dramatically influenced by the absence of CD11a, such that both the CD127+ and CD127− fractions were significantly reduced in CD11a−/− mice (Fig. 4C, 4G). In addition, using a gating strategy reported by Saran et al. (33), we discovered an overall decrease in the Flt3highCD27+ population in CD11a−/− mice (Fig. 4D, 4I). Moreover, the development of CD127+ CLPs, a subset of the Flt3highCD27+ population in CD11a−/− mice, was severely impaired in CD11a−/− mice (Fig. 4E, 4F). The frequency of CLPs also can be assessed using an alternative gating strategy whereby Lin− Sca-1+ c-kit+ cells that also express CD127 are defined as CLPs (Supplemental Fig. 1). Not only did we note a distinct reduction in Sca-1+c-kit+ cells in CD11a-deficient BM, we also found a substantial reduction in the frequency of CD127+ CLPs compared with WT control mice, consistent with the data shown in Fig. 4E and 4I. Because both CLPs and LMPPs are believed to be able to enter the circulation and seed the thymus (34), the impaired development of these populations in the BM may explain the reduced number of ETP cells in the thymus of CD11a−/− mice.

**Cell-intrinsic CD11a expression is required for optimal T cell development in vitro**

Next, we asked whether the reduced development of T cells in CD11a−/− mice was the result of an intrinsic defect of CD11a-deficient progenitor cells to efficiently generate T cells. Thus, to compare the developmental potential of WT and CD11a−/− BM cells, we used the in vitro OP9-DL1 stromal cell coculture system (35). Equal numbers of LSK BM cells were sorted from either WT or CD11a−/− mice and

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**Table II. Absolute numbers (×10^6) of lymphocytes and progenitors in the thymus of WT and CD11a−/− mice**

<table>
<thead>
<tr>
<th>Population</th>
<th>WT</th>
<th>CD11a−/−</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+CD8− (DN)</td>
<td>3.088 ± 0.4</td>
<td>1.673 ± 0.8</td>
<td>0.03</td>
</tr>
<tr>
<td>DN1</td>
<td>0.150 ± 0.03</td>
<td>0.0375 ± 0.8</td>
<td>0.005</td>
</tr>
<tr>
<td>DN2</td>
<td>0.0175 ± 0.01</td>
<td>0.0150 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>DN3</td>
<td>0.975 ± 0.2</td>
<td>1.028 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>DN4</td>
<td>1.950 ± 0.29</td>
<td>0.9525 ± 0.8</td>
<td>0.004</td>
</tr>
<tr>
<td>ETP</td>
<td>0.006 ± 0.001</td>
<td>0.002 ± 0.003</td>
<td>0.02</td>
</tr>
<tr>
<td>c-kit+DN2</td>
<td>0.015 ± 0.005</td>
<td>0.006 ± 0.0006</td>
<td>0.09</td>
</tr>
<tr>
<td>DP</td>
<td>67.87 ± 10.76</td>
<td>31.87 ± 3.697</td>
<td>0.0195</td>
</tr>
<tr>
<td>CD4 SP</td>
<td>5.12 ± 0.73</td>
<td>1.31 ± 0.23</td>
<td>0.0015</td>
</tr>
<tr>
<td>CD8 SP</td>
<td>1.31 ± 0.13</td>
<td>0.35 ± 0.07</td>
<td>0.0006</td>
</tr>
<tr>
<td>TCRγδ</td>
<td>0.136 ± 0.014</td>
<td>0.184 ± 0.028</td>
<td>NS</td>
</tr>
</tbody>
</table>

DN (CD3+ TCRγδ− CD4+ CD8−) cells were separated based on CD44 and CD25 expression to identify DN1–4 subsets or by c-kit and CD25 expression to identify ETP and c-kit− DN2 subsets. CD3+ TCRγδ− cells were subdivided into CD4+ (CD4 SP) and CD8+ (CD8 SP) thymocytes. Data are inclusive of five mice/group. Mice ranged in age from 8 to 12 wk. Two individual experiments were performed.
cocultured with OP9-DL1 stromal cells for various times. On day 9 after seeding, substantially reduced numbers of Thy1.2+ T cells were generated from CD11a<sup>−/−</sup> BM cells compared with WT (Fig. 5A, 5B). By day 12, total Thy1.2+ frequencies were still reduced in cultures seeded with CD11a<sup>−/−</sup> LSKs (Fig. 5B). Interestingly, at this time point we also observed an increased frequency of DN2 (Thy1.2+CD44+CD25+) cells in the CD11a-deficient progenitor cultures, suggesting that, in WT progenitors, CD11a might have enhanced the developmental progression of thymocytes beyond the DN2 stage in vitro (Fig. 5C, 5D). However, by day 15 of culture, this difference was no longer present, despite a continued reduction in the total percentage of Thy1.2+ cells in CD11a<sup>−/−</sup>-derived cells (Fig. 5B, 5D). Thus, these results indicated that CD11a expression by BM progenitors regulated T cell developmental potential, especially at the early stages.

**Competitive BM reconstitution reveals an obligate requirement for CD11a in lymphoid development**

Thus far, the results suggested that cell-intrinsic expression of CD11a was important for optimal T cell development. However, despite dramatic alterations in BM precursors in CD11a<sup>−/−</sup> mice, overall T cell development was only marginally affected. To test whether this finding was due to compensatory mechanisms available in the complete absence of CD11a, we generated mixed BM chimeric mice to determine the competitive fitness of CD11a<sup>−/−</sup> progenitors to give rise to mature T cells. We transplanted equal ratios (1:1) of unfractionated BM cells from WT and CD11a<sup>−/−</sup> donor mice into irradiated WT hosts, using congenic expression of CD45 to differentiate each donor population from the recipient. After allowing >12 wk for full reconstitution to take place, chimerism was assessed in the spleen and thymus of recipient mice. Surprisingly, CD11a<sup>−/−</sup> BM was virtually unable to generate ETPs, mature thymocytes, or mature peripheral T and B cells, whereas NK cell development was impaired to a lesser degree (Fig. 6A–C). Similar trends were observed in chimeric hosts that had been allowed to reconstitute for a longer period of time (24 wk; data not shown).

**FIGURE 4.** CD11a<sup>−/−</sup> mice have normal frequencies of LSKs but severe depletion of downstream T cell progenitors in the BM. (A) BM was isolated from femurs and tibias of adult WT B6 mice and assessed for the expression of CD11a in hematopoietic progenitor cells. Representative graphs show CD11a expression in total Lin<sup>−</sup> cells compared with the FMO control of Lin<sup>−</sup> BM cells (left panel). CD11a expression on individual populations of lymphoid progenitors compared with the negative control (right panel). Gating strategy for progenitors was as follows: LSK, Lin<sup>−</sup>Sca-1<sup>−</sup>c-kit<sup>+</sup>; Flt3<sup>high</sup>LSKs; CLP, Lin<sup>−</sup>c-kit<sup>−</sup>Sca-1<sup>−</sup>CD127<sup>+</sup>Flt3<sup>high</sup>CD127<sup>−</sup>. (B–E) BM was isolated from WT and CD11a<sup>−/−</sup> mice to compare frequencies of the T cell progenitor populations. Representative zebra plots show LSK (Lin<sup>−</sup>c-kit<sup>−</sup>Sca-1<sup>−</sup>) gating from Lin<sup>−</sup> cells (B); LSKs were subdivided into CD127<sup>−</sup>Flt3<sup>high</sup> and CD127<sup>−</sup>Flt3<sup>high</sup> cells (C). Lin<sup>−</sup> BM cells that expressed Flt3 and CD27 (D) were subsequently gated to identify CLPs (Lin<sup>−</sup>Flt3<sup>high</sup>CD27<sup>−</sup>CD127<sup>−</sup>c-kit<sup>−</sup>/CD127<sup>+</sup>Flt3<sup>high</sup>CD27<sup>−</sup>CD127<sup>−</sup>c-kit<sup>−</sup>) (E). (F–I) The frequency of each population is shown in the bar graphs. Five animals/group were analyzed in three individual experiments. *p < 0.05.
CD11a is essential for development of lymphoid and myeloid progenitors

Lastly, we directly addressed our hypothesis that CD11a expression was intrinsically critical for the generation of hematopoietic intermediates within the BM compartment. To this end, we analyzed chimeric mice to examine progenitor development downstream of LSKs in the absence of CD11a expression. Overall, these results suggested that, in addition to acting as a crucial element in controlling lymphoid progenitor development, CD11a expression contributed to myeloid progenitor development.

Discussion

Earlier work suggested that CD11a plays a role in T lymphocyte development, but the precise stage(s) of development in which CD11a participated was unclear. During the complex process of adult T cell lymphopoiesis, T cell progenitors from the BM enter the circulation and consequently home to the thymus for successful development based on alterations in ETPs and mature thymic and peripheral T cell subsets. Moreover, in intact CD11a-deficient and WT bone marrow donors, although the frequency of the cells was reduced ∼2-fold in the absence of CD11a expression (Fig. 7C).

Given that innate myeloid cells also originate from HSCs in the BM, we wondered whether hematopoietic intermediates required for myelopoiesis were similarly compromised in the absence of CD11a expression. Thus, we analyzed chimeric mice to examine macrophage and dendritic cell progenitor (MDP) and common dendritic cell precursor (CDP) populations using a previously illustrated gating strategy (36). Briefly, MDPs are identified based on the expression of Lin−c-kithighCD115+Flt3+ (37), whereas CDPs, which are downstream of MDPs and can only give rise to classical dendritic cells and plasmacytoid dendritic cells, have an identical phenotype except for lower overall expression of c-kit (i.e., c-kitlow) (Fig. 8A) (36, 38). Interestingly, MDPs and CDPs derived from CD11a−/− BM were significantly reduced in both the 1:1 and 1:3 chimeras (Fig. 8B, 8C), suggesting an aberration in myeloid development downstream of LSKs in the absence of CD11a expression. Overall, these results suggested that, in addition to acting as a crucial element in controlling lymphoid progenitor development, CD11a expression contributed to myeloid progenitor development.
CD11a deficiency resulted in a significant decrease in the frequencies of downstream lymphoid progenitor subsets: Flt3+LSKs (which includes the LMPP subset) and CLPs. One explanation for this phenomenon is that CD11a expression is critical for proper localization of progenitors within specific BM niches. If this is the case, the absence of CD11a may result in poor survival as a result of the inability of the progenitors to localize to areas rich in cytokines that promote homeostasis. Alternatively, the absence of CD11a could potentially disrupt interactions between progenitors and BM stromal elements that are needed to drive progressive development. In the absence of these interactions, progenitors could be liberated into the circulation. Previously, other investigators provided evidence to support this notion using anti-β2 integrin treatment (39, 40). However, these studies showed that blocking of β2 integrins alone was not sufficient to induce mobilization into the blood; rather, cotreatment with anti-α4 integrin was required to facilitate their release. Therefore, poor survival or defective generation of LMPPs and CLPs is a more likely scenario that could explain the reduced frequencies of these subsets in CD11a−/− mice. Regardless, limited availability of these two subsets likely leads to reduced numbers of TSPs in the thymus. Indeed, frequencies of ETPs were reduced in the thymus of CD11a−/− mice. However, the possibility remains that the absence of CD11a may also diminish the ability of TSPs to traverse through thymic endothelium when entering the thymus, and a role for β2 integrins in thymic settling of progenitors was suggested (21). Nonetheless, our in vivo and in vitro data suggested that CD11a expression by BM progenitors plays a cell-intrinsic role in driving T cell generation. However, we were unable to directly test an additional defect in thymic entry in CD11a−/− mice because two of the candidates of TSPs (LMPPs and CLPs) were both greatly reduced in the BM of CD11a−/− mice.

By generating mixed BM chimeras, we were able to highlight the requisite role that CD11a played in lymphocyte development. In this setting, CD11a-deficient BM progenitors were forced to compete with WT BM progenitors for niches and survival signals that are key for optimal lymphocyte generation. This competition resulted in the nearly complete inability of CD11a−/− precursors to produce either immature thymocytes or mature T cells and B cells. Although chimerism of TCR γδ T cells was not equivalent to CD11a−/− and WT cells, development of mature TCR γδ T cells appeared to be less dependent on CD11a expression compared with TCR αβ T cells. Interestingly, NK cells, considered closely related to T cells and also generated from CLPs (10), were reduced but not completely absent in the chimeras.

FIGURE 6. BM cells from CD11a−/− donors are unable to reconstitute multiple lymphocytic subsets within the thymus and periphery of mixed chimeras. Unfractionated BM cells from WT and CD11a−/− mice were mixed in either an equal ratio (1:1) or at a ratio of 1:3 (WT/CD11a−/−) before transplantation into lethally irradiated WT hosts. Reconstitution was allowed to take place for 13–24 wk before hosts were assessed for chimerism using CD45.1 and CD45.2 congenic markers in 1:1 chimeras (A–C) and 1:3 chimeras (D–F). (A and D) Thymocytes in chimeric hosts were assessed for the presence of either WT-derived cells (black bars) or CD11a−/−-derived cells (white bars). Lin−CD3+ TCRγδ−CD4−CD8− cells were separated based on CD44 and CD25 expression to identify DN1–4 subsets or by c-kit and CD25 expression to identify ETPs and the c-kit+DN2 subset. (B and E) Bar graphs show the contribution of WT-derived cells (black bars) or CD11a−/−-derived cells (white bars) to total lymphocytes (total), NK cells, CD19+ B cells, and various CD3+ T cell subsets within the spleens of both sets of chimeras. Data are representative of four or five mice/ratio. Three individual experiments were performed. *p < 0.05.
LSKs failed to contribute equally to the total LSK subset in the 1:1 chimeras. In addition, CLPs did not develop from CD11a<sup>2</sup>/<sup>2</sup> progenitors. The production or maintenance of LSKs did not require CD11a, because in chimeras in which 3-fold greater numbers of CD11a<sup>2</sup>/<sup>2</sup> BM cells were mixed with WT BM cells and infused into irradiated recipients, CD11a<sup>2</sup>/<sup>2</sup> LSK development was slightly greater from CD11a<sup>2</sup>/<sup>2</sup> BM than from WT BM. Importantly, even in this case, development of CLPs derived from CD11a<sup>2</sup>/<sup>2</sup> BM progenitors was essentially absent. Thus, CD11a was absolutely required for development of CLPs in the BM.

A recent study demonstrated that the absence of only one lymphoid precursor population fails to completely abolish T cell development, indicating that multiple precursor subsets can contribute to T cell development (33). Thus, in the case of CD11a<sup>2</sup>/<sup>2</sup> mice, although the frequencies of Flt3<sup>hi</sup>LSKs and CLPs were reduced, both populations together apparently provided a sufficient source of progenitors for T cell generation in the adult thymus. This could explain why only a mild defect in T cell populations was observed in the periphery of intact CD11a<sup>2</sup>/<sup>2</sup> mice. An interesting possibility to be considered in future work is

**FIGURE 7.** CD11a<sup>2</sup>/<sup>2</sup> LSKs are unable to generate CLPs in irradiated chimeric hosts. Chimerism was assessed in the BM of the WT:CD11a<sup>2</sup>/<sup>2</sup> chimeras at 13–24 wk after reconstitution. (A) Bar graphs show contribution of WT donors (black bars) or CD11a<sup>2</sup>/<sup>2</sup> donors (white bars) to the BM LSK population in either 1:1 or 1:3 chimeras. Flt3<sup>hi</sup> LSKs and CLPs in 1:3 chimeras are also shown. (B) Representative dot plot shows gating and equal chimerism of the LSK population in 1:3 chimeras. (C) LSKs as shown in (B, right panel) were subdivided to identify Flt3<sup>hi</sup>CD127<sup>+</sup> and Flt3<sup>lo</sup>CD127<sup>−</sup> cells. Dot plots show the absence of Flt3<sup>hi</sup>CD127<sup>+</sup> cells from both WT and CD11a<sup>2</sup>/<sup>2</sup> donors in 1:3 chimeras. (D) Lin<sup>−</sup>CD27<sup>−</sup>Flt3<sup>−</sup> BM cells of 1:3 chimeras were gated as in Fig. 4D and separated based on congenic markers to identify WT and CD11a<sup>2</sup>/<sup>2</sup> donor populations (data not shown). Representative dot plot shows CD127<sup>−</sup>c-kit<sup>lo</sup>− CLPs within each gated donor population. Five chimeras/ratio of donors were used in two individual experiments. *p < 0.05.

**FIGURE 8.** CD11a<sup>2</sup>/<sup>2</sup> BM exhibits defective generation of myeloid progenitors in chimeric mice. Chimerism of myeloid progenitor populations was also determined in the BM of 1:1 and 1:3 chimeras. (A) Representative contour plots show the gating strategy for MDP and CDP identification. Briefly, the c-kit<sup>hi</sup>Flt3<sup>−</sup> population of Lin<sup>−</sup>Sca-1<sup>−</sup> BM cells was subdivided into c-kit<sup>lo</sup>CD115<sup>+</sup>MDPs and c-kit<sup>hi</sup>CD115<sup>+</sup>CDPs. (B) Representative dot plots show chimerism of gated MDPs (left panel) and CDPs (right panel) in 1:1 chimeras. (C) Total chimerism of MDP and CDP populations in 1:1 and 1:3 chimeras pooled from three individual experiments, with a total of 12 mice for 1:1 ratio chimeras and 10 mice for 1:3 ratio chimeras at 13–24 wk after reconstitution. *p < 0.05.
that the requirements for CD11a during hematopoiesis and entry into the thymus during embryonic and neonatal life are unique from our findings in adult animals. Acquisition of this knowledge, irrespective of whether differential requirements for CD11a adhesion exist, would contribute to advancing understanding of the fundamental mechanisms used during fetal and adult hematopoiesis and T cell development.

Of particular interest in the mixed BM chimeric mice was our finding that there was minimal, if any, development of the Flt3

CD127

CD117

LSK subset that includes LMPPs, even from WT BM. Although the reason for this phenomenon is not clear, irradiation was reported to cause thymic stromal damage and alterations in the BM microenvironment (41–43). Perhaps such effects lead to the loss of the BM niche required for development of this lymphoid progenitor. In any case, this fortuitous finding indicated that the LMP subset was not essential to lymphocyte development and was not an essential precursor to CLPs.

Collectively, we showed that CD11a expression is required for T and B cell development, although compensatory mechanisms could partially overcome this requirement in the absence of competition from WT progenitors. The nearly complete dependence of CLP development on CD11a is unique among known regulators of hematopoietic development, and our results provide new insight into this process. Future studies will seek to identify the cellular interactions mediated by CD11a that promote the generation of BM progenitors.

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stem cell mobilization by antibodies against the beta 2 integrins LFA-1 and Mac-1. Blood 100: 327–333.