Experimental Hematology

Experimental Hematology 2015;43:44–52

ADAM17 limits the expression of CSF1R on murine hematopoietic progenitors

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(Received 5 April 2014; revised 5 August 2014; accepted 1 October 2014)

All-lymphoid progenitors (ALPs) yield few myeloid cells in vivo, but readily generate such cells in vitro. The basis for this difference remains unknown. We hypothesized that ALPs limit responsiveness to in vivo concentrations of myeloid-promoting cytokines by reducing expression of the corresponding receptors, potentially through posttranscriptional mechanisms. Consistent with such a mechanism, ALPs express higher levels of *CSF1R* transcripts than their upstream precursors, yet show limited cell-surface protein expression of colony-stimulating factor 1 receptor (CSF1R). All-lymphoid progenitors and other hematopoietic progenitors deficient in A disintegrin and metalloproteinase domain 17 (ADAM17), display elevated cell surface CSF1R expression. *ADAM17*$^{-/-}$ ALPs, however, fail to yield myeloid cells upon transplantation into irradiated recipients. Moreover, *ADAM17*$^{-/-}$ ALPs yield fewer macrophages in vitro than control ALPs at high concentrations of macrophage colony stimulating factor. Mice with hematopoietic-specific deletion of *ADAM17* have normal numbers of myeloid and lymphoid progenitors and mature cells in vivo. These data demonstrate that ADAM17 limits CSF1R protein expression on hematopoietic progenitors, but that compensatory mechanisms prevent elevated CSF1R levels from altering lymphoid progenitor potential.

Hematopoietic stem cells traverse through a series of developmental intermediates, termed progenitors, en route to lineage commitment and maturation [1]. As differentiation progresses, these progenitors lose their ability to undergo self-renewing divisions. At specific developmental branch points, progenitors also lose their ability to generate specific subsets of mature blood lineages. At each of these branches, these progenitors are considered to be committed to the remaining blood lineages that they can still generate.

Complicating the definition and analysis of lineage commitment, in vivo assays can yield different results from in vitro experiments [2]. For example, common-lymphoid progenitors (CLPs) or all-lymphoid progenitors (ALPs) that yield primarily lymphocytes and dendritic cells in vivo can readily generate macrophages and neutrophils in vitro with high efficiencies [2–10]. These data demonstrate that CLPs and ALPs have not epigenetically silenced their myeloid programs [11], yet myeloid cells are infrequently generated from these progenitors under physiologic conditions [4]. Thus, there is substantial disagreement on whether CLPs and ALPs should be considered lymphoid-committed.

As highlighted by this disagreement, the mechanisms by which lymphoid progenitors limit myeloid output in vivo remain incompletely understood. One possibility is that lymphoid progenitors home to distinct niches in vivo in which local concentrations of myeloid cytokines are low [12,13]. Another non–mutually exclusive possibility is that ALPs reduce the expression of myeloid cytokine receptors such that they are unresponsive to the in vivo concentrations of such factors. Yet by providing excess amounts of myeloid lineage-promoting cytokines in vitro, lymphoid progenitors can still generate macrophages and neutrophils. Indeed, ectopic expression of certain cytokine receptors allows for robust myeloid cell production by lymphoid progenitors [14]. Endogenous cytokine receptor expression can be regulated by both transcriptional and

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Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.exphem.2014.10.001.

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http://dx.doi.org/10.1016/j.exphem.2014.10.001

posttranscriptional mechanisms. For example, A disintegrin and metalloproteinase domain 17 (ADAM17) can cleave colony stimulating factor 1 receptor (CSF1R) an essential and instructive cytokine receptor for macrophage colony stimulating factor (M-CSF), which mediates macrophage commitment and homeostasis [15–19].

ADAM17 belongs to a family of metalloproteases with broad target specificities and essential roles in many biological processes. It is best known for its role in cell-intrinsic processing of tumor necrosis factor a (TNFα) to its secreted form, and is often referred to as TNFα-converting enzyme (TACE) [20,21]. However, many studies have identified other ADAM17 targets in addition to TNFα, including CSF1R [18]. Although the role of ADAM17 in mature myeloid cells and responses to bacterial endotoxin challenge has been studied [22–26], to our knowledge there have been no reports describing its function during hematopoietic development. Given similar roles in other cell types, we hypothesized that ADAM17 limits CSF1R expression on lymphoid progenitors, thereby preventing macrophage and granulocyte production in vivo. Here we demonstrate that ALPs express *CSF1R* transcripts, and that ADAM17 does indeed limit cell surface expression of CSF1R on ALPs and other hematopoietic progenitors. Yet despite its role in limiting CSF1R on the surface of ALPs, ADAM17 is not required for preventing myeloid cell production by lymphoid progenitors in vivo.

Materials and methods

Mice

We purchased *ADAM17*$^{fl/fl}$ [22], *Vav1-iCre* [27], C57BL/6, and B6.SJL mice from The Jackson Laboratory (Bar Harbor, ME) and subsequently housed and maintained them in our animal care facility. The genotype of ADAM17-knockout mice in all experiments was *ADAM17*$^{fl/fl}$ *Vav1-iCre*$^{+}$, whereas wild-type mice were *ADAM17*$^{fl/+}$ *Vav1-iCre*$^{-}$ or *ADAM17*$^{fl/fl}$ *Vav1-iCre*$^{-}$. All studies were carried out according to the Institutional Animal Care and Use Committee at Washington University (St. Louis, MO).

Microarray and quantitative real-time polymerase chain reaction

CSF1R expression levels were analyzed for lymphoid-primed multipotent progenitors (LMPPs) and ALPs from previously published microarrays [5]. For quantitative real-time polymerase chain reaction (PCR) analysis, cells were double-sorted into TRIzol reagent (Invitrogen, Carlsbad, CA) using a BD FACSAria (BD Biosciences, San Jose, CA). SuperScript III First Strand Kit (Invitrogen) was used to generate cDNA using random hexamers, per the manufacturer's instructions. SybrGreen PCR master mix (Applied Biosystems, Foster City, CA) was used for real-time PCR assays per the manufacturer's instructions. The ABI 7000 Sequence Detection System (Applied Biosystems) was used to quantify expression. Primer sequences were: *CSF1R*, 5′-ACACGCACGGGCCACCATGAA-3′ and 5′-GCAT GGACCGTGAGGATGAGGC-3′; and *GAPDH*, 5′-GGCAAA TTCAACGGCACAGT-3′ and 5′-GATGGTGATGGGCTTCCC-3′.

M-CSF enzyme-linked immunosorbent assay

Epiphyses were removed, and dissected femurs from wild-type mice were flushed with 1 mL phosphate-buffered saline (PBS). Cells were pelleted, and M-CSF levels were quantified from bone marrow supernatant or blood serum using a mouse M-CSF enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's instructions (Sigma-Aldrich). Bone marrow M-CSF concentrations were calculated by dividing the total amount of M-CSF in the bone marrow supernatant by the marrow volume of a mouse femur, estimated to be 9.4 μL. This estimate is based upon the approximations that a mouse femur is a normal cylinder, the cross-sectional marrow radius r is 0.46 mm [28], the length l is 15 mm [28], and the volume can be calculated as $\pi r^2 l$.

Flow cytometry and cell sorting

Staining buffer consisted of 2% adult bovine serum (Hyclone, Logan, UT) or PBS with 1 mmol/L ethylenediaminetetraacetic acid. Dead cells were gated out using propidium iodide (Sigma-Aldrich). Cells were acquired and sorted on the FACSAria (BD Biosciences) or analyzed on a LSRII (BD Biosciences). Data were analyzed using FlowJo software (TreeStar, Ashland, OR). For a list of antibodies used in the experiments, see Supplementary Table E1 (online only, available at www.exphem.org).

In vitro differentiation assay

We double-sorted 500 ALPs into culture media that consisted of 10% Defined fetal bovine serum (Hyclone) in Dulbecco's Modified Eagle Medium: Nutrient Mixture F12 (DME-F12) + 10 mmol/L Hepes (Sigma Aldrich) and supplemented with nonessential amino acids (Lonza, Basel, Switzerland), sodium pyruvate (Lonza), penicillin/streptomycin (Sigma-Aldrich), Glutamax (Invitrogen), and 50 μmol/L 2-mercaptoethanol (Invitrogen). We added M-CSF (Peprotech, Rocky Hill, NJ) at the indicated concentration. Cells were cultured for 4 days before staining and flow cytometric analysis on a BD LSRII. To quantify total numbers of cells, software acquisition and recording was initiated before the sample was loaded and continued until the sample was completely consumed, and no additional live events were observed. Viability was quantified by the percentage of cells incorporating propidium iodide.

In vivo differentiation assay

We double-sorted 5,000 ADAM17 wild-type or knockout ALPs (CD45.2^{+}) into PBS and then injected into each 800 cG–irradiated B6.SJL (CD45.1^{+}) recipient mice via retro-orbital injection. Ten days postinjection, bone marrow and spleens were harvested and mechanically dissociated in staining buffer. Cells were stained and analyzed as described in Results.

Results

Cytokine signaling in hematopoietic progenitors can play an instructive role in directing fate decisions [14,17,19,29]. Thus, we hypothesized that lymphoid progenitors display reduced expression of myeloid-promoting cytokine receptors relative to their uncommitted multipotent precursors. To test this hypothesis, we examined global gene expression profiles of ALPs, which generate only lymphocytes in vivo [5], and LMPPs, which can generate macrophages in vivo through CSF1R-expressing progeny [5,30–32].

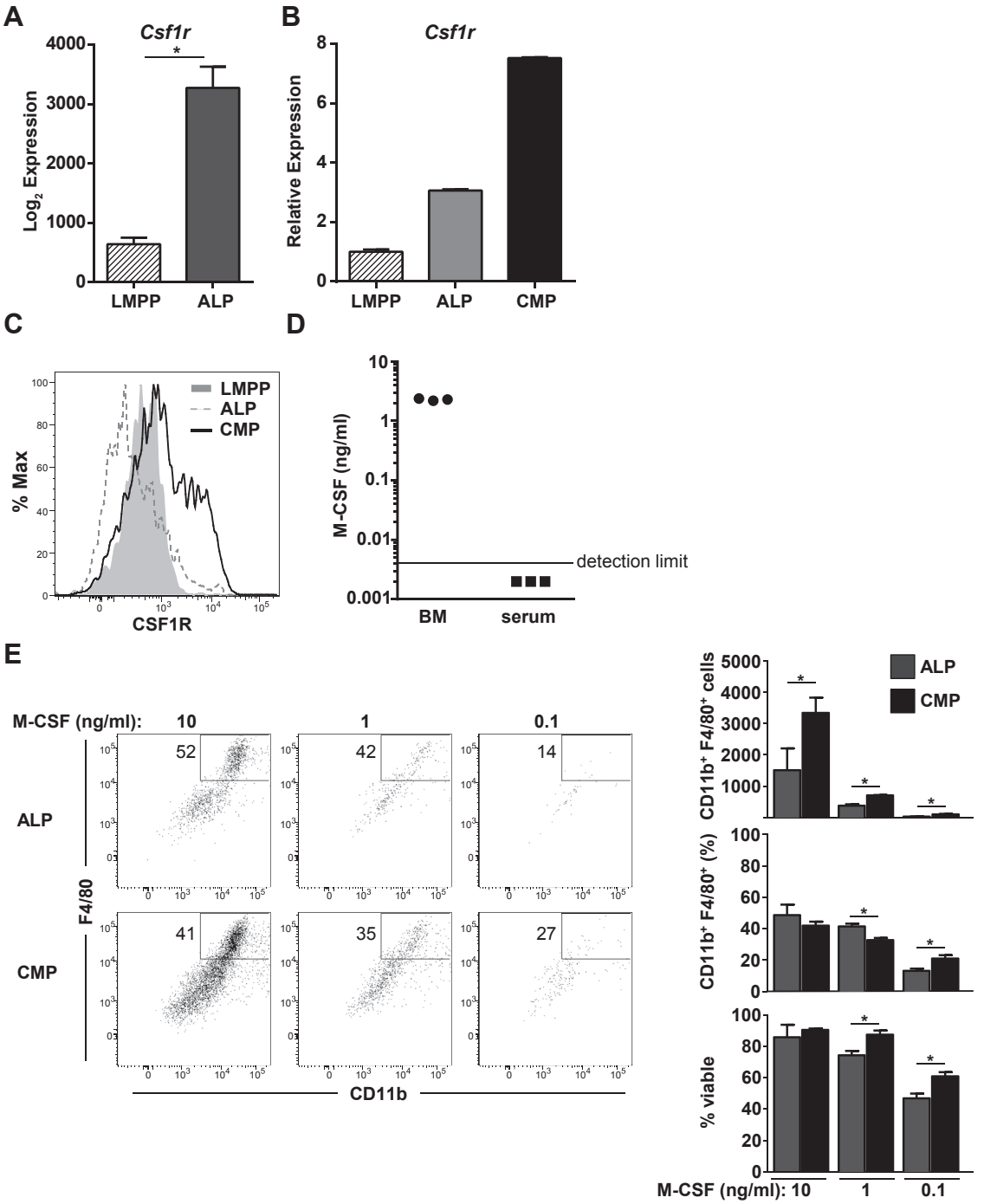

Figure 1. ALPs express low levels of CSF1R and differentiate into monocytes in vitro with exogenous M-CSF administration. (**A**) Microarray data obtained from Inlay et al. [5] showing log2 expression values for *CSF1R*. Signals from probeset 1419872_at are shown. Three biologically distinct samples were analyzed for each population. (**B**) Quantitative real-time PCR analysis of *CSF1R* for LMPPs, ALPs, and CMPs. *CSF1R* expression was normalized to *GAPDH* expression. Data are representative of 2 independent experiments. (**C**) Flow cytometry plots show the surface levels of CSF1R expressed on LMPPs, ALPs, and CMPs. These data are representative of three independent experiments. (**D**) ELISA analysis of BM and serum M-CSF levels. Femurs were flushed with 1 mL of PBS, levels of M-CSF were quantified in the supernatant, and BM concentrations were estimated assuming a femur volume of 9.4 μL (see Materials and Methods for details). Data are cumulative from two independent experiments. (**E**) Flow cytometric analysis of ALP output. We double-sorted 500 ALPs or CMPs and cultured them in the presence of M-CSF for 4 days. Flow cytometric plots in the left panel depict representative data from ALP and CMP cultures. Values within the plots depict the percentage of $F4/80^{+}CD11b^{+}$ macrophages generated. Column graphs in the right panel show cumulative data for the absolute number or frequency of macrophages or the frequency of viable cells. Data are inclusive of four independent experiments and represent mean values ± SEM. $*p \leq 0.05$, calculated by Student's unpaired, two-tailed *t* test. *BM* = Bone marrow.

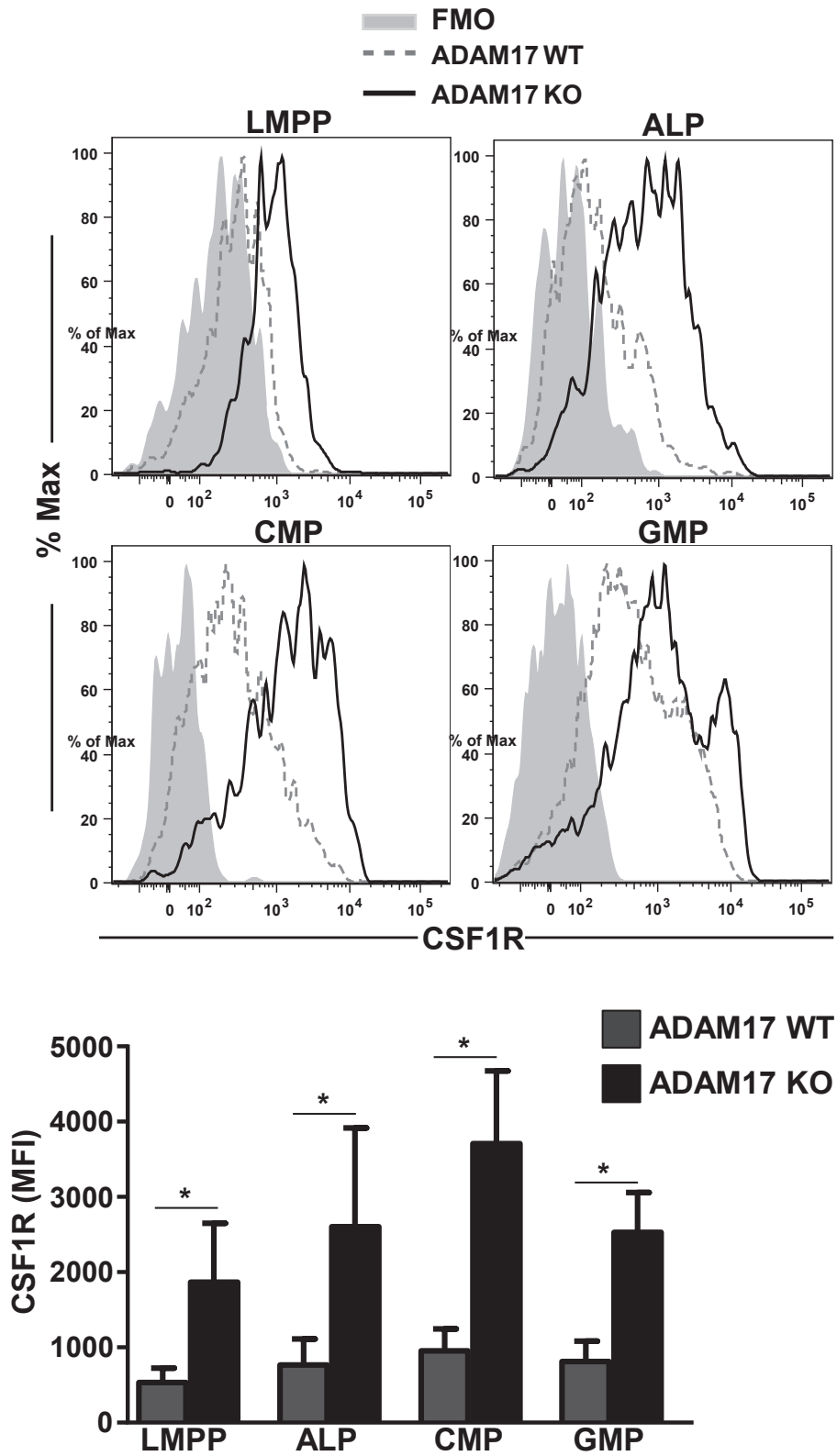

Figure 2. ADAM17 regulates the surface expression of CSF1R on hematopoietic progenitor cells. Flow cytometric analysis of CSF1R expression on wild-type and ADAM17-deficient progenitors. Histograms are representative plots from three independent experiments. Bottom panels show an average of CSF1R MFI ± SEM for the same three experiments. *$p \leq 0.05$, calculated by Student's unpaired, two-tailed t test. *MFI* = Mean fluorescence intensity.

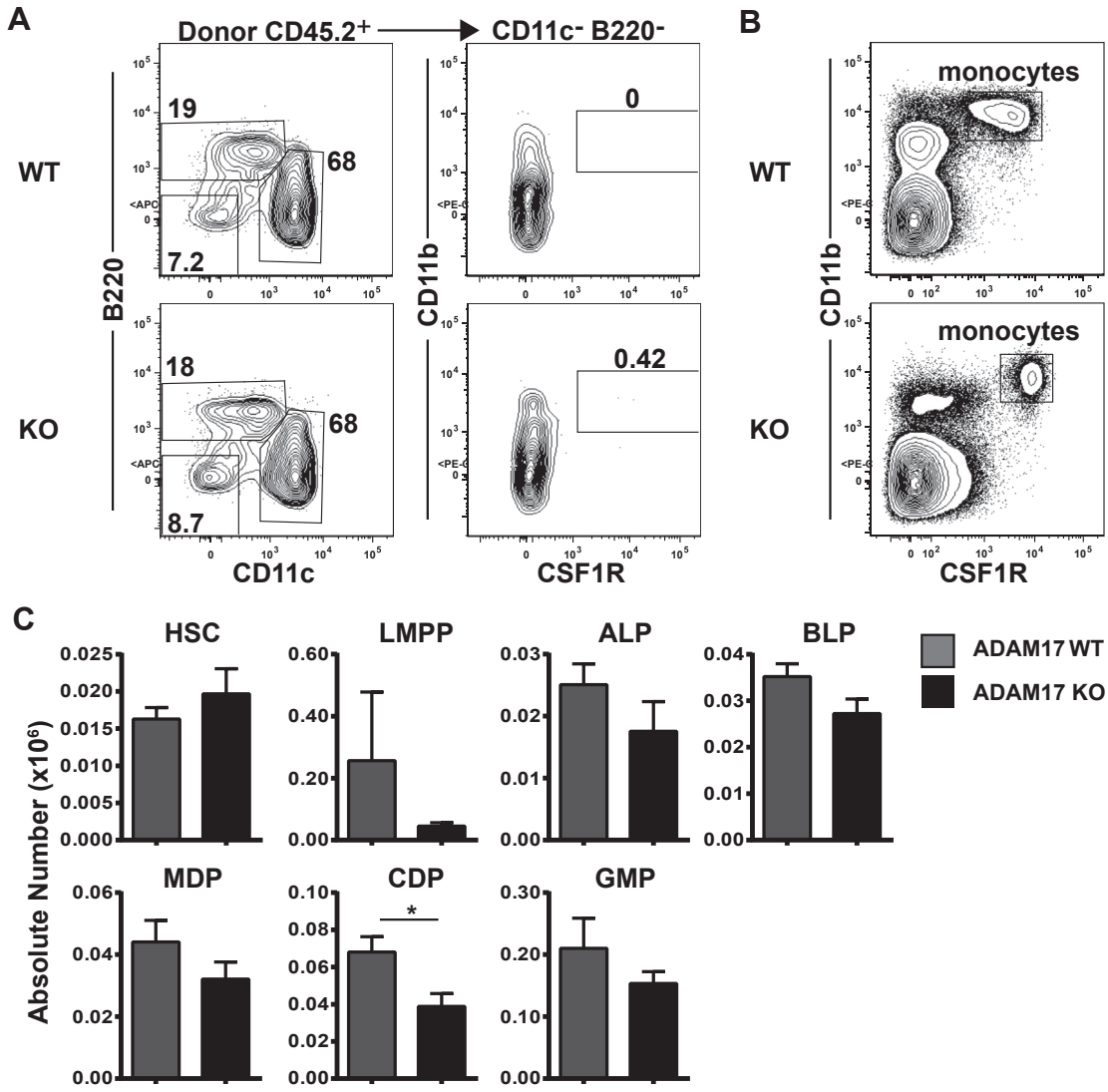

Figure 3. ADAM17-deficient ALPs do not produce monocytes in vivo. (**A**) Flow cytometric analysis of ALP output in vivo. We double-sorted 5,000 ALPs from ADAM17 wild-type or knockout mice. Cells were injected into sublethally irradiated congenic recipients. Ten days postinjection, spleens were harvested and analyzed for donor-derived ($CD45.2^+CD45.1^-$) monocytes ($B220^-CD11c^-CD11b^+CSF1R^+$), B cells ($B220^+$), and dendritic cells ($CD11c^+$). (**B**) Flow cytometric analysis of CSF1R expression on splenic monocytes (pregated on $B220^-Ly6G^-CD11c^-$ cells) from ADAM17 wild-type or knockout mice. Flow cytometric analysis of (**C**) hematopoietic stem and progenitor cells or (**D**, **E**) mature progeny in (**C**, **E**) the bone marrow or (**D**) spleens of ADAM17 wild-type or knockout mice. Bar graphs show the mean absolute number cells in each tissue ± SEM. Data are cumulative from three independent experiments. *$p \leq 0.05$, calculated by Student's unpaired, two-tailed t test.

Unexpectedly, the expression of CSF1R, which promotes monocyte and macrophage development [15], was increased in ALPs relative to LMPPs (Fig. 1A). This increase was also observed through quantitative real-time PCR analysis (Fig. 1B). Thus, ALPs express more *CSF1R* transcripts than do LMPPs, but less than do CMPs (Fig. 1B).

Protein levels of CSF1R can be regulated through several posttranscriptional mechanisms [33]. Therefore, increased transcript levels may not strictly correlate with increased cell surface protein levels or responsiveness to cytokines. To test cell surface expression of CSF1R in LMPPs and ALPs, we performed flow cytometric analysis. On ALPs, CSF1R surface expression was low, similar to that seen in LMPPs and markedly less than that observed in CMPs (Fig. 1C; gating strategies shown in Supplementary Figure E1, online only, available at www.exphem.org). Thus, ALPs use posttranscriptional mechanisms to limit CSF1R expression.

We hypothesized that the diminished levels of surface CSF1R on ALPs would render these cells insensitive to physiologic concentrations of M-CSF. To quantify the endogenous levels of M-CSF, we performed ELISA analyses. These data demonstrated that the bone marrow M-CSF concentration is ~2 ng/mL (Fig. 1D).

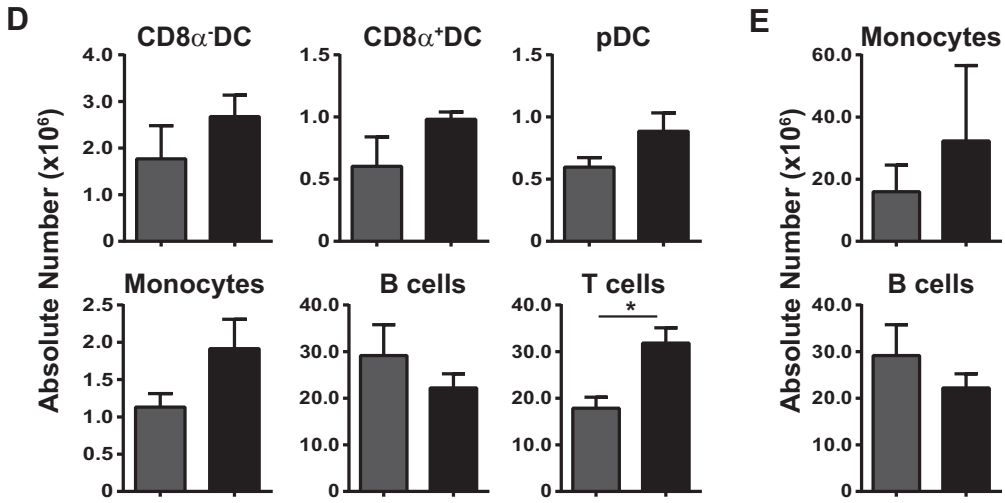

Figure 3. (*continued*)

Interestingly, M-CSF was undetectable in the serum of these same animals (Fig. 1D). These data demonstrate that M-CSF levels are locally restricted. Local concentrations of M-CSF could potentially also vary greatly within distinct marrow regions from the average value of ~2 ng/mL. Thus, to determine whether the low levels of cell surface CSF1R expression on ALPs would allow for macrophage development, we cultured purified ALPs in the presence of a broad range of M-CSF concentrations. All-lymphoid progenitors readily generated macrophages at high concentrations (10 ng/mL) of M-CSF, but this output fell sharply at lower doses (Fig. 1E). At all doses, CMPs generated more macrophages than did ALPs, potentially due to higher levels of surface CSF1R expression (Fig. 1E). The proportion of macrophages generated was comparable between ALPs and CMPs, with some small differences at the lower concentrations of M-CSF (Fig. 1E). Lowering the concentration of M-CSF led to a progressive decline in viability, although CMPs were less sensitive to death than were ALPs (Fig. 1E). These data demonstrate that ALPs express relatively little cell surface CSF1R despite transcription, and they robustly respond to M-CSF only at high doses.

Previous studies have shown that the metalloprotease ADAM17 can cleave CSF1R protein in activated macrophages [18]. We thus hypothesized that a similar mechanism limits CSF1R expression in ALPs. To test this hypothesis, we generated *ADAM17*$^{fl/fl}$ *Vav1-iCre* mice, which selectively lack ADAM17 in the hematopoietic compartment [24]. We then assessed ALPs and other progenitors for cell surface CSF1R levels. Indeed, ALPs, along with granulocyte macrophage progenitors, CMPs, and LMPPs, all showed elevated expression of surface CSF1R (Fig. 2; gating strategies shown in Supplementary Figure E1, online only, available at www.exphem.org). These data demonstrate that hematopoietic-intrinsic ADAM17 limits the expression of CSF1R in vivo.

We next sought to determine the functional consequences of ADAM17 deficiency on ALP behavior in vivo. We purified ALPs from *ADAM17*$^{fl/fl}$ *Vav1-Cre* or control littermates and transferred them into sublethally irradiated recipients. For both wild-type and ADAM17-deficient ALPs, mean splenic donor chimerism was identical at 1.9% (data not shown), and B cells and dendritic cells were readily generated from ALPs of both genotypes (Fig. 3A). However, we observed no evidence of monocyte production by ADAM17-deficient ALPs (Fig. 3A). At this same time point, CMPs readily generate monocytes [31,34]. We cannot exclude the possibility that ALPs generate mature cells such as monocytes with much different kinetics than do CMPs. However, this possibility seems unlikely, as both of these progenitors yield mature dendritic cells with similar kinetics [34–36]. As monocytes are commonly identified using CSF1R expression as a marker, we were concerned that this strategy would not be faithful for ADAM17-deficient monocytes. To address this, we examined wild-type and ADAM17-deficient splenocytes. Although ADAM17-deficient monocytes did indeed express elevated levels of CSF1R and required slightly different gates for quantification, they could still be readily identified (Fig. 3B). A similar proportion of wild-type and ADAM17-deficient monocytes expressed lymphocyte antigen complex 6c (Ly6C), a marker of inflammatory monocytes, thus further validating the gating strategy. Therefore, in this adoptive transfer system, elevated levels of CSF1R are not sufficient to confer in vivo myeloid potential to ALPs.

Because irradiation can alter in vivo concentrations of cytokines and homing properties of progenitors [37–39], we next sought to determine whether ADAM17 deficiency led to any changes in the numbers of progenitors or mature cells under steady-state conditions. Despite elevated surface levels of CSF1R, no significant defects were observed in the numbers of lymphoid or myeloid progenitors in

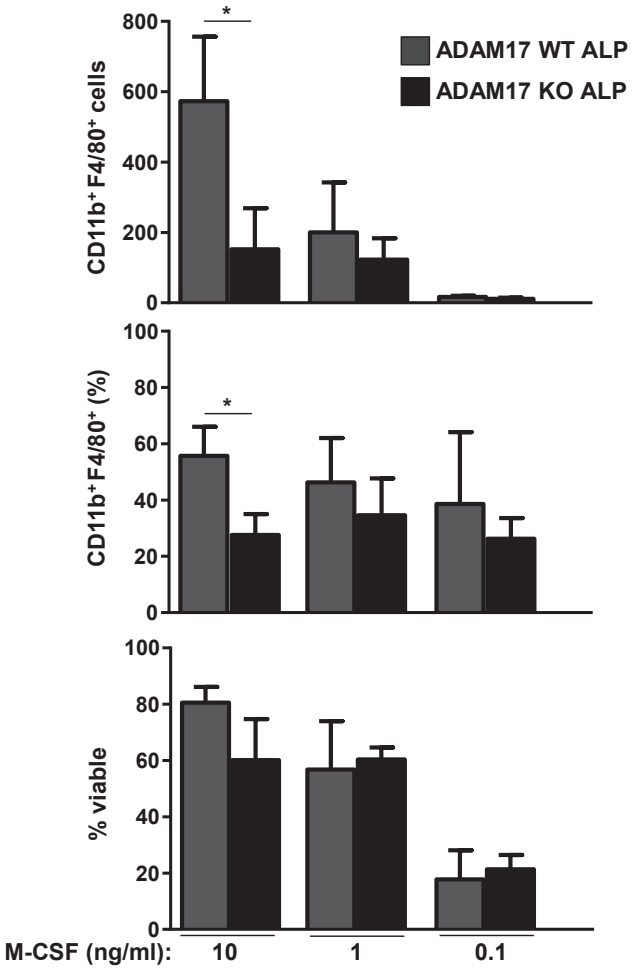

Figure 4. ADAM17-deficient ALPs yield few macrophages at high M-CSF doses. Flow cytometric analysis of wild-type and *ADAM17*$^{-/-}$ ALPs in vitro. We cultured 500 ALPs from wild-type or ADAM17-deficient donors in the presence of varying concentrations of M-CSF for 4 days. CD11b^{+} F4/80^{+} macrophages and propidium iodide^{+} dead cells were quantified by flow cytometry. Three ADAM17-deficient animals and wild-type siblings were analyzed. Bar graphs show mean values ± SEM. *$p < 0.05$, calculated by Student's unpaired, two-tailed t test.

ADAM17-deficient animals, aside from a modest reduction in common dendritic-cell progenitors (Fig. 3C; gating strategies shown in Supplementary Figure E1, online only, available at www.exphem.org). Similarly, no defects were observed in the numbers of mature dendritic cells, monocytes, or lymphocytes in the spleen (Fig. 3D) or bone marrow (Fig. 3E), except for a slight increase in splenic *ADAM17*$^{-/-}$ T-cell numbers (gating strategies shown in Supplementary Figure E2, online only, available at www.exphem.org).

To explore the basis for the lack of dramatic in vivo effects of ADAM17 deficiency, we compared the in vitro responsiveness of wild-type and *ADAM17*$^{-/-}$ ALPs to M-CSF. Unexpectedly, ADAM17-deficient ALPs yielded fewer macrophages in vitro than did wild-type ALPs at the highest doses of M-CSF (Fig. 4), despite expressing higher levels of CSF1R (Fig. 2). At lower M-CSF doses, the numbers and frequencies of macrophages generated were similar between wild-type and ADAM17-deficient ALPs (Fig. 4). Overall viability was also similar between genotypes at all doses of M-CSF (Fig. 4, bottom panel). We cannot exclude the possibility that a distinct proteolytic target of ADAM17 somehow prevents monocyte and macrophage development. However, given that M-CSF is the only cytokine included in these in vitro cultures, our data suggest that excessive M-CSF signaling may either prevent the differentiation of macrophages from lymphoid progenitors or selectively kill those which have already formed. Thus, negative feedback signaling through CSF1R may prevent major changes from occurring in vivo in ADAM17-deficient animals. This may also help explain the modest reduction in ADAM17-deficient common-dendritic cell progenitors (Fig. 3C), which already express high levels of CSF1R, even in ADAM17-sufficient animals [40,41]. Together, these data demonstrate that, although ADAM17 limits CSF1R expression in progenitors, it is not necessary for lymphoid lineage commitment or myeloid-cell homeostasis under steady-state conditions.

Discussion

Fate decisions during hematopoietic differentiation are regulated by complex interactions between cell-extrinsic and cell-intrinsic cues. At certain intermediates, progenitor cell differentiation in vivo is often more restricted than their epigenetic profiles or in vitro potentials might predict [2,11]. In these cases, it is likely that extrinsic factors dictate cellular outcomes in vivo, either through instructive actions or by selectively permitting the survival or proliferation of specific downstream lineages. By tuning their sensitivities to these extrinsic factors, progenitors could regulate outcomes in vivo.

We hypothesized that one way in which lymphoid progenitors regulate their sensitivities to myeloid cytokines is by ADAM17-mediated cleavage of CSF1R. Indeed, CSF1R surface levels were significantly higher in *ADAM17*$^{-/-}$ ALPs and other progenitors relative to their *ADAM17* $^{fl/fl}$ counterparts. Thus, it seemed reasonable to expect that cells expressing elevated CSF1R levels would increase macrophage or monocyte production in vivo. Contrary to our hypothesis, however, we observed no differences in *ADAM17*$^{-/-}$ ALP output in vivo compared with controls, and there were no major differences in mature cell subsets in the spleen or bone marrow. This may, in part, be attributable to negative feedback inhibition of CSF1R signaling, since *ADAM17*$^{-/-}$ ALPs generated relatively few macrophages at the highest doses of M-CSF in vitro compared with *ADAM17* $^{fl/fl}$ ALPs.

The physiologic importance of elevated CSF1R transcription in ALPs relative to LMPPs is unclear, as is the latent myeloid potential of these cells. Lymphoid

progenitors can alter their lineage output when exposed to pathogen-associated molecular patterns, such as Toll-like receptor ligands [42]. By maintaining a reservoir of *CSF1R* transcripts, it is possible that ALPs can rapidly contribute to emergency myelopoiesis upon systemic infection. Yet, under steady-state conditions, this monocyte and macrophage potential is not utilized.

The full mechanisms by which lymphoid progenitors restrict myeloid output in vivo thus remain unresolved. Specific lymphoid niches and consequent restriction of access to M-CSF are possible explanations as to why ALPs generate so few myeloid cells in vivo [13,43–45]. Our data are consistent with this mechanism and justify further studies on specialized niches and cytokine gradients in vivo.

Acknowledgments

We thank E Lantelme and D Brinja for assistance with flow cytometry and L. Hursey for animal colony maintenance. Dr. D Bhattacharya is a New York Stem Cell Foundation Robertson Investigator. This work was supported by National Institutes of Health grants to Dr AM Becker (no. T32CA009547) and Dr. D Bhattacharya (no. K01DK078318).

Conflict of interest disclosure

No financial interest/relationships with financial interest relating to the topic of this article have been declared.

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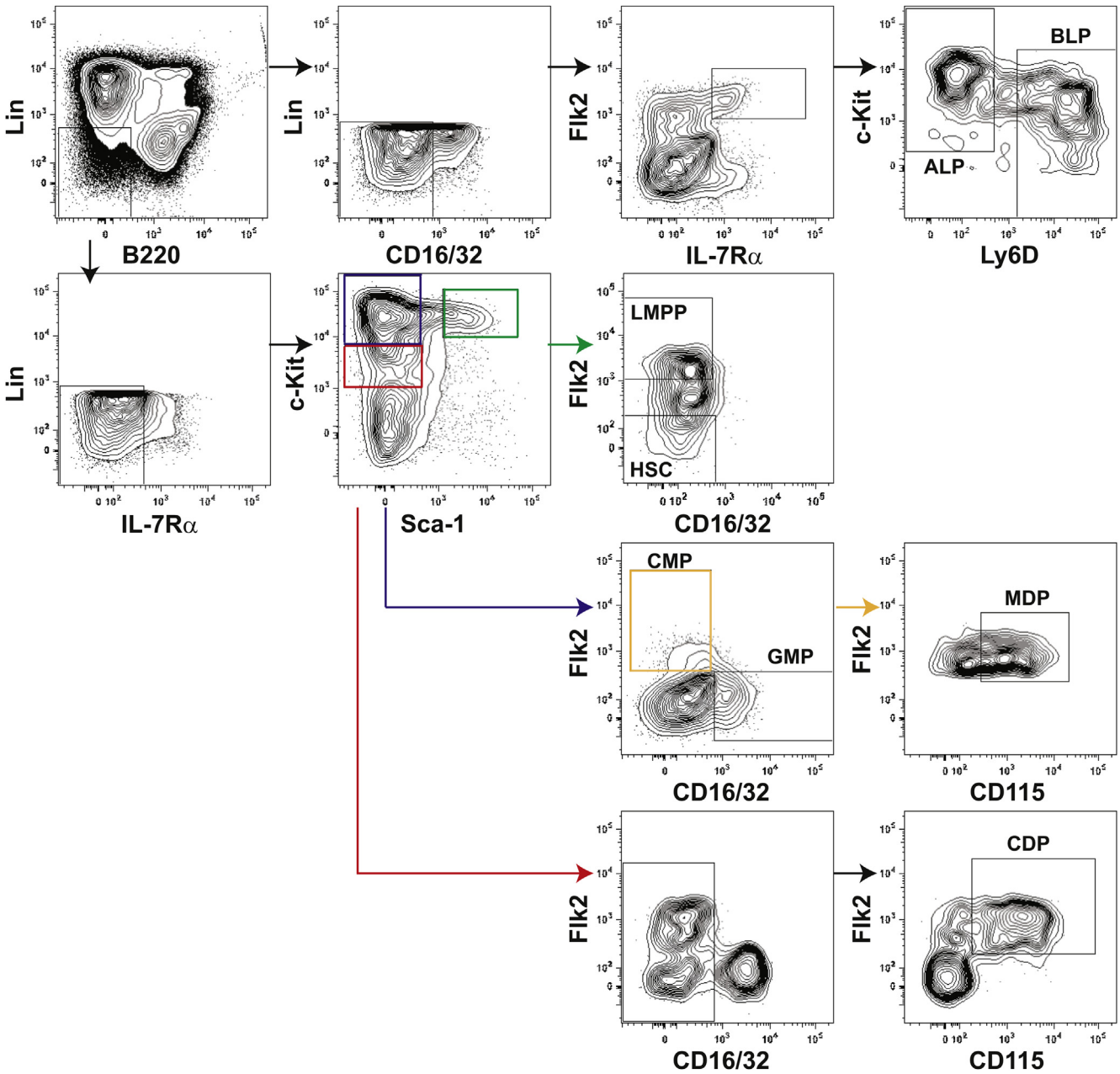
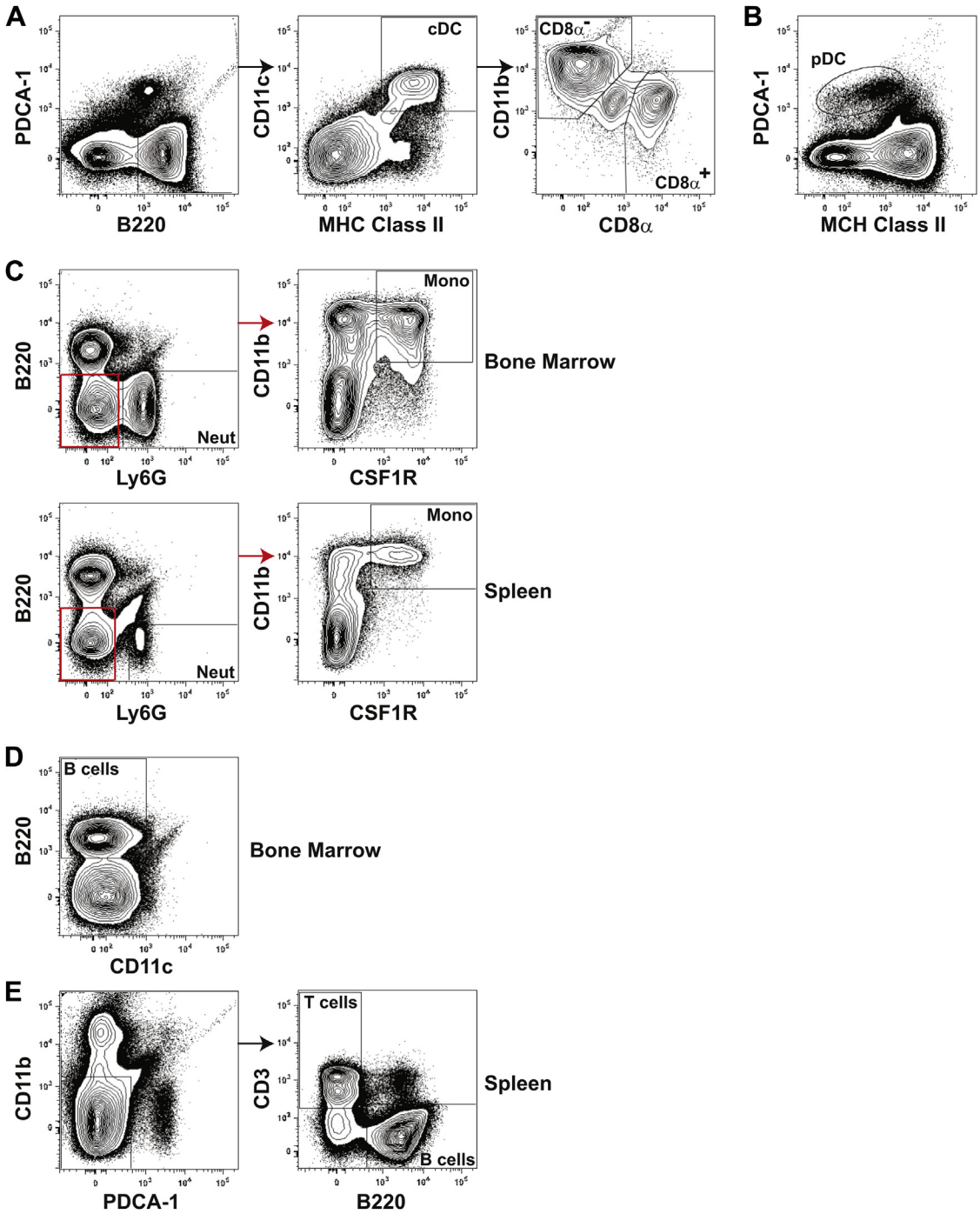
Supplementary Table E1. Antibodies

Antibody	Conjugate	Clone	Source
Flk2/CD135 PE	PE	N418	eBiosciences
CD27	APC	LG.7F9	eBiosciences
CD115	APC	AFS98	eBiosciences
PDCA-1	APC	eBio10129C	eBiosciences
c-kit/CD117	Pe-Cy7	2B8	eBiosciences
Gr-1	Pe-Cy7	RB6-8C5	eBiosciences
B220	APC0eFluo780	RA3-6B2	eBiosciences
CD11c	PerCP-Cy5.5	N418	eBiosciences
CD16/32	PerCP-Cy5.5	93	eBiosciences
MHC class II	Biotin	M5/114.15.2	eBiosciences
CD11b	PeCy7	M1/70	BD Biosciences
CD8	PE	53-67	BD Biosciences
Ly6G	PE	1A8	Miltenyi Biotec
CD3	Purified/AlexaFluor 488	145-2C11	Bio X-Cell/Bhattacharya
CD3	Purified/Pacific Blue	145-2C11	Bio X-Cell/Bhattacharya
CD4	Purified/Pacific Blue	GK1.5	Bio X-Cell/Bhattacharya
CD8	Purified/Pacific Blue	53-6.72	Bio X-Cell/Bhattacharya
CD11b	Purified/Pacific Blue	M1/70	Bio X-Cell/Bhattacharya
CD11c	Purified/Pacific Blue	N418	Bio X-Cell/Bhattacharya
CD19	Purified/Pacific Blue	1D3	Bio X-Cell/Bhattacharya
B220	Purified/Pacific Blue	6B2	Bio X-Cell/Bhattacharya
Terr119	Purified/Pacific Blue	Terr119	Bio X-Cell/Bhattacharya
Gr-1	Purified/Pacific Blue	8C5	Bio X-Cell/Bhattacharya
Sca-1	Purified/Pacific Blue	E13-161-70	Bio X-Cell/Bhattacharya
CD45.1	Purified/AlexaFluor 680	A20.1.7	Bio X-Cell/Bhattacharya
CD45.2	Purified/AlexaFluor 488	AL1-4A2	Bio X-Cell/Bhattacharya
Ly6D	Purified/AlexaFluor 488	ThB	Bio X-Cell/Bhattacharya
IL7Rα	Purified/Biotin	A7R34	Bio X-Cell/Bhattacharya
Streptavidin	Qdot605		Invitrogen



Supplementary Figure E1. Gating strategy for hematopoietic stem and progenitor cells. Lineage markers include CD3, CD4, CD8, CD11b, CD11c, CD19, Gr-1, and Ter119. *BLP* = B-lymphocyte progenitor; *CDP* = common dendritic-cell progenitor; *GMP* = granulocyte-macrophage progenitor; *HSC* = hematopoietic stem cell; *Lin* = Lineage; *MDP* = macrophage-dendritic cell progenitor.



Supplementary Figure E2. Gating strategy for mature cells in the bone marrow and spleen. Gating strategy for (**A**) CD8α$^+$ and CD8α$^-$ cDCs in the spleen, (**B**) pDCs in the spleen, (**C**) monocytes in the bone marrow and spleen, (**D**) B cells in the bone marrow, and (**E**) T cells and B cells in the spleen. *cDC* = Classical dendritic cell; *mono* = monocytes; *neut* = neutrophils; *pDC* = plasmacytoid dendritic cells.