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Coactivator-Associated Arginine Methyltransferase 1 Regulates Fetal Hematopoiesis and Thymocyte Development

Jia Li,*† Ziqin Zhao, † Carla Carter,§ Lauren I. R. Ehrlich,‡,† Mark T. Bedford,∗,† and Ellen R. Richie∗,†

Coactivator-associated arginine methyltransferase 1 (CARM1) is a protein arginine methyltransferase that methylates histones and transcriptional regulators. We previously reported that the absence of CARM1 partially blocks thymocyte differentiation at embryonic day 18.5 (E18.5). In this study, we find that reduced thymopoiesis in Carm1−/− mice is due to a defect in the fetal hematopoietic compartment rather than in the thymic stroma. To determine the cellular basis for impaired thymopoiesis, we examined the number and function of fetal liver (FL) and bone marrow cells. Despite markedly reduced cellularity of hematopoietic progenitors in E18.5 bone marrow, the number of long-term hematopoietic stem cells and downstream subsets was not reduced in Carm1−/− E14.5 or E18.5 FL. Nevertheless, competitive reconstitution assays revealed a deficit in the ability of Carm1−/− FL cells to contribute to hematopoiesis. Furthermore, impaired differentiation of Carm1−/− FL cells in a CARM1-sufficient host showed that CARM1 is required cell autonomously in hematopoietic cells. Coculture of Carm1−/− FL cells on OP9-DL1 monolayers showed that CARM1 is required for survival of hematopoietic progenitors under conditions that promote differentiation. Taken together, this report demonstrates that CARM1 is a key epigenetic regulator of hematopoiesis that affects multiple lineages at various stages of differentiation. 

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Hematopoietic stem cells (HSC) can self-renew throughout life and differentiate into all myeloid and lymphoid lineages (1). Epigenetic modifications are a driving force of this cellular differentiation. Because the genetic information of cells pre- and postdifferentiation is identical, epigenetic marks like DNA methylation and histone methylation are critical for facilitating the lock-in of a differentiated state (2). Epigenetic mechanisms function like a ratchet, allowing lineage-specific differentiation, but generally not dedifferentiation. There is an element of plasticity to cellular differentiation that can be forcibly reversed by small molecule epigenic regulators and/or the overexpression of a few specific genes to generate an induced pluripotent stem cell state (3). Perhaps one of the best biological systems to study epigenetic changes that correlate with differentiation is hematopoietic cell development. Indeed, recently, genome-wide DNA methylation patterns were analyzed at each major stage of hematopoiesis, revealing clear epigenetic signatures for each cell lineage (4). Like DNA methylation, there are a number of reports that arginine methylation also plays a critical role in lymphocyte development and signal transduction (5).

Arginine methylation is a common posttranslational modification that subtly alters the function of its substrates (6). It does this in a number of ways: 1) Arginine methylation provides a docking site for Tudor domain–containing effector molecules (7–10); 2) it can also block protein–protein interactions, as in the case of certain SH3 domain–driven interactions (11); 3) arginine methylation can negatively regulate AKT-mediated phosphorylation, because the AKT consensus motif contains key arginine residues (12, 13); and similarly, 4) lysine methylation can also be blocked by adjacent arginine methylation events (14, 15). The substrates for protein arginine methyltransferases (PRMTs) are both nuclear and cytoplasmic, and in the nucleus, histones are a major target of these enzymes. Histone methylation allows the PRMTs to feed into the epigenetic code and contribute to key molecular switches that dictate cell fate.

The mammalian PRMT family of enzymes consists of nine members, PRMT1–9; the majority of these enzymes target the N-terminal tails for histones H3, H4, and H2A for methylation (16). Coactivator-associated arginine methyltransferase 1 (CARM1)/PRMT4 was the first family member to be identified as a transcriptional coactivator, which methylates the H3R17 and H3R26 sites, respectively. PRMT1 and PRMT4 are both nuclear and cytoplasmic, and in the nucleus, histones are a major target of these enzymes. Histone methylation allows the PRMTs to feed into the epigenetic code and contribute to key molecular switches that dictate cell fate.

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Abbreviations used in this article: BM, bone marrow; CARM1, coactivator-associated arginine methyltransferase 1; CLP, common lymphoid progenitor; DN, double-negative; DP, double-positive; E, embryonic day; FL, fetal liver; GMP, granulocyte-macrophage progenitor; HSC, hematopoietic stem cell; KLS, Lineage−/C211−; LT-HSC, long-term HSC; MEP, megakaryocyte/erythroid progenitor; MPP, multipotent progenitor; PRMT, protein arginine methyltransferase; SP, single-positive; ST-HSC, short-term HSC.

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Materials and Methods

Mice

Carm1−/− embryos were described previously (18) and generated by crossing Carm1+/− breeders. Timed pregnancies were established, and the day of vaginal plug was designated embryonic day 0.5 (E0.5). Embryos were genotyped using the primers 5′-CCCCCTCTGTCATCCTCTTTG-3′ and 5′-TAACCTAAAAGAAATGGAATG-3′. Transgenic for EFGR under control of the β-actin promoter were kindly provided by Dr. Irving Weissman (25). C57BL/6 and Rag2−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Thymic NCCsinos mice were obtained from NCI (Frederick, MD). Mice were maintained in the Vivarium at the MD Anderson Cancer Center Science Park in accordance with guidelines established by the Association for the Accreditation of Laboratory Animal Care. All protocols using these mice were reviewed and accepted by the MD Anderson Animal Care and Use Committee.

Immunofluorescence microscopy

Serial sections (5 μm) from OCT-embedded frozen tissue were air dried and fixed in cold acetone for 5 min at room temperature. After washing in TNT (0.1 M Tris pH 7.5, 0.15 M NaCl, 0.05% Tween 20), sections were blocked for 15 min in TNB (0.1 M Tris-HCl pH 7.5, 0.15 M NaCl, 0.5% blocking reagent from TSA Biotin System [NEN Life Science Products, Boston, MA]). The slides were incubated at room temperature with pri-

Flaw cytometry

Single-cell suspensions were prepared from E18.5 thymi, spleen, and FL by dissociation of tissues through a 70-μm strainer (Fisher). BM cells were obtained by finely mincing E18.5 femur, tibia, humerus, radius, and ulna with a single-edge razor blade to release cells into FACS buffer. The dissociated cells and fragments were filtered through a 70-μm strainer (Fisher). BM and spleen cells were treated with RBC lysis buffer (17 mM Tris, 160 mM NH4Cl, pH 7.3) to remove RBCs, then resuspended in FACS buffer (PBS pH 7.2, 0.005 M EDTA, 2% FBS). Cells were stained with fluorochrome-conjugated Abs in FACS buffer for 30 min on ice and washed with FACS buffer. Propidium iodide (Invitrogen) containing 10% FBS (Atlanta Biologicals), 1% penicillin–streptomycin (Invitrogen), 2 mM L-glutamine (Invitrogen), 1 mM sodium pyruvate (Invitrogen), and 1.35 mM 2′-deoxyguanosine for 5 d. The thymocyte-depleted lobes were transplanted under the kidney capsule of athymic recipient NCCinos mice.

Competitive repopulation assay

C57BL/6 E14.5 embryos or controls (Carm1−/− × EFGR or Carm1−/− × littermates) were mixed 1:1 with competitor EFGR FL cells from wild-type C57Bl/6 E14.5 embryos and transplanted into sublethally irradiated (4.5 Gy) B6.D2-Cy−/− mice by retroorbital injection. The recipient mice were analyzed 8 or 16 wk after BM transplantation.

Results

Carm1 deficiency results in a block at the DN1-DN2 transition in thymopoiesis

We previously observed that Carm1 deficiency results in a reduction in thymic cellularity, associated with an accumulation of CD44+CD25−CD4+CD8+ thymocytes at E18.5. This pop-
ulation is heterogeneous, containing both T and non-T lineage progenitors. c-Kit is expressed on the most immature T cell progenitors, DN1 cells, within this population (DN1: c-Kit+CD44+CD25-CD4-CD8-)(26, 27). DN1 thymocytes give rise to DN2 progenitors (c-Kit+CD44+CD25+CD4-CD8-) that subsequently downregulate CD25 to become DN3 cells (c-Kit+CD44+CD25-CD4-CD8-) that are committed to the T cell lineage. To further characterize the block in thymocyte development observed in Carm1-/- embryos (24), we analyzed c-Kit expression within the CD44+CD25-CD4-CD8- compartment to distinguish the earliest T cell progenitor population. Consistent with our previous report, there was an increased frequency of CD44+CD25- thymocytes in the CD4-CD8- compartment in Carm1-/-E18.5 embryos (Fig. 1A). Furthermore, analysis of c-Kit expression revealed an increase in the frequency of DN1 versus DN2 progenitors in Carm1-/-E18.5 embryos, consistent with a block in differentiation of the earliest thymocyte subset.

The absolute number of DN1 thymocytes was not significantly different in Carm1-/- E18.5 versus control embryos. However, the DN2 subset was significantly reduced, consistent with a block in the DN1-DN2 transition (Fig. 1B). Carm1 deficiency resulted in a >90% reduction in the number of DN2, DN3, and DN4 (c-Kit+CD4-CD8-CD44+CD25-) thymocytes. DN4 cells are the immediate precursors of double-positive (DP) thymocytes (CD4+CD8+), which then give rise to mature CD4 single-positive (SP; CD4+CD8-) and CD8SP (CD4-CD8+) subsets. These latter subsets were reduced by ∼75% in the absence of CARM1 (Fig. 1B). We also observed a significant reduction of 85% in γδ T cells, as might be expected from a reduction in their progenitors, DN2 cells (Fig. 1B). Together, these data indicate that CARM1 is required for continued maturation of thymocytes beyond the DN1 stage.

Reduced thymopoiesis in Carm1-/- mice is not due to thymic stromal defect

Thymopoiesis requires input from stromal cells in the thymic microenvironment. Thus, the block in thymocyte development observed in Carm1-/- embryos could be an indirect consequence of a defect in the thymic stromal compartment. To determine whether Carm1 is required for proper thymic stromal function, we transplanted E15.5 2-deoxyguanosine–treated Carm1-/- versus control fetal thymic lobes under the kidney capsule of athymic nude recipients. Eight to 12 wk after transplantation, thymic grafts were recovered and analyzed by flow cytometry. In contrast with the developmental block observed in Carm1-/- E18.5 thymi (Fig. 1), thymopoiesis was not impaired when CARM1 deficiency was restricted to thymic stromal cells (Fig. 2). The cellularity was comparable for all thymocyte subsets regardless of whether they developed in a control or Carm1-/- stromal environment. Engrafted Carm1-deficient lobes were smaller than controls at the outset of the transplantation experiment; this initial difference in thymic size could account for the slight decreases in thymocyte numbers observed in transplanted Carm1-/- lobes (Fig. 2). In addition, comparable numbers of CD4SP and CD8SP T cells were recovered from the spleens of athymic recipients transplanted with Carm1-/- or control fetal thymus (Supplemental Fig. 1). Altogether, the normal differentiation of thymocyte progenitors in a Carm1-deficient microenvironment, in contrast with the severely reduced cellularity in Carm1-/-E18.5 thymi, indicates that loss of CARM1 predominantly affects thymocyte progenitors as opposed to the thymic stromal microenvironment.

CARM1 deficiency affects thymocyte cellularity in the E12.5 thymic rudiment

Given the defect in thymic cellularity at E18.5, we examined cryosections from E12.5–E18.5 from Carm1-/- embryos to determine whether the reduction in thymocyte cellularity was apparent earlier in ontogeny. At E12.5, there was a striking paucity in the number of CD45+ hematopoietic progenitors in the Carm1-/- thymic rudiment (Fig. 3A). This deficiency was also apparent E13.5–E17.5 in sections stained for c-Kit and CD25, markers of...
of thymic organogenesis (Fig. 3), we hypothesized that the absence of Carm1 might affect prethymic hematopoietic progenitors. Because hematopoietic progenitors are present in the fetal BM by E18.5 (28), we compared E18.5 BM from Carm1−/− and control littermates for hematopoietic progenitor subset composition. Cellularity was reduced in Carm1−/− BM compared with littermate controls (data not shown). Interestingly, the proportion and number of c-Kit+ cells within the lineage− fraction was greatly reduced in Carm1-deficient embryos (Fig. 4A, left panels, 4B). The KLS progenitors, which contain the most undifferentiated hematopoietic progenitors, are reduced in both frequency and absolute number in the E18.5 Carm1−/− BM (Fig. 4). The KLS population consists of several progenitor subsets: long-term HSCs (LT-HSCs: Slamf1+Flk2+ KLS), short-term HSCs (ST-HSCs: Slamf1− Flk2− KLS), and lymphoid-biased multipotent progenitors (Flk2+MPP: Flk2+Slamf1− KLS). All three of these early hematopoietic progenitors are significantly reduced in the E18.5 Carm1−/− BM. Flk2+MPP are reduced by 95%, whereas LT-HSCs and ST-HSCs were reduced by ~80% (Fig. 4).

In addition to the decrease in oligopotent hematopoietic progenitors in E18.5 Carm1−/− BM, downstream lineage-restricted progenitors were also diminished. The lineage−/CD27+Flk2+ BM subset contains all progenitors with thymocyte differentiation.
or GFP+ littermate controls were mixed with GFP
(CLP: Lineage-CD27+Flk2+IL-7R+), which was reduced by 85%
tains the lymphoid-restricted common lymphoid progenitor subset
eloid progenitors (Lineage-CD34+FcgR+ (Fig. 4). These progenitors can be subdivided into common my-
stream granulocyte-macrophage progenitors (GMP: Lin-CD34+ FcgR+MP), and megakaryocyte/erythroid progenitors (MEP: Lin- CD34- FcgR- MP). Common myeloid progenitors and MEPs were significantly reduced by 92 and 66%, respectively, whereas neither BM GMPs nor splenic granulocytes were significantly diminished
(Fig. 4, Supplemental Fig. 2). The reduction in LT-HSCs and subsequent progenitors through CLPs, together with our observ-
ations that E18.5 thymocytes and splenic B cells are reduced in
Carm1−/− embryos (Fig. 1, Supplemental Fig. 2), suggest that
CARM1 is required for early stages of hematopoiesis and con-
tinued lymphoid differentiation.

Cellularity of hematopoietic progenitors is not decreased in
Carm1−/− FL
Although hematopoiesis is shifting to the BM by E18.5, hema-
topoietic progenitors are still present in the FL (28). Therefore, we
analyzed the hematopoietic progenitor compartments from E18.5
FL in Carm1-deficient mice and littermate controls. In contrast
with E18.5 BM, the number of FL cells in Carm1−/− mice is comparable with controls (data not shown). Interestingly, the
number of LT-HSCs was slightly increased in Carm1−/− FL,
resulting in an increase in overall KLS cells (Fig. 5). This indi-
cates that CARM1 is not required to maintain HSC cellularity in
the FL. All other hematopoietic progenitors, from ST-HSCs
through lymphoid committed CLPs and myeloid committed
GMPs, as well as MEPs, were present in similar numbers to
controls (Fig. 5). Although the lack of CARM1 did not reduce cellularity of LT-HSCs and subsequent hematopoietic progenitors (Fig. 5), we observed a striking decrease in hematopoiesis in the fetal BM, thymus, and spleen at the same time point. Therefore, we questioned whether the hematopoietic potential of Carm1−/−
FL progenitors was impaired.

Carm1−/− FL cells have impaired hematopoietic potential in vivo
To assess the functional potential of E18.5 FL progenitors, we
performed a competitive reconstitution experiment. We first crossed
Carm1−/− mice with an actin-driven EGFP transgenic line (25).
Similar to E18.5 FL, we did not observe a decrease in cellularity or
in hematopoietic progenitor subsets in E14.5 FL (data not shown).
Equal numbers of E14.5 FL cells from GFP+ Carm1−/− embryos or
GFP− littermate controls were mixed with GFP− wild-type FL
cells from E14.5 C57BL/6 embryos. This mixture was injected into
sublethally irradiated Rag2−/− γc−/− mice. A schematic of the
experiment is shown in Fig. 6A. Recipient thymi and BM were
analyzed for donor chimerism in all hematopoietic subsets 8–12
wk after transfer. Carm1−/− FL cells were dramatically impaired in
their ability to give rise to all thymocyte subsets, from DN1
through CD4SP and CD8SP, when in competition with control FL
cells (Fig. 6B). Furthermore, in this competitive setting, Carm1−/−
FL cells failed to contribute efficiently in establishing hematopoietic progenitor chimerism in the BM, with the exception of
MEP (Fig. 6C). Thus, despite the fact that hematopoietic progen-
itors were present at near-normal numbers in E18.5 Carm1−/− FL,
they were severely impaired in their ability to contribute to he-
matopoiesis of all lineages. Given that the recipient mice were
CARM1 sufficient, these data also demonstrate that CARM1 is
required cell autonomously in hematopoietic progenitors.

Carm1−/− FL cells are capable of responding to Notch-driven
T cell differentiation signals, but display reduced viability in vitro
The impaired ability of Carm1−/− FL cells to contribute to
hematopoietic lineages, including T cells, suggests that they are
compromised either in their differentiation potential or in their
response to survival cues. Although there were insufficient num-
bers of Carm1−/− fetal BM progenitors to compare the frequency
of apoptotic cells relative to controls, we observed a slight in-
crease in apoptosis in ex vivo Carm1−/− FL KLS progenitors (data not shown). Therefore, to functionally assess the ability of
Carm1−/− FL progenitors to respond to differentiation and survival
cues, we used the OP9-DL1 coculture system, which robustly
promotes T cell differentiation from hematopoietic progenitors,
largely through activation of the Notch1 signaling pathway (30, 31).
Equal numbers of KLS progenitors from control or
Carm1−/− E14.5 FL were sorted into triplicate wells containing monolayers of OP9-DL1 stroma. After 6 d of culture in the presence of IL-7 and Flt3L, the cells were harvested and analyzed by flow cytometry to
determine their ability to commit to the T cell lineage. Both control and
Carm1−/− progenitors were capable of T cell commitment, as evidenced by differentiation to the DN3 developmental stage. However, we found a significant reduction in the number of DN1, DN2, DN3, and DN4 cells recovered from wells plated with Carm1−/− progenitors compared with controls (Fig. 7A). Neither control nor Carm1−/− cells progressed to the DP stage in this timeframe. Although we consistently observed a block at the DN1 to DN2 transition in vivo (Fig. 1) (23, 24), this was not observed in the in vitro OP9-DL1 coculture system. Instead, there was a consistent decrease of ∼4-fold for all subsets derived from Carm1−/− relative to control progenitors. To clarify the basis for the reduction in cellularity, we assessed whether Carm1−/− progenitors were defective in survival or proliferation in culture. We analyzed the frequency of cycling cells in the OP9-DL1 cultures, using Vybrant Dye Cycle to assess DNA content. We did not find a significant difference in the percentage of cycling cells between Carm1−/− and control cells (Fig. 7B). However, there was a significant increase in the frequency of apoptotic cells in wells seeded with

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**FIGURE 6.** Competitive reconstitution reveals a cell-autonomous requirement for CARM1 in hematopoiesis. (A) Schematic of competitive reconstitution assay. Equal numbers of EGFP+ C57Bl6/J FL cells were mixed with EGFP− FL cells from Carm1−/− or control E14.5 embryos. The cell mixture was injected i.v. into sublethally irradiated (4.5 Gy) RAG-2−/− γc−/− mice. Eight to 12 wk postinjection, recipient thymocytes and BM cells were analyzed for the relative EGFP+ versus EGFP− chimism. (B) The ratio of EGFP+ to EGFP− chimism within each thymocyte subset in competitive reconstitution recipients was determined by flow cytometric analysis. Results are from four independent experiments with two or more animals per experiment. (C) The ratio of EGFP+ to EGFP− chimism within each BM progenitor subset in competitive reconstitution recipients was determined by flow cytometric analysis. Results are from three independent experiments with two or more animals per experiment. Error bars show ± SEM. *p < 0.05, **p < 0.01.

**FIGURE 7.** CARM1 deficiency impairs survival of E14.5 FL progenitors during in vitro T cell differentiation. Five hundred sorted KLS from E14.5 Carm1−/− or control FL were cultured on OP9-DL1 stroma in the presence of 5 ng/ml IL-7 and Flt3L for 6 d. (A) Cellularity of DN1, DN2, DN3, and DN4 subsets was determined by flow cytometric analysis. Each point represents an average of three replicate wells plated from a single embryo. Mean values are indicated by horizontal bars. Data are combined from six independent experiments. (B) Percentage of cycling cells was determined using flow cytometric analysis of DNA content in recovered cells. Each point represents an average of three replicate wells plated from a single embryo. (C) Percentage of Annexin V+ cells was determined by flow cytometric analysis of recovered cells. Each point represents an average of three replicate wells plated from a single embryo. (D) Percentage of Annexin V+ cells was determined by flow cytometric analysis of recovered cells. Each point represents an average of three replicate wells plated from a single embryo. **p < 0.01, ***p < 0.001. (D) Representative flow cytometric histograms of IL-7R expression on E18.5 FL and thymocyte progenitors. IL-7R expression is comparable on FL Flk2+ MPP, CLP, and on thymocyte DN3 subsets in Carm1−/− versus control embryos. IL-7R expression is reduced on the Carm1−/− CD44+CD25− thymocyte progenitor population, including the c-Ki+ DN1 subset. FL data are representative of three separate experiments, including a total of six control and five Carm1−/− embryos. Thymocyte data are representative of four separate experiments, including a total of five control and six Carm1−/− embryos.

Carm1−/− progenitors (Fig. 7C). Because IL-7 is a critical survival factor during T cell differentiation, we considered the possibility that reduced IL-7R expression could account for the survival defect
of Carm1−/− progenitors. IL-7R expression was not reduced in E18.5 Carm1−/− CLPs in the FL. However, IL-7R expression was diminished on the entire CD44+CD25+ thymocyte subset, including the c-Kit+ DN1 progenitors in Carm1−/− compared with wild-type controls (Fig. 7D). After T lineage commitment and progression to the DN3 stage, there was little or no difference in IL-7R expression. Taken together, these data suggest that CARM1 is required for survival of hematopoietic progenitors, particularly at the earliest stages of T cell differentiation.

**Discussion**

Protein arginine methyltransferase is a posttranslational modification involved in various cellular functions, including signal transduction, subcellular protein localization, transcriptional regulation, protein–protein interactions, and DNA repair (6). We previously reported that the absence of CARM1 results in impaired fetal thymopoiesis (24). In this study, we demonstrate that this defect is due not only to a requirement for CARM1 in T cell development, but also to a much earlier requirement for CARM1 in oligopotent fetal hematopoietic progenitors. Although the number of LT-HSCs and downstream KLS subsets was not reduced in Carm1−/− E14.5 or E18.5 FL, competitive reconstitution assays revealed a deficit in the ability of Carm1−/− FL cells to contribute to hematopoiesis.

CARM1 is required cell autonomously in hematopoietic cells, as revealed by impaired differentiation of Carm1−/− FL cells in a CARM1-sufficient host, as well as by unimpeded differentiation of wild-type thymocyte progenitors in a Carm1−/− thymic stromal microenvironment.

Our previous observation of reduced thymic cellularity in E18.5 Carm1−/− mice (24), together with our current finding of fewer thymocyte progenitors in thymic rudiments as early as E12.5 (Fig. 3), suggested that there might be a block in prethymic hematopoiesis. During fetal development, hematopoiesis transitions from the liver to the BM, such that at E18.5, hematopoiesis could occur in both compartments (28, 32, and this study). Therefore, we analyzed the frequency and number of hematopoietic progenitors in both the E18.5 FL and BM. Interestingly, although a severe reduction in all c-Kit+ progenitors was observed in the BM, the FL did not recapitulate this phenotype. Indeed, there was a small, but significant, increase in LT-HSCs and downstream KLS subsets was not reduced in Carm1−/− FL. In addition, at E14.5, when the FL is the major site of hematopoiesis, lack of CARM1 did not result in altered FL progenitor numbers or frequencies (data not shown). However, the competitive FL reconstitution assays did reveal a functional defect in these E14.5 FL progenitors (Fig. 6). There are at least three possible explanations that could reconcile normal numbers of FL hematopoietic progenitors with their impaired function and the reduction in BM progenitor cellularity in Carm1−/− embryos. First, CARM1 deficiency could impair the ability of hematopoietic progenitors to emigrate from the FL, thus resulting in an accumulation of these progenitors in the FL and a reduction in the BM. However, this possibility is unlikely because on FL transplantation, a functional defect in these progenitors is revealed despite their manual release from the FL. Although this does not rule out a possible emigration defect, this mechanism is not sufficient to account for functional defects in hematopoiesis. Second, the ability of FL hematopoietic progenitors to home to the BM could be impaired. Chemotactant and integrins are known to regulate cellular trafficking and localization of hematopoietic cells, including LT-HSCs. CARM1 could control expression of these molecules, impacting the ability of Carm1−/− FL HSCs to migrate to the fetal BM (33). Finally, CARM1 deficiency could alter the ability of HSCs in the fetal BM to respond to molecular cues in the HSC BM niche, which regulate self-renewal, survival, and/or differentiation (34).

Reduced functionality of fetal hematopoietic progenitors could be sufficient to explain the reduction in thymic cellularity; however, our data suggest that CARMI plays an additional role in regulating thymocyte differentiation and/or survival. At E18.5, DN1 thymocyte progenitors are not significantly reduced in Carm1−/− embryos. However, DN2 and subsequent stages of thymocyte differentiation are severely impaired (Fig. 1). These data indicate that CARM1 is required for efficient transition between DN1 and DN2 stages in vivo, in keeping with our previous report (24). Interestingly, whereas CARM1 deficiency results in a marked deficit in E18.5 BM progenitors with the potential to seed the thymus (note the reduction in Flk2+CD27+ progenitors in Fig. 4) (29), there is not a significant reduction in DN1 numbers. Thus, DN1 niches may be extremely limiting, so that the reduced number of CLPs in the BM would still provide an adequate number of thymic seeding cells to saturate this niche. In addition, homeostatic mechanisms may be in place to maintain DN1 cellularity. Absence of such homeostatic factors in the OP9-DL1 culture system could account for the reduced DN1 cellularity in the Carm1−/− versus control cultures (Fig. 7A). Furthermore, increased apoptosis of Carm1−/− progenitors in vitro supports a role for CARM1 in maintaining survival during T cell differentiation (Fig. 7C). Because IL-7 is known to be such a potent survival cue during T cell differentiation, the reduction in IL-7R expression on ex vivo Carm1−/− DN1 thymocytes could account for the impaired survival and reduced cellularity in conditions promoting T cell differentiation (Fig. 7D). We note that CARM1 is not required for IL-7R expression in all hematopoietic progenitors because IL-7R expression is not impaired in FL CLP or lineage committed DN3 thymocytes. In contrast with its influence on survival, CARM1 is not essential for Notch1-driven T cell commitment, as evidenced by the ability of surviving DN1 progenitors to progress through subsequent DN2 and DN3 maturation stages (Fig. 7B). Taken together, our findings indicate that CARM1 influences hematopoiesis both in BM and FL progenitors, as well as in thymocyte progenitors, consistent with a role for CARM1 in differentiation or survival, or both, of multiple cell types (6).

As a member of the PRMT family of arginine methyltransferases, CARM1 contributes to epigenetic regulation of differentiation in many cell types (6). The enzymatic activity of CARM1 is required for thymocyte development, as well as for embryonic survival, adipocyte differentiation, and transcriptional coactivator activity (23). Thus, arginine methylation is a critical epigenetic modification that contributes to proper differentiation of hematopoietic lineages. Indeed, epigenetic regulation of HSC self-renewal and differentiation has been demonstrated by dynamic changes in the methylation status of both DNA and histones during hematopoietic lineage progression (35–37). In this study, we demonstrate a novel function for the epigenetic modifier CARM1 in the regulation of fetal hematopoiesis in BM and FL, as well as in thymopoiesis. Given this impact of CARM1 on early hematopoiesis and thymocyte development, CARM1-mediated epigenetic regulation may contribute to lymphoid and myeloid leukemogenesis. In this light, small molecule inhibitors of CARM1 may have therapeutic potential. The feasibility of this approach is suggested by the finding that treatment of Th cells with broad-spectrum PRMT small molecule inhibitors partially blocks cytokine production (38). In conclusion, CARM1 is required at multiple stages of hematopoietic differentiation, identifying it as a key epigenetic regulator of a cellular differentiation process that occurs throughout life. Future investigations will further elucidate the role of CARM1 at specific stages of hematopoiesis and determine the potential for therapeutic modulation of CARM1, which could be beneficial for hematopoietic disorders.
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Disclosures

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