Acat1 Gene Ablation in Mice Increases Hematopoietic Progenitor Cell Proliferation in Bone Marrow and Causes Leukocytosis

Li-Hao Huang, Jingang Gui, Erika Artinger, Ruth Craig, Brent L. Berwin, Patricia A. Ernst, Catherine C.Y. Chang, Ta-Yuan Chang

Objective—To investigate the role of acyl-CoA:cholesterol acyltransferase 1 (ACAT1) in hematopoiesis.

Approach and Results—ACAT1 converts cellular cholesterol to cholesteryl esters for storage in multiple cell types and is a potential drug target for human diseases. In mouse models for atherosclerosis, global Acat1 knockout causes increased lesion size; bone marrow transplantation experiments suggest that the increased lesion size might be caused by ACAT1 deficiency in macrophages. However, bone marrow contains hematopoietic stem cells that give rise to cells in myeloid and lymphoid lineages; these cell types affect atherosclerosis at various stages. Here, we test the hypothesis that global Acat1−/− may affect hematopoiesis, rather than affecting macrophage function only, and show that Acat1−/− mice contain significantly higher numbers of myeloid cells and other cells than wild-type mice. Detailed analysis of bone marrow cells demonstrated that Acat1−/− causes a higher proportion of the stem cell–enriched Lin−Sca-1−c-Kit+ population to proliferate, resulting in higher numbers of myeloid progenitor cells. In addition, we show that Acat1−/− causes higher monocytosis in Apoe−/− mouse during atherosclerosis development.

Conclusions—ACAT1 plays important roles in hematopoiesis in normal mouse and in Apoe−/− mouse during atherosclerosis development. (Arterioscler Thromb Vasc Biol. 2013;33:2081-2087.)

Key Words: acyl-CoA cholesterol acyltransferase 1    atherosclerosis    leukocytosis    monocytes

Atherosclerosis remains the leading cause of death and disability in the developed world. Statin drugs, potent inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase, are effective in reducing serum cholesterol levels. However, the residual risk for atherosclerosis after statin treatment remains significant, and alternative approaches have been developed to further combat atherosclerosis. One approach is to inhibit acyl-CoA:cholesterol acyltransferases (ACATs).1 ACATs are membrane-bound enzymes that use long-chain fatty acyl-coenzyme A and cholesterol as substrates to form cholesteryl esters. Mammals contain 2 isoenzymes, ACAT1 and ACAT2, which are encoded by 2 different genes.1–3 The expression of ACAT1 is ubiquitous,4 whereas the expression of ACAT2 is tissue restricted.5 Both enzymes are drug targets for treating atherosclerosis. Macrophages play important roles in atherosclerosis. ACAT1 is the major isoenzyme in macrophages.6 An ACAT1-specific small molecule inhibitor K604 inhibits macrophage foam cell formation in cell culture; when fed to the atherosclerotic Apoe−/− mouse at low concentration, K604 significantly diminishes the presence of lesion macrophages.7 In addition, an isoform-nonspecific ACAT inhibitor F1394 at low concentration reduces progression of advanced atherosclerotic lesions in mice without plaque or systemic toxicity.8 These studies suggest that partial inhibition of ACAT1 may be beneficial to treat atherosclerosis. On the contrary, mouse genetic experiments showed that in atherosclerotic Ldlr−/− or Apoe−/− mice, germline Acat1 gene ablation actually enlarged the lesion size of the plaque.9,10 These results raise caution against using ACAT1 inhibitors at high doses to treat atherosclerosis. Further investigation showed that when bone marrow (BM) isolated from Acat1−/− or Acat1−/− animals was transplanted individually to lethally irradiated Ldlr−/− or Apoe−/− recipient mice, the atherosclerotic lesions were larger in mice receiving BM from the Acat1−/− donor. Because BM contains myeloid progenitor cells that differentiate into monocytes/macrophages, these results suggest that the lack of Acat1 in macrophages may enlarge the lesions.10 However, BM contains hematopoietic stem cells (HSCs) that give rise to cells in the myeloid lineages (eg, monocytes/macrophages, neutrophils, and dendritic cells) and cells in the lymphoid lineages (eg, T cells and B cells). Germline Acat1 loss may affect the function of HSC and the function of various
cells derived from HSC. Recently, adenosine triphosphate-binding cassette (ABC) transporters A1 and G1 (ABCA1 and ABCG1). 2 key proteins involved in cellular cholesterol efflux, were shown to affect proliferation of HSCs and other progenitor cells in mouse BM.11 In addition, lysosomal acid lipase, a key enzyme that produces cholesterol and free fatty acids from cholesteryl esters present in late endolysosomes, is shown to affect HSC proliferation.12 In the current work, we test the hypothesis that germine Acat1 loss may affect HSC and other progenitor cell proliferation in BM.

Materials and Methods
Materials and Methods are available in the online-only Supplement.

Results

Acat1 Knockout Mice Exhibit Leukocytosis
To test the hypothesis that global Acat1 loss may affect hematopoiesis, we first compared the numbers of monocytes, neutrophils, B cells, and T cells in peripheral blood of age-matched wild-type (WT) and Acat1−/− mice. The results show that Acat1−/− mice contain significantly higher numbers of monocytes (CD11b+), neutrophils (Gr1+), and B cells (CD19+) in their blood compared with WT littermates (Figure 1A). The Acat1−/− mice also contain slightly higher numbers of T cells than WT mice. In addition, their spleen size is bigger, and more cells are present in the lymph nodes compared with WT mice (Figure I in the online-only Data Supplement). The body weights of WT and Acat1−/− mice are the same for both males and females (Figure I in the online-only Data Supplement). The increase in leukocytes in Acat1−/− mouse could be caused by a cell-autonomous proliferation, or survival difference of cells within BM, and by change(s) in tissue microenvironment in these mice. To address this issue, we performed BM transplant experiments. BM cells from WT or Acat1−/− mice were transplanted into lethally irradiated recipient WT mice to produce chimeric mice. Seven weeks after transplantation, Acat1 gene expression in leukocytes isolated from the chimeric mice was measured by real time-polymerase chain reaction and by Western blot. The results confirm that expression of both Acat1 mRNA and ACAT1 protein in mice transplanted with Acat1−/− BM is <10% of that of mice transplanted with Acat1+/+ BM (Figure IIA in the online-only Data Supplement, left and middle). We next monitored blood leukocyte numbers in chimeric mice at different time points, from 7 to 11 weeks after transplantation. The results show that leukocyte numbers in mice transplanted with Acat1−/− BM are significantly higher than in mice transplanted with WT BM (Figure 1B). We also performed a parallel experiment using lethally irradiated Acat1−/− mice as recipients of BM cells from either WT mice or Acat1−/− mice. Again, as expected, Western blot and real-time–polymerase chain reaction analyses show that the expression of both Acat1 mRNA and ACAT1 protein in mice transplanted with Acat1−/− BM is <10% of that found in mice transplanted with Acat1+/+ BM (Figure IIB in the online-only Data Supplement, left and middle). We then monitored leukocyte numbers in chimeric mice from 7 to 11 weeks after transplantation. The results show that leukocyte numbers in Acat1−/− recipient mice (Figure 1C) were higher than those found in WT recipient mice (Figure 1B) when donor BM came from either the WT mice (comparing solid circles in Figure 1B and 1C) or the Acat1−/− mice (comparing open circles in Figure 1B and 1C). Additional analysis shows that when WT BM donor cells were used, Acat1−/− recipient mice had significantly higher leukocyte numbers than WT recipient mice (comparing solid circles in Figure 1B and 1C), suggesting the possibility that Acat1−/− background/microenvironment plays a significant role in causing leukocytosis. Other results show that the spleen weights of the chimeric mice with BM from Acat1−/− mice are higher than those with BM from WT mice (Figure IIA and IIB in the online-only Data Supplement, right). Together, these results suggest that Acat1 loss causes higher leukocyte numbers in the blood by affecting both cell-autonomous proliferation in BM and the microenvironment that supports hematopoiesis. Acat1 loss may also affect cell differentiation/self renewal or cell death.13 The rest of the work presented here focuses on the cell-intrinsic effects of Acat1−/− in hematopoietic cells in BM.
**Acat1 Loss Increases Hematopoietic Progenitor Cell Proliferation**

To examine the effects of Acat1 loss on homeostasis of BM cells, we compared the number and the percentage of myeloid cell (Gr1+CD11b+) and B-cell (CD19+) lineages in the BM, isolated from age-matched (8-week-old) WT and Acat1−/− mice. The results show that Acat1−/− BM contains higher numbers of cells in myeloid and B-cell lineages (Figure 2A), whereas the percentages of these cells in WT and Acat1−/− BM remain the same (Figure 2B). These results show that the Acat1−/− BM has more cells of all lineages, suggesting that Acat1−/− may affect HSCs or progenitor cell populations that differentiate into myeloid cells and other cell types. To test this possibility, we compared the number and percentage of the following progenitor cells: LSK (Lin−Sca-1−c-Kit+), common myeloid progenitor (CMP) (Lin−Sca-1−c-Kit−CD34+FcRⅡ/Ⅲ+), granulocyte/macrophage progenitor (GMP) (Lin−Sca-1−c-Kit+CD34+FcRⅡ/Ⅲ+), megakaryocyte/erythrocyte-restricted progenitor (Lin−Sca-1−c-Kit−CD34+FcRⅡ/Ⅲ+), and common lymphoid progenitor (CLP; IL7Rα+Lin−Sca-1−c-Kit−) cells in BM isolated from WT and Acat1−/− mice. The results show that the numbers of CMPs and GMPs in the Acat1−/− BM were increased, whereas the numbers of LSKs, megakaryocyte/erythrocyte-restricted progenitors, and CLPs in WT and Acat1−/− BM were similar (Figure 3A), suggesting that Acat1−/− causes selective expansion of CMP and GMP progenitor populations.14 We cultured these purified cells in medium with 10% fetal bovine serum supplemented with IL3 for 3 days and then performed the BrdU incorporation assay to monitor cell proliferation in WT and Acat1−/− BM. In an effort to confirm the finding that Acat1−/− enhances specific LSK cell proliferation at the in vitro level, we isolated various progenitor cells by cell sorting, including LSKs, CMPs, and GMPs, from BM of WT and Acat1−/− mice. The cytokine interleukin 3 (IL3) supports proliferation in several myeloid progenitor populations.14 We cultured these purified cells in medium with 10% fetal bovine serum supplemented with IL3 for 3 days and then performed the BrdU incorporation assay in vitro. The results (Figure IV in the online-only Data Supplement) show that when compared with cells from WT BM, only the LSK cells, but not the CMP or GMP cells from the Acat1−/− BM, exhibit a significantly higher percentage of proliferating cells. 

**Figure 2.** Acat1−/− increases myeloid cells and B cells in bone marrow (BM). BM leukocytes were analyzed by flow cytometry. A, BM myeloid cells (CD11b+Gr1+); and B, B cells (CD19+). Results were reported as total cell number (left) and percentage in BM (right). Results are mean±SEM. For total BM cells, 24 wild-type (WT) mice and 24 Acat1−/− mice in 5 experiments; for myeloid cells, 13 WT mice and 13 Acat1−/− mice in 3 experiments; for B cells, 24 WT mice and 24 Acat1−/− mice in 5 experiments.

**Figure 3.** Acat1−/− increases proliferation of hematopoietic progenitor cells in bone marrow (BM). A, Lin−Sca-1−c-Kit+ (LSK), common myeloid progenitor (CMP), granulocyte/macrophage progenitor (GMP), megakaryocyte/erythrocyte-restricted progenitor (MEP), and common lymphoid progenitor (CLP) cells in BM were quantified by flow cytometry; results are reported as total number (left) and as percentage within the BM (right). Proliferation (B) and apoptosis (C) of various progenitors were quantified by bromodeoxyuridine (BrdU) and Annexin V staining assays. Results are mean±SEM. A, Fifteen wild-type (WT) mice and 15 Acat1−/− mice in 4 experiments for LSK, CMP, MEP, and GMP determinations; 6 WT mice and 6 Acat1−/− mice in 2 experiments for CLP determinations. B and C, Eight WT mice and 8 Acat1−/− mice in 2 experiments.
Brdu incorporation. These results are in accord with the in vivo data and demonstrate that Acat1−/− LSK cells proliferate more frequently in vivo and in vitro. Further analysis of LSK cell subpopulations, including long-term HSCs (LSK/CD34−CD135−), short-term HSCs (LSK/CD34−CD135+), and multi-potent progenitors (LSK/CD34+CD135+), showed that Acat1−/− causes a small but statistically significant selective increase in multi-potent progenitor subpopulation within LSK cells (Figure V in the online-only Data Supplement).

**Acat1−/−** Increases BM Cell Proliferation by Activating the ERK Pathway

Yvan-Charvet et al11 showed that on stimulation by IL3 for 72 hours in vitro, BM isolated from ABCA1/ABCG1 double knockout (DKO) mouse exhibited increased cell proliferation, both in LSK cell subpopulations, including long-term HSCs (LSK/CD34−CD135−), short-term HSCs (LSK/CD34−CD135+), and multi-potent progenitors (LSK/CD34+CD135+), showed that Acat1−/− caused a small but statistically significant selective increase in multi-potent progenitor subpopulation within LSK cells (Figure V in the online-only Data Supplement).

![Image](https://example.com/image1.png)

**Figure 4.** Cultured Acat1−/− bone marrow (BM) cells proliferate faster through activated IL3Rβ/phospho-extracellular signal-regulated kinase (p-ERK) pathway. Wild-type (WT) and Acat1−/− BM cells were cultured in Iscove’s Modified Dulbecco’s Medium (IMDM) media containing 10% fetal bovine serum in the presence of indicated growth factors with or without specific inhibitors as indicated for 72 h. Concentrations of growth factors used are as follows: 100 ng/mL for stem cell factor, 6 ng/mL for IL3, and 2 ng/mL for granulocyte-macrophage-colony stimulating factor. Concentration of inhibitors used are as follows: 10 μmol/L for U0126; 1 μmol/L for PP2. **A.** Proliferation was measured by performing 3 h 3H-thymidine incorporation to DNA in intact cells. Expression of p-ERK protein under IL3 treatment as detected by Western blot analysis (B) or by flow cytometry (C). Results are means±SEM of duplicate cultures in 3 experiments. SCF indicates stem cell factor.

![Image](https://example.com/image2.png)

**Figure 5.** Acat1−/− Lin-Sca-1+ c-Kit+ (LSK) cells have higher IL3Rβ expression in vivo and in culture. Cell surface IL3Rβ expression was monitored by using flow cytometry as described in Methods in the online-only Data Supplement. **A.** Expression in LSK cells and in total bone marrow (BM) cells as indicated in vivo. Results are from 8 wild-type (WT) mice and 8 Acat1−/− mice in 2 experiments. **B.** Expression in BM cell culture under IL3 treatment for 72 h. Results are from WT and Acat1−/− BM cell culture in duplicate dishes in 3 experiments. Results are means±SEM.
ACAT1 converts cholesterol to cholesteryl esters and prevents the accumulation of free cholesterol in various cell membranes.\textsuperscript{1} IL3R\(\beta\) is mainly expressed at the plasma membrane.\textsuperscript{16} Signaling of IL3 through IL3R\(\beta\) is affected by cellular membrane lipid composition. The effect of Acat1\(^{-/-}\) on IL3 signaling may be attributed to a change in the cholesterol content in BM cells. To test this possibility, we used a small molecule squalene synthase inhibitor CP-340868, which blocks the biosynthesis of squalene from farnesyl pyrophosphate. At micromolar concentrations, this inhibitor effectively shuts down cholesterol biosynthesis without inhibition of nonsterol polyisoprenoid biosynthesis and protein prenylation.\textsuperscript{17} We incubated WT and Acat1\(^{-/-}\) BM cells with IL3 in the presence or absence of CP-340868 and found that treating cells with CP-340868 reversed the enhanced cell proliferation observed in Acat1\(^{-/-}\) BM cells (Figure VI in the online-only Data Supplement; lanes 2 and 3). The result of a parallel experiment showed that adding ApoA1, a cellular cholesterol efflux mediator (at 50 or 100 \(\mu\)g/mL), to the cell culture did not diminish the enhanced proliferation.

**Acat1\(^{-/-}\) Animals Exhibit an Increase in B-Cell Progenitors**

We showed earlier (Figure 1A) that in Acat1\(^{-/-}\) mouse blood, in addition to the myeloid cell number, the B-cell number (CD19\(^{+}\)) is also increased. The CLP cell is the major progenitor for B lineage–restricted cells. However, no difference in either absolute number or percentage of CLPs can be found between WT and Acat1\(^{-/-}\) BM (Figure 3A). These results imply that Acat1 loss may affect B-cell progenitor populations downstream of CLPs. To test this possibility, we monitored specific B-cell progenitor populations and found that when compared with WT, the pre-B–cell population, but not the pro-B–cell population, was increased in Acat1\(^{-/-}\) BM (Figure 6A, left). The percentage of B-cell progenitors in WT and Acat1\(^{-/-}\) BM was the same (Figure 6A, right). We next found that the BrdU incorporation in vivo was slightly but statistically higher in Acat1\(^{-/-}\) pro-B cells than their WT counterparts (Figure 6B, left; \(P\) value <0.01). The result of a parallel experiment showed that apoptotic cells in vivo (as quantified by Annexin V staining) were reduced in the Acat1\(^{-/-}\) pro-B–cell population when compared with the WT counterpart (Figure 6B, right). In B-cell progenitors, the IL7 receptor \(\alpha\) subunit plays a key role both in survival and in proliferation.\textsuperscript{13} We compared the expression of cell surface IL7R\(\alpha\) in BM B cells and found that its expression is elevated in Acat1\(^{-/-}\) pro-B cells compared with WT pro-B cells (Figure 6C). This result suggests that Acat1 loss facilitates proliferation of pro-B cells in part through elevated cell surface IL7R\(\alpha\) expression, similar to our findings with the IL3R\(\beta\) in the myeloid lineage.

**Acat1\(^{-/-}\) Causes Higher Monocytosis in Apoe\(^{-/-}\) Mouse During Atherosclerosis Development**

Monocytes and macrophages play key roles in atherosclerosis development. In mouse models for atherosclerosis, global Acat1 gene ablation enlarged the lesion size of the plaque.\textsuperscript{9,10} On the basis of results described above, we suspect that Acat1\(^{-/-}\) may affect blood monocyte counts during atherosclerosis development. To test this possibility, we crossed Apoe\(^{-/-}\) mice with Acat1\(^{-/-}\) mice to generate Apoe\(^{-/-}\)/Acat1\(^{-/-}\) (DKO) mice. We then fed age-matched male Apoe\(^{-/-}\) and DKO mice with modified Paigen’s diet for 0 to 6 weeks and monitored blood myeloid (CD11b\(^{+}\)Gr1\(^{+}\)) cell numbers at various time points. The results showed that from 0 to 2 weeks blood myeloid cell numbers in the DKO mice tended to be higher than those in the Apoe\(^{-/-}\) mice, but the difference did not reach statistical significance. From 4 to 6 weeks, in the Apoe\(^{-/-}\) mice blood myeloid cell numbers began to increase; this finding is consistent with an earlier published result demonstrating that Apoe\(^{-/-}\) promotes monocytosis in mice;\textsuperscript{15} in the DKO mice, the increase in monocytes was much higher (Figure VII in the online-only Data Supplement). These results show that global
Acat1−/− causes higher monocytoyis in the Apoe−/− mouse during atherosclerosis development. Accelerated monocytes may account for the previous observation that global Acat1−/− exaggerates lesion formation in atherosclerotic mice.

**Discussion**

Here, we showed that in normal mice on normal diet, Acat1−/− in BM increases peripheral blood leukocytes. The monocytes, neutrophils, and B cells in Acat1−/− mice are significantly higher in absolute number than those in the normal, Acat1+/+ mice. The leukocytosis phenotype is attributed to both cell-autonomous mechanism and environmental influences. We performed additional experiments to explore the cell-autonomous mechanism(s). The results show that Acat1 loss causes leukocytosis mainly by increasing LSK and pro-B-cell proliferation, through upregulation of their cell surface IL3Rα and IL7Rα expression. Recently, 2 other studies involving cholesterol homeostasis have reported similar findings. Yvan-Charvet et al11 showed that ABCA1 and ABCG1 DKO in mice cause structural and functional changes in lipid rafts. Our study and the work of others11,12 support the concept that lipid rafts play important roles in atherosclerosis.

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**References**

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Supplemental Figure I. Spleen weight (A), Inguinal lymph node (LN) cell number (B), and body weight (C) of WT and Acat1/-/- mice. 7 to 9 week old age matched WT and Acat1/-/- mice with comparable numbers in males and females were employed. Results are means ± SEM. For A, 16 WT mice and 21 Acat1/-/- mice in 6 separate experiments. For B, 7 WT mice and 10 Acat1/-/- mice in 2 separate experiments. For C, 5 WT and 7 Acat1/-/- male mice, 12 WT and 13 Acat1/-/- female mice in 2 separate experiments. **P<0.01
Supplemental Figure II. Acat1 mRNA (left panels) and protein expression (middle panels) in blood leukocytes were examined from WT or Acat1-/ recipient mice transplanted with WT or Acat1-/ BM 7 week after BMT (A), (B). Spleen weight (right panels) from both recipient mice 6 and 11 week after BMT were also measured. Results are means ± SEM. For (A), 4 WT BM and 4 Acat1-/ BM in 1 experiment for RT-PCR and western blot analysis; 4 WT and 4 Acat1-/ mice in 1 experiment for mouse spleen weight determination post BMT 6 week, and 6 WT and 10 Acat1-/ mice in 2 experiments for mouse spleen weight determination post BMT 11 week. For (B), 4 WT BM and 4 Acat1-/ BM in 1 experiment for RT-PCR and western blot analysis; 4 WT and 4 Acat1-/ mice in 1 experiment for mouse spleen weight determination post BMT 6 week, and 6 WT and 6 Acat1-/ mice in 2 experiments for mouse spleen weight determination post BMT 11 week. *P<0.05
Supplemental Figure III.

Acat1-/- BM produces more colonies in vitro. Colony forming unit assay and delineation of various different progenitor types were according to procedures described in Materials and Methods, using WT and Acat1-/- mouse BM as indicated. Abbreviations used: Meg/E, megakaryocyte/erythroid; BFU-E, burst formation unit-erythroid; G, granulocyte; M, macrophage; GM, granulocyte/macrophage; GEMM, granulocyte, erythroid, macrophage, megakaryocyte colony.

Results are means ± SEM. 8 WT mice and 8 Acat1-/- mice were used in 2 separate experiments. ***P<0.001
Supplemental Figure IV.

Acat1/-/ increases proliferation of purified LSK Cells under IL3 treatment. LSK, CMP, and GMP cells from WT and Acat1/-/ BM were sorted and cultured individually under IL3 for 72hr. Proliferation was measured by BrdU incorporation using flow cytometry. Results are means ± SEM. 8 WT mice and 8 Acat1/-/ mice in 2 separate experiment. **P<0.01
Supplemental Figure V.
Quantification of LSK subpopulations (LT-HSCs, ST-HSC, and MPPs) analyzed by flow cytometry. Results are means ± SEM from 2 separate experiments, using 4 mice per group for each experiment. *P<0.05
Supplemental Figure VI.
Inhibition of de novo cholesterol synthesis by squalene synthase inhibitor (SSI) attenuates the increased proliferation phenotype in Acat1-/- BM cells. WT and Acat1-/- BM cells were cultured under 6ng/mL IL3 without or with 1µM SSI, or with 50 or 100 µg/ml of ApoA1 as indicated for 72hr. Proliferation was measured by H-thymidine incorporation. Results are means ± SEM of 3 separate experiments. *P<0.05
Supplemental Figure VII.

Blood myeloid cells in Apoe-/-, and Apoe-/-/Acat1-/- mice. Mice at 2 months of age were fed with modified Paigen’s diet for 0 to 6 weeks. At various time points as indicated, blood myeloid cell (CD11b+Gr1+) number was measured by using flow cytometry as described in Materials and Methods. Results are means ± SEM; 6 male Apoe-/- and 4 Apoe-/-/Acat1-/- mice were tested. *P<0.05; ***P<0.001
Materials and Methods

Animal Care
Wild-type and Acat1−/− mice employed are in C57BL/6 background. Apoe−/− mice were from Jackson Laboratories (ID number 007067). Modified Paigen’s diet (without cholate) was from Research Diets (99020201). Animal protocols were approved by Dartmouth Animal Care Committee (protocol number 08.05.01).

Cell Isolation and Counting
Peripheral blood cells or BM cells were isolated from age- and sex-matched mice using methods previously described. Total cells were counted with a Coulter cell counter. Blood cells or BM cells were incubated with red blood cell lysis buffer (e-Bioscience) for 15 min at ambient temp. After washing once with phosphate-buffered saline (PBS) containing 3% FBS, cells were re-suspended in the same buffer. Leukocyte number was calculated after gating out red blood cells using flow cytometry. Inguinal lymph nodes (LN) cell numbers were counted after LN were removed, homogenized, and filtered with 40μm cell strainers.

Flow Cytometry Analysis
The method described in was used to gate cell populations. Peripheral blood or BM leukocytes from age- and sex-matched mice were filtered using 40μm cell strainers and stained with various specific antibodies for 15 min at 4°C followed by a wash with PBS containing 3% FBS. For peripheral leukocytes, monocyte and neutrophil populations were quantified by staining with CD11b (1/70)-APC and with Gr1 (RB6-8C5)-PE; B and T populations were quantified by staining with CD3 (145-2C11)-PE and with B220 (RA3-6B2)-APC; antibodies were from Biolegend. For hematopoietic populations, the following lineage antibodies from Biolegend were used: c-Kit (2B8)-APC, Sca-1 (D7)-PE, CD34 (HM34)-Biotin, Streptavidin-APC-Cy7, Streptavidin-PE-Cy5, FcgRII/III (93)-FITC, IL7Rα (A7R34)-FITC, and CD135 (A2F10)-PE. Lineage antibodies, p-ERK1/2-PE and CD131 (IL3Rβ) (JRO50)-PE were from BD Biosciences. Cells were analyzed on a BD FAScan machine (BD Biosciences) installed with a 25mW diode red laser and Rainbow software (Cytek Development, Fremont, CA), or sorted on a BD FACSARia Cell Sorter (BD Biosciences). Data were analyzed by using FlowJo software from Tree Star, Inc.

Bone Marrow Transplantation
The experiments were carried out as described previously, using 8 week old female WT, Acat1−/− or Apoe−/− mice as recipients. Donor BM cells were prepared from two or more female WT or Acat1−/− mice as two separate pools. After lysis of red blood cells, BM cells (2×10^6) from each pool were transplanted into lethally irradiated recipient WT, Acat1−/−, or Apoe−/− mice through retro-orbital injection. Lethal irradiation was achieved using a Cs137 irradiator using a split dose of 950 rads.
Colony-Forming Assay
The experiments were carried out as described previously. Twenty thousand BM cells isolated from each 8 week old WT or Acat1-/- mouse were cultured individually in methylcellulose media (MethoCult 3434; Stem Cell Technologies) at 37°C. Colonies were counted after 7 days. Various progenitor types were counted based on colony morphology as described in 5.

Proliferation Assays
For cell proliferation measurement \textit{in vivo}, the manufacturer’s protocol (BD Pharmingen; BrdU flow kit) was used. Age- and sex-matched mice of both genotypes were injected intraperitoneally twice, once every 12 hr, with 0.5 mg bromodeoxyuridine (BrdU)/injection/mouse. Twenty-four hours after the first injection, BM progenitor cells were stained and sorted as described earlier. Proliferation of each of the sorted cell populations was measured by measuring BrdU incorporation using the BrdU flow kit (BD Pharmingen). For proliferation measurement of BM-derived myeloid cells in culture, the method described in 4 was used. Briefly, after lysis of red blood cells from crude BM, cells were cultured in IMDM (Gibco) containing 10% fetal bovine serum (FBS) for 2 hr to remove attached cells. The unattached cells were re-plated for 72 hr in various conditions as described in Figure 4. Stem cell factor IL3 and GM-CSF were from R&D Systems. U0126, PP2, and ApoA1 were from Sigma. Squalene synthase inhibitor (SSI) compound CP-340868 was from Pfizer. Cells were pulsed with 2µCi/mL \textit{3}H-thymidine for 3 hr. \textit{3}H-thymidine incorporation was monitored using a liquid scintillation counter.

Annexin V Staining
Apoptotic cell populations were measured using FITC-annexin V staining (BD Pharmingen). The staining procedure was according to the manufacturer’s protocol. The analysis of annexin V positive cells was performed using a BD FAScan machine (BD Biosciences).

Western Blot and RT-PCR Analyses
To prepare BM-derived myeloid cells in culture for western blotting, cells were washed two times with PBS; cell pellets were lysed in RIPA buffer containing 0.15M NaCl, 0.01M Tris-Cl (pH 7.4), 1% NP-40, and protease inhibitors, incubated at room temp for 30 min, followed by spinning at 14,000rpm for 5 min to remove cell debris. Supernatants were collected and adjusted to 0.1M DTT, 2% SDS and 1X sample buffer. Samples were resolved by SDS-PAGE and transferred to 0.22 µm nitrocellulose membranes. After blocking with nonfat milk dissolved in Tris buffer containing 0.1% Tween 20 (TBST) at room temp for 30 min, the primary antibodies, p-ERK or ERK (Cell Signaling) in TBST were incubated with the membrane filter, followed by incubation with secondary antibodies and detected by ECL chemiluminescence (Pierce). Acat1 gene and protein expressions in leukocytes were analyzed by western blotting and by RT-PCR as previously described.
**Statistical Analysis**

Results are reported as means ± SEM. Two-tailed parametric student’s t test and two-way ANOVA analysis with Bonferroni multiple comparison post-test were used to evaluate the statistical significance among various study groups. Analysis was performed and plotted using GraphPad Prism 5.0. The difference between two sets of values was considered significant when the *p* value was < 0.05 (*p*<0.05, **p**<0.01, ***p**<0.001).

**References**


