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Regulator of Fatty Acid Metabolism, Acetyl Coenzyme A Carboxylase 1, Controls T Cell Immunity

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Fatty acids (FAs) are essential constituents of cell membranes, signaling molecules, and bioenergetic substrates. Because CD8$^+$ T cells undergo both functional and metabolic changes during activation and differentiation, dynamic changes in FA metabolism also occur. However, the contributions of de novo lipogenesis to acquisition and maintenance of CD8$^+$ T cell function are unclear. In this article, we demonstrate the role of FA synthesis in CD8$^+$ T cell immunity. T cell–specific deletion of acetyl coenzyme A carboxylase 1 (ACC1), an enzyme that catalyzes conversion of acetyl coenzyme A to malonyl coenzyme A, a carbon donor for long-chain FA synthesis, resulted in impaired peripheral persistence and homeostatic proliferation of CD8$^+$ T cells in naive mice. Loss of ACC1 did not compromise effector CD8$^+$ T cell differentiation upon listeria infection but did result in a severe defect in Ag-specific CD8$^+$ T cell accumulation because of increased death of proliferating cells. Furthermore, in vitro mitogenic stimulation demonstrated that defective blasting and survival of ACC1-deficient CD8$^+$ T cells could be rescued by provision of exogenous FA. These results suggest an essential role for ACC1-mediated de novo lipogenesis as a regulator of CD8$^+$ T cell expansion, and may provide insights for therapeutic targets for interventions in autoimmune diseases, cancer, and chronic infections. ***The Journal of Immunology*****, 2014, 192: 3190–3199.**

Upon Ag recognition, CD8$^+$ T cells undergo rapid phenotypic changes involving metabolism, survival, and differentiation. These changes, characterized by increased cell size, proliferation, and acquisition of effector functions during differentiation into cytotoxic T cells, depend on optimal cell–cell interactions and cross talk between multiple signaling pathways (1). Fatty acids (FAs), in the form of triglycerides, phosphoglycerides, or sphingolipids, are directly involved in these cellular processes as key components of cell membranes, as signaling molecules, and as energy-yielding substrates (2–5). Evidence shows that modifications in FA metabolism at both cellular and whole-organism levels can influence immunity. The polyunsaturated FAs eicosapentaenoic acid and docosahexaenoic acid have immunoregulatory roles through influence on both immune and nonimmune cells (6). Polyunsaturated FAs reduce production of proinflammatory cytokines and activate the NLRP3 inflammasome in macrophages (7, 8), and have been demonstrated to have a beneficial role in a variety of inflammatory diseases, including diabetes, atherosclerosis, Crohn's disease, and arthritis (9). Also, modification of FA composition of the cell membrane through diet (10) or genetic manipulation (11) modulates T cell function partly through alteration of lipid raft structure and the translocation of signaling molecules. We previously demonstrated that pharmacologically enhancing FA oxidation drives CD8$^+$ T cells toward a memory fate (12). These results show a key role for FA metabolism as a potential cell-intrinsic determinant of immune outcomes. Despite these findings, it remains unclear how direct regulation of intracellular FA homeostasis affects CD8$^+$ T cell activation, proliferation, and effector differentiation because the upstream molecular regulators have not yet been investigated.

Acetyl coenzyme (CoA) carboxylase (ACC) catalyzes conversion of acetyl CoA to malonyl CoA, which regulates both biosynthesis and breakdown of long-chain FAs. Two isozymes, ACC1 and ACC2, mediate distinctive physiological functions within the cell, with ACC1 localized primarily to the cytosol and ACC2 to the mitochondria (13). Malonyl CoA produced in the cytosol by ACC1 serves as a carbon donor for long-chain FA synthesis mediated by FA synthase (FAS) (14), whereas malonyl CoA synthesized by ACC2 anchored along the mitochondria surface works as an inhibitor of carnitine palmitoyl transferase 1, regulating transport of long-chain FAs into mitochondria for subsequent β-oxidation (15–18).

Because of its role in FA metabolism, ACC1 has been considered a good target for intervention in metabolic syndromes and cancers. Earlier studies showed that specific deletion of ACC1 in liver (19) or adipose tissues (20) resulted, respectively, in reduced de novo FA synthesis and triglyceride accumulation, or skeletal growth retardation, suggesting functional importance of ACC1 for both lipogenesis and cellular homeostasis. Also, aberrantly increased ACC1 or FAS expression/activity have been observed in metastatic cancer (14, 21–23), and effective interventions against

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Abbreviations used in this article: ACC, acetyl coenzyme A carboxylase; FA, fatty acid; FAS, fatty acid synthase; FSC, forward scatter; LmOVA, attenuated *Listeria monocytogenes* OVA; MFI, mean fluorescence intensity; mLN, mesenteric lymph node; pLN, peripheral lymph node; SSC, side scatter; WT, wild-type.

tumorigenesis with ACC1 and FAS inhibitors (24, 25) imply ACC1 may regulate cell differentiation, transformation, or fate. Combined, previous studies support a key role for ACC1 in lipid metabolism and cell fate regulation, but the role of ACC1 in lymphocyte biology is completely unknown.

In this article, we have demonstrated the crucial role for ACC1 in processes involved in the acquisition and/or maintenance of T cell fate. T cell–specific deletion of ACC1 impaired T cell persistence in the periphery and homeostatic proliferation in naive mice. ACC1 appeared dispensable for acquiring $CD8^+$ T cell effector functions upon listeria infection, but played an indispensable role in Ag-specific $CD8^+$ T cell accumulation by influencing survival of proliferating cells. Further, in vitro analysis demonstrated that de novo lipogenesis is necessary for blastogenesis and sustaining proliferation of $CD8^+$ T cells under mitogenic conditions. Provision of exogenous FA was sufficient to rescue defective cell growth and accumulation of ACC1-deficient $CD8^+$ T cells, emphasizing the importance of de novo lipogenesis for regulating optimal T cell blastogenesis and survival.

Materials and Methods

Mice

ACC1$^{f/f}$ mice (from Dr. David E. James, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia) on C57BL/6 background were crossed to *Cd4*-Cre mice. *ACC1$^{f/+}$Cd4*-Cre$^+$ or *ACC1$^{f/f}$Cd4*-Cre$^-$ littermates (WT) were used as controls in all experiments. In addition, *ACC1$^{f/f}$ Cd4*-Cre mice (ACC1ΔT) were crossed with Tg(*TcraTcrb*)1100Mjb/J (OT-I) mice to generate *ACC1$^{f/f}$ Cd4*-Cre OT-I ($CD45.2^+$, ACC1ΔT OT-I) mice. A further cross with $CD45.1^+$ or $CD90.1^+$ C57BL/6 mice produced $CD45.1^+/2^+$ or $CD90.1^+$ WT OT-I mice. B6.Ly5.2/Cr (CD45.1 congenic) mice were purchased from the National Cancer Institute (Frederick, MD). All mice were maintained in specific pathogen-free facilities at the University of Pennsylvania. Mice and tissues collected from mice were maintained in strict accordance with University of Pennsylvania policies on the humane and ethical treatment of animals.

Mononuclear cell isolation, cell purification, and flow cytometry

Mononuclear cells were prepared and stained for flow cytometric analysis as described elsewhere (26). Thymus, spleen, peripheral lymph nodes (pLNs), and mesenteric lymph nodes (mLNs) were removed and homogenized through 70 μM of nylon mesh, and the resultant cell suspension was pelleted by centrifugation. RBCs were lysed, and remaining cells were washed three times and counted. Absolute cell numbers were calculated based on the percentage of specific T or B cells from the total cell population acquired as determined by flow cytometric analysis.

For intracellular cytokine staining, single-cell suspensions from spleens were cultured at 37˚C in complete RPMI 1640 supplemented with Golgiplug (BD Biosciences) in the presence of SIINFEKL for 5 h. After surface staining with anti-CD44 and anti-CD8 Abs, cell suspensions were fixed and permeabilized by Cytofix/Cytoperm solution (BD Biosciences), followed by staining with anti–IFN-γ (XMG1.2) Abs. Anti-mouse CD4 (RM4-5), anti-CD8 (53-6.7), anti-CD25 (PC61.5), anti-CD71 (R17217), anti-CD98 (RL388), anti-CD127 (A7R34), anti–Thy-1.2 (53-2.1), anti-CD107a (1D4B), anti–T-bet (4B10), anti-emoes (Dan11mag), anti–IFN-γ, and anti-CD107a (1D4B) Abs were purchased from eBioscience. Anti-CD45.2 (104), anti-CD44 (IM7), anti–KLRG-1 (2F1), and anti-CD62L (MEL14) Abs were from Biolegend. Anti–granzyme B Ab was purchased from Invitrogen. Stained cells were collected with an LSRII (BD Biosciences) and analyzed with FlowJo software (Tree Star).

For naive cell purification, mononuclear cells from spleen and lymph nodes were enriched for CD8 using magnetic separation beads (Miltenyi Biotec). After MACS enrichment, cells were stained with anti-CD44, -CD62L, -CD25, -CD8, and -CD4 Abs to further sort out naive $CD8^+$ T cells ($CD44^{low}CD62L^{high}CD25^{neg}$) by FACSAria (BD Biosciences).

Quantification of newly synthesized long-chain FAs

FACS-purified naive WT and ACC1ΔT $CD8^+$ T cells were activated with PMA and ionomycin for 24 h in the presence of deuterium oxide (D_2O, 5% final concentration). Culture medium was collected, cells were harvested and counted, and lipids were extracted to analyze newly synthesized FAs as described in detail elsewhere (27). In brief, C-17 heptadecanoic acid was added to cell pellets as an internal standard for FAs. Cells were dried and lipids extracted using chloroform:methanol. The lipid extract was saponified with 1 ml 0.3N KOH-ethanol at 60˚C for 1 h. FAs were derivatized to methyl ester forms with methanolic boron trifluoride, extracted into hexane, and injected into an Agilent 7890A/5975 gas chromatography–mass spectrometry fitted with a DB-5MS column. The FA methyl esters were run in split mode (1 μl at a split of 1:10) with the following settings: inlet temperature, 250˚C; flow rate, 1 ml/min; transfer line 280˚C; MS quadrupole, 150˚C; MS source, 230˚C; oven set at 150˚C initially, ramped to 200˚C at a rate of 5˚C/min, and then ramped to 300˚C at a rate of 10˚C/min (22 min total run time). A palmitate standard was used to quantitate palmitate, stearate, and oleate after applying a response correction. Quantification of the area under the curve for selected ions was done with Chemstation software.

Lymphopenia-induced proliferation

FACS-purified naive $CD8^+$ T cells from *ACC1$^{f/f}$Cd4*-Cre (ACC1ΔT) and their wild-type (WT) littermates or from $CD90.1^+$ congenic mice were labeled with CFSE, and 0.8×10^6 cells of WT or ACC1ΔT $CD8^+$ T cells per mouse were injected i.v. into hosts irradiated 1 d earlier with 750 rad. The same number of $CD90.1^+$ $CD8^+$ T cells was cotransferred per mouse. After 14 d, host spleen and lymph node cells were analyzed by flow cytometry.

Attenuated Listeria monocytogenes OVA *infection*

A total of 1×10^3 ACC1ΔT OT-I ($CD45.2^+$) and their WT littermate ($CD45.2^+$ or $CD45.1/2^+$) cells were transferred i.v. to 6- to 8-wk-old $CD45.1^+$ recipient mice. Mice were then infected i.v. with 1×10^6 CFU recombinant attenuated *L. monocytogenes* (LmOVA) (12, 28).

BrdU labeling

For in vivo labeling, BrdU (1 mg/mouse) was injected i.p. into mice. For in vitro labeling, BrdU (1 mM/ml) was added to cell culture for 1 h. Cells were stained and analyzed by flow cytometry according to the manufacturer's protocol (BD Biosciences).

Cell culture and FA preparation

FACS-purified naive $CD8^+$ T cells ($CD25^{neg}CD44^{low}CD62L^{high}$) were cultured in RPMI supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, 100 μg/ml penicillin and streptomycin, and 50 μM 2-ME in 96-well plates. Cells were activated with Dynabeads mouse T-activator CD3/28 (Invitrogen) according to the manufacturer's protocol in the presence 100 U/ml human IL-2 (Peprotech).

Sodium palmitate and oleic acid (Sigma) were dissolved in methanol at 25 mM; 25-mM stocks were diluted 10-fold in PBS containing 0.9% FA free BSA (Roche). These 2.5-mM (100×) stocks were thoroughly mixed by vortexing and incubated at 37˚C for 1 h before use.

Statistical analysis

All data are presented as mean ± SD. The mixed-effect model or the two-tailed unpaired Student *t* test was used for comparison of the two groups using customized routines written in the statistical programming language R (version 2.15.0). In all cases, $p < 0.05$ was considered statistically significant.

Results

ACC1 deficiency compromises de novo lipogenesis

Mitogenic signals, like those encountered by $CD8^+$ T cells during pathogenic infections, induce lipogenesis in T cells (29–31). To begin to explicitly characterize the functional importance of FA metabolism to $CD8^+$ T cell function, we first assayed gene induction of the ACCs, ACC1 and ACC2, during primary expansion of $CD8^+$ T cells. Quantitative PCR analysis of purified naive versus effector $CD8^+$ T cells 6 d after listeria infection showed significant induction of *ACC1*, but not *ACC2* mRNA (Fig. 1A). This result suggests a potentially important role for ACC1 in $CD8^+$ T cell activation and effector differentiation. Therefore, we chose to focus on investigating the T cell–intrinsic role of ACC1 in immune responses.

Complete lack of ACC1 in mice is lethal at approximately embryonic day 8.5 (32). To study the role of ACC1 specifically in

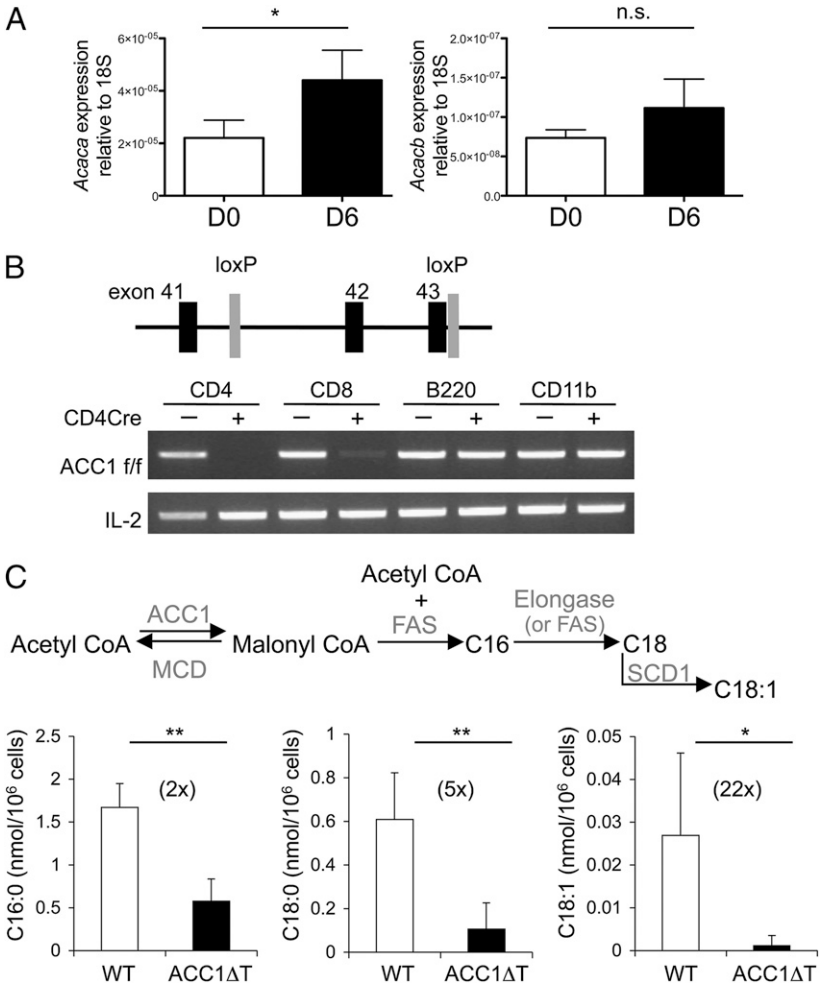

FIGURE 1. ACC1 deficiency compromises de novo lipogenesis in T cells. (**A**) Real-time quantitative PCR analysis of *ACC1* and *ACC2* gene expression from naive and effector CD8$^+$ T cells after LmOVA infection. Mice were infected with LmOVA 1 d after transfer of 1×10^3 OT-I cells per mouse, and donor cells were FACS sorted 6 d later. Results are presented relative to 18S ($n = 4$). (**B**) Generation of mice with T cell–specific deletion of the *ACC1* gene and confirmation of gene deletion in CD4$^+$ and CD8$^+$ T cells. Schematic presentation of the floxed allele and PCR analysis of ACC1 deletion in genomic DNA in FACS-purified CD4$^+$, CD8$^+$, B220$^+$, CD11b$^+$ cells from *ACC1$^{f/f}$* (WT) and *ACC1$^{f/f}$ Cd4-cre* (ACC1ΔT) mice. IL-2 was an internal control. (**C**) Quantification of newly synthesized long-chain FAs in WT and ACC1ΔT CD8$^+$ T cells by gas chromatography–mass spectrometry 24 h after activation in vitro. WT: $n = 6$; ACC1ΔT: $n = 4$. $^*p < 0.05$, $^{**}p < 0.001$.

T cells in vivo, we crossed mice in which exons 42 and 43 of the gene encoding ACC1, *Acaca*, are flanked by *lox*P sites to mice carrying the *Tg(Cd4-Cre)1Cwi* transgene to induce T cell–specific deletion (Fig. 1B). Efficient and specific deletion of targeted exons 42 and 43 in peripheral T cells was demonstrated by PCR of genomic DNA (Fig. 1B). In addition, we observed that ACC1 deletion did not affect mRNA expression of *ACC2*, *FAS*, or *SCD-1* in naive CD8$^+$ T cells (data not shown). We next analyzed the de novo lipogenesis capacity of activated T cells isolated from ACC1$^{f/f}$-*Cd4Cre* mice (ACC1ΔT) by quantifying newly synthesized long-chain FAs by gas chromatography–mass spectrometry analysis (Fig. 1C). New synthesis of C16:0 and C18:1 was reduced 2- and 22-fold, respectively, in activated ACC1ΔT compared with WT T cells, demonstrating that deletion of ACC1 in T cells has functional effects on de novo lipogenesis. However, the total quantity of each FA, C16:0, C18:0, or C18:1, was not significantly different between WT and ACC1ΔT T cells at 24 h postactivation, possibly because of the small proportion of newly synthesized FAs compared with the total amount of each FA.

Loss of ACC1 impairs T cell homeostasis in the periphery

To examine the effects of ACC1 deletion on peripheral T cell homeostasis, we analyzed the frequency and numbers of T cells in thymus, spleen, and pLNs isolated from 7-wk-old ACC1ΔT and WT littermate mice. Although the CD4$^+$ and CD8$^+$ profiles of thymocytes from ACC1ΔT mice were unremarkable, frequencies and numbers of CD8$^+$ T cells in spleens and pLNs were dramatically reduced in ACC1ΔT compared with WT controls (Fig. 2A, 2B). Much less, but still significant, reduction in CD4$^+$ T cells was also observed in the ACC1ΔT mice, whereas peripheral ACC1ΔT B cell numbers were normal (Fig. 2A, 2B). To determine whether ACC1 deletion resulted in cellular phenotypic differences, we analyzed expression of various surface markers. Expression of activation markers CD69, CD25, and CD127, as well as expression of transferrin (CD71) and amino acid transporter (CD98) on ACC1ΔT CD8$^+$ T cells was similar to levels expressed by cells from littermate control cells (Fig. 2C). However, further phenotypic analysis of peripheral T cells in ACC1ΔT mice showed a significantly lower proportion of activated-memory phenotype (CD44^{hi}) in CD8$^+$ T cells compared with littermate controls (Fig. 2D), suggesting that ACC1 is necessary to acquire and/or maintain an activated phenotype. Together, these observations suggest a general role for ACC1 in peripheral T cell maintenance, with particular importance to the CD8$^+$ T cell compartment.

Loss of ACC1 impairs CD8$^+$ T cell persistence and homeostatic proliferation

To account for possible cell nonautonomous and/or thymic feedback effects on ACC1ΔT CD8$^+$ T cell hypocellularity, we examined the persistence of naive CD8$^+$ T cells in the periphery. FACS-sorted naive (CD44^{low}CD62L^{high}CD25^{neg}) WT congenic (CD90.1$^+$CD45.2$^+$) and ACC1ΔT CD8$^+$ T cells (CD90.2$^+$CD45.2$^+$) were cotransferred into naive recipient mice (CD45.1$^+$) in equal numbers, and donor-derived CD8$^+$ T cells were longitudinally analyzed to measure the ratio of the WT and ACC1ΔT CD8$^+$ T cells in the blood. ACC1ΔT CD8$^+$ T cell numbers decayed faster than those of WT CD8$^+$ T cells; thus, the ratio of WT CD8$^+$ T cells to ACC1ΔT CD8$^+$ T cells increased over time (Fig. 3A).

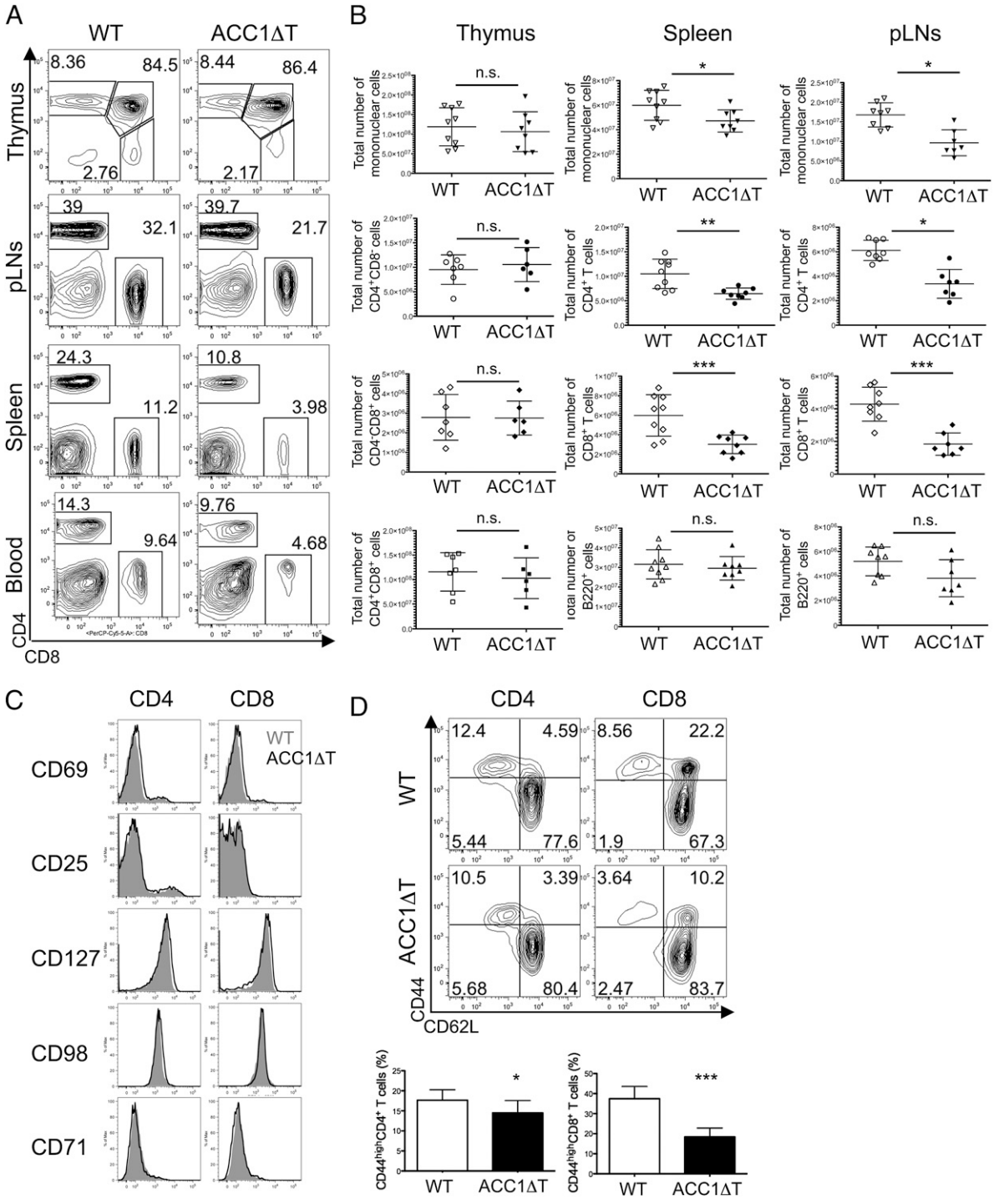

FIGURE 2. Loss of ACC1 impairs T cell homeostasis in the periphery. (**A**) Frequency of $CD4^+$ and $CD8^+$ T cells in the thymus, spleen, pLNs, and blood from naive ACC1ΔT and WT littermate mice (7 wk old). Shown are representative dot plots from five independent experiments. (**B**) Numbers of isolated cells in the spleen and pLNs from ACC1ΔT mice and WT littermates (means ± SD). (**C**) Expression of various surface markers in splenic $CD4^+$ and $CD8^+$ T cells from WT and ACC1ΔT mice at 7 wk old. Results are representative of at least nine mice per group analyzed. (**D**) CD44 and CD62L expression profiles of $CD4^+$ and $CD8^+$ T cells and their frequencies in the spleen; results are representative of at least nine mice per group. $*p < 0.05$, $**p < 0.001$, $***p < 0.0001$.

Also, consistent with previous observations of naive ACC1ΔT mice (Fig. 2B), five times fewer ACC1ΔT than WT $CD8^+$ T cells were recovered from spleens 8 wk posttransfer (Fig. 3B), suggesting defective survival and/or turnover of naive ACC1ΔT $CD8^+$ T cells when in a T cell–replete (competitive) environment. We next examined the capacity of ACC1ΔT $CD8^+$ T cells to persist and expand in a lymphopenic environment. CFSE-labeled, FACS-sorted naive WT or ACC1ΔT $CD8^+$ T cells ($CD90.2^+CD45.2^+$) were transferred into irradiated congenic recipient mice ($CD90.2^+$ $CD45.1^+$) along with reference cells (naive $CD90.1^+CD45.2^+$ $CD8^+$ T cells) and harvested 14 d later. Consistent with the previous result (Fig. 3A), 10 times fewer ACC1ΔT $CD8^+$ T cells were recovered from the spleen (Fig. 3C). These results, along with the diminished CFSE dilution (Fig. 3D), suggest a defect in survival and/or proliferation of ACC1ΔT $CD8^+$ T cells under lymphopenic condition.

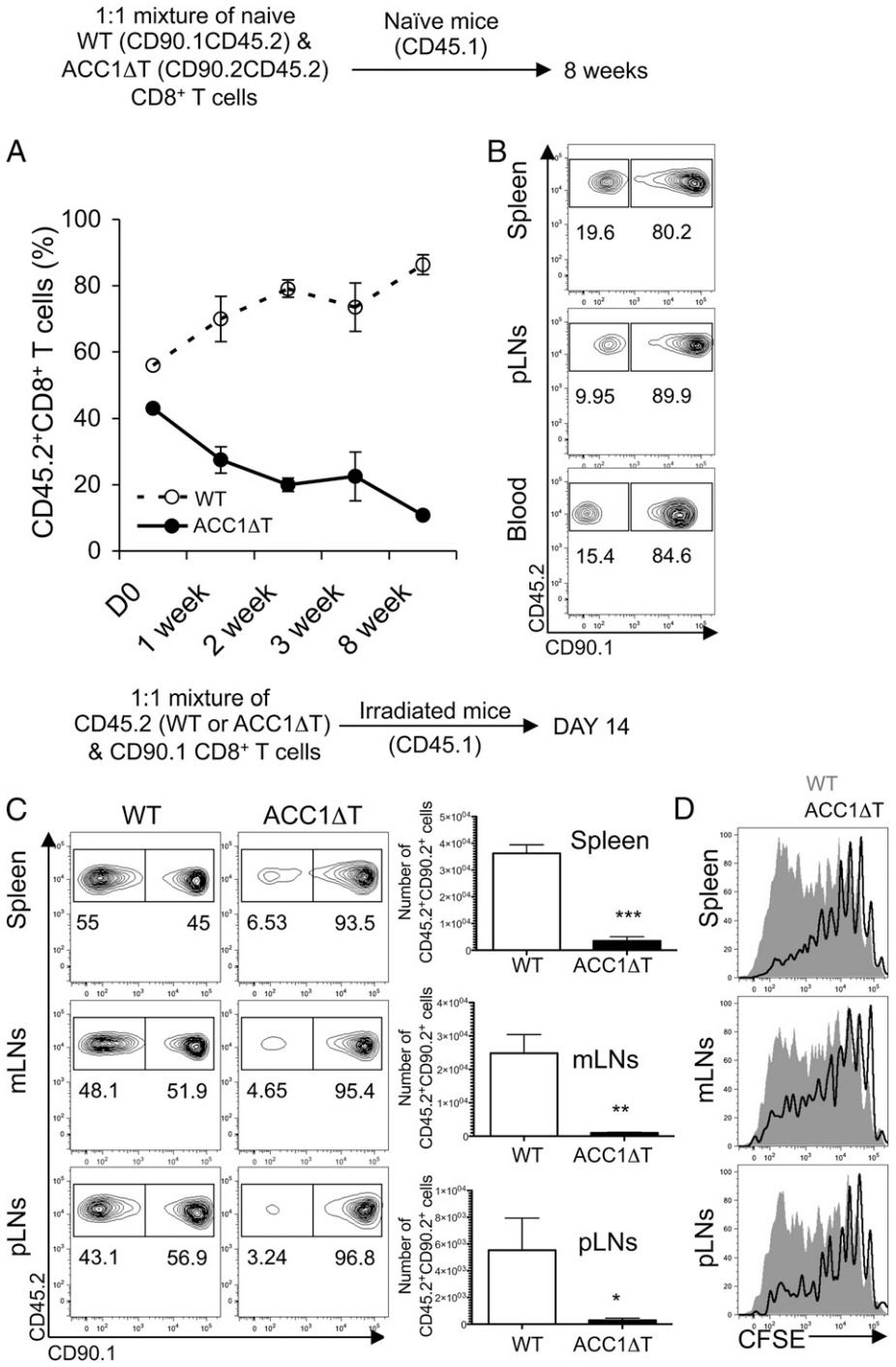

FIGURE 3. Loss of ACC1 impairs $CD8^+$ T cell persistence and homeostatic proliferation in the periphery. (**A** and **B**) 1:1 mixture of 2×10^6 sorted naive ($CD44^{low}CD62L^{high}CD25^{neg}$) WT ($CD90.1^+CD45.2^+$) and ACC1ΔT ($CD90.2^+CD45.2^+$) $CD8^+$ T cells were transferred into naive congenic recipient mice ($CD45.1^+$). Mice were bled at indicated time points, and mononuclear cells were surface stained ($n = 3$). (A) Longitudinal analysis of the frequency of WT and ACC1ΔT $CD8^+$ T cells in the blood and (B) in various tissues 8 wk after cell transfer. (**C**) Lymphopenia-induced proliferation of WT or ACC1ΔT $CD8^+$ T cells from the spleen, mLNs, and pLNs was measured 14 d after transfer of CFSE-labeled naive WT or ACC1ΔT $CD8^+$ T cells ($CD45.2^+$) into irradiated host mice ($CD45.1^+$). Naive $CD90.1^+$ $CD8^+$ T cells were cotransferred as reference cells. Dot plots show the frequency of WT or ACC1ΔT $CD8^+$ T cells in comparison with that of reference cells from the same recipient mouse ($n = 5$, one representative result shown from three independent experiments). (**D**) Histograms show CFSE dilution of transferred WT (grayed area) and ACC1ΔT $CD8^+$ T cells (black line) from the spleen, mLNs, and pLNs. Graphs represent numbers of isolated WT and ACC1ΔT $CD8^+$ T cells from indicated tissues (means ± SD, $n = 5$). One representative result shown from three independent experiments. $^*p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$.

ACC1 is required for accumulation of Ag-specific $CD8^+$ T cells during LmOVA infection

To investigate whether ACC1 is required for $CD8^+$ T cell responses during infection, we adoptively transferred chicken OVA-specific TCR transgenic (OT-I) $CD8^+$ T cells isolated from either WT or ACC1ΔT mice into recipient mice and examined their responses to listeria-OVA (LmOVA) infection. LmOVA infection results in robust expansion of $CD8^+$ T cells, accompanied by effector differentiation (12). On day 7 postinfection, donor-derived WT and ACC1ΔT OT-I cells were identified by costaining for K^b/OVA tetramer and the donor congenic marker CD45.2. Splenic frequency of ACC1ΔT OT-I within the $CD8^+$ T cell population was five times lower than for WT controls, with eight times fewer total ACC1ΔT OT-I cells recovered, demonstrating a severe defect in accumulation of Ag-specific $CD8^+$ T cells upon LmOVA infection (Fig. 4A). A similar result was observed in the blood (data not shown), suggesting that reduced accumulation of ACC1ΔT OT-I cells in the spleen was not caused by preferential sequestration in other tissues. In addition, we confirmed that defective accumulation of ACC1ΔT OT-I cells after LmOVA infection was cell-intrinsic, and not related to differences in abundance of Ag or other environmental factors affecting $CD8^+$ T cell responses, by cotransferring WT and ACC1ΔT OT-I cells into the same recipient mice in equal numbers (Supplemental Fig. 1).

Despite diminished Ag-specific ACC1ΔT $CD8^+$ T cell numbers, ACC1ΔT effector differentiation on a per-cell basis, as determined by expression of the degranulation marker CD107a, the inflammatory cytokine IFN-γ, and granzyme B, was similar to WT OT-I

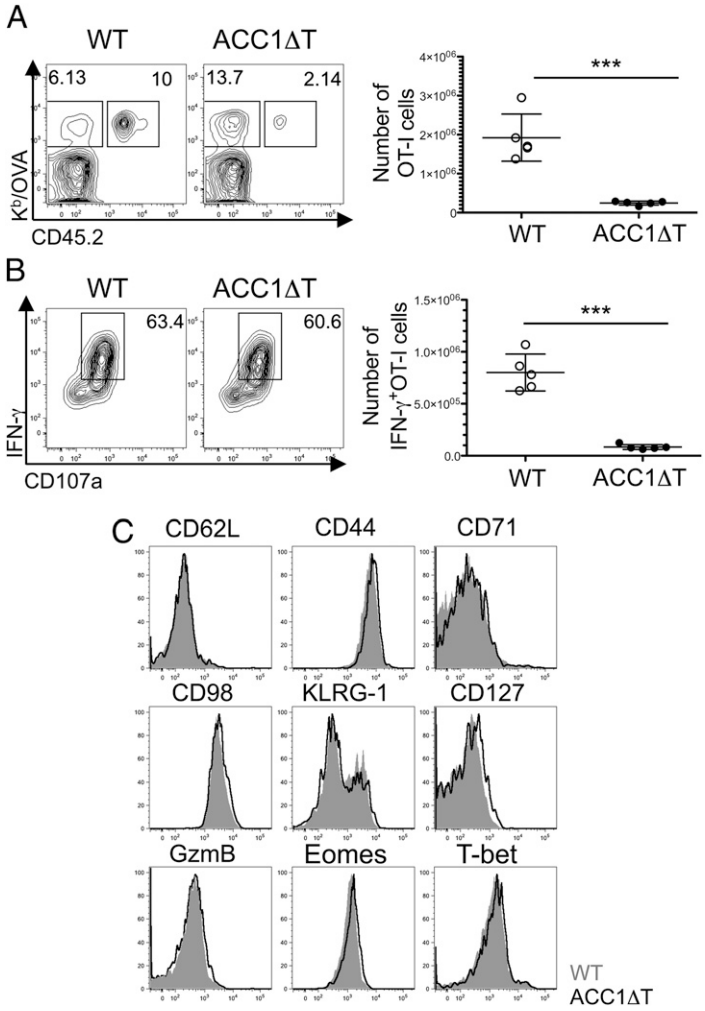

FIGURE 4. ACC1 is required for accumulation of Ag-specific CD8$^+$ T cells during LmOVA infection. A total of 1×10^3 WT OT-I or ACC1ΔT OT-I (CD45.2$^+$) cells was transferred into CD45.1$^+$ recipients and infected with LmOVA. Seven days postinfection, single cell suspensions were prepared from spleens and stained for various surface markers, cytokines, and transcription factors. Data are representative of two independent experiments ($n = 8$). (**A**) Dot plots show donor cells by CD45.2 and K^b/OVA tetramer (numbers indicate percent of total CD8$^+$ T cells that are host- or donor-derived). Graph represents number of WT and ACC1ΔT OT-I cells (means ± SD). (**B**) Dot plots show frequency of IFN-γ–producing WT and ACC1ΔT OT-I cells. Graphs represent number of IFN-γ–producing WT and ACC1ΔT OT-I cells (means ± SD). (**C**) The expression of surface markers, granzyme B, and transcription factors was determined in WT (grayed area) and ACC1ΔT OT-I (black line) cells. ***$p < 0.0001$.

cells (mean fluorescence intensity [MFI] of IFN-γ WT OT-I: 2194 ± 474; ACC1ΔT OT-I: 1772 ± 581; $p = 0.2$; MFI of granzyme B WT OT-I: 280 ± 22; ACC1ΔT OT-I: 286 ± 18; $p = 0.6$; Fig. 4B, 4C). In addition, T-bet and eomes, the T-box transcription factors essential for acquiring effector CD8$^+$ T cell functions (33), were expressed normally in ACC1ΔT OT-I cells (Fig. 4B). Further phenotypic analysis of surface marker expression showed normal upregulation of CD62L, CD44, CD71, CD98, and KLRG-1, but slightly less downregulation of CD127 (MFI of WT OT-I: 118.98 ± 17, ACC1ΔT OT-I 137.6 ± 7, $p = 0.003$; Fig. 4C). These results suggest that ACC1 is indispensable for Ag-specific CD8$^+$ T cell accumulation during infection, but is dispensable for acquiring CD8$^+$ T cell effector functions.

ACC1 is essential for survival of proliferating CD8$^+$ T cells

To directly address the proliferation and survival capacity of ACC1ΔT CD8$^+$ T cells in vivo, we analyzed transferred WT or ACC1ΔT OT-I cells 5 d post-LmOVA infection, at the peak of Ag-specific CD8$^+$ T cell proliferation, by injecting mice with BrdU to pulse proliferating cells. Although the frequency of ACC1ΔT OT-I cells within the total CD8$^+$ T cell population was two times lower than for WT, the frequency of BrdU incorporation by WT and ACC1ΔT OT-I cells was similar (Fig. 5A). This result suggests that although ACC1ΔT OT-I cells are capable of synthesizing DNA, most of the BrdU-incorporating daughter cells fail to survive. To further examine whether ACC1 is directly involved in the survival of CD8$^+$ T cells, we analyzed the number of live cells under both nonmitogenic and mitogenic conditions in vitro. Naive ACC1ΔT CD8$^+$ T cells exhibited normal survival in the presence of IL-7 for 3 d (Fig. 5B). Furthermore, no substantial differences were observed in live cell counts up to 24 h postactivation with anti-CD3 and anti-CD28 Abs (before the first cell division; Fig. 5C). However, at 72 h postactivation, when all the cells have undergone several cycles of proliferation, significant defects were observed in both cell numbers and the dilution profile of proliferation dye (Fig. 5D). The average ACC1ΔT CD8$^+$ T cell underwent fewer cycles of proliferation compared with WT CD8$^+$ T cells. These results demonstrated that deletion of ACC1 renders CD8$^+$ T cells sensitive to cell death upon mitogenic stimulation.

Exogenous FAs rescue survival and proliferation of ACC1ΔT CD8$^+$ T cells under mitogenic conditions

Lipid macromolecules are a major physical constitute of cells (2, 4). Therefore, it is logical to speculate that limiting these "building block" molecules may have negative effects on survival during cell division. We tested whether FA supplementation could rescue survival and proliferation of ACC1ΔT CD8$^+$ T cells under mitogenic conditions. We provided exogenous long-chain FAs to proliferation dye-labeled WT and ACC1ΔT CD8$^+$ T cells during activation with anti-CD3 and anti-CD28 Abs, and analyzed cell expansion 60 h later. Cells were also pulsed with BrdU for 1 h before harvest to determine the frequency of proliferating cells in a set period. Consistent with in vivo results (Fig. 5A), significantly fewer ACC1ΔT CD8$^+$ T cells were recovered when cultured without FA despite frequencies of BrdU-incorporating cells similar to WT CD8$^+$ T cells. However, overall cell expansion of ACC1ΔT

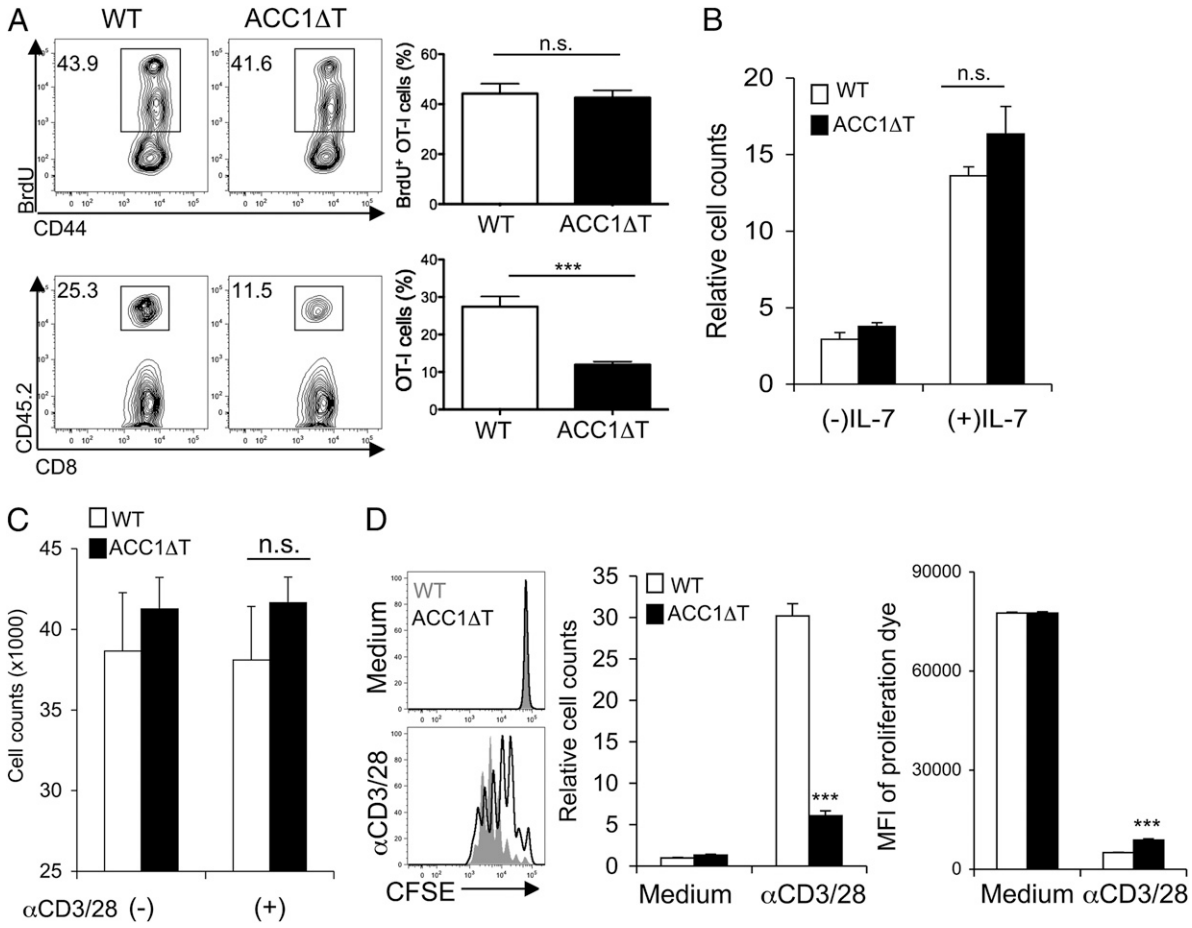

FIGURE 5. ACC1 is essential for survival of proliferating CD8$^+$ T cells. (**A**) OT-I cells (1×10^4) from WT and ACC1ΔT (CD45.2$^+$) mice were transferred into CD45.1$^+$ recipients and infected with LmOVA 1 d later. Mice were injected with BrdU i.p. 5 d postinfection. One hour after BrdU injection, spleens were harvested and CD8$^+$CD45.2$^+$ donor-derived cells were analyzed for BrdU incorporation. Numbers indicate the percentage of BrdU$^+$ cells among the donor CD45.2$^+$ cells or donor CD45.2$^+$ cells among CD8$^+$ T cells. Data are representative of three independent experiments (n = 3–5/group/experiment). (**B**) FACS-sorted naive WT and ACC1ΔT CD8$^+$ T cells (CD44^{low}CD62L^{high}CD25^{neg}) were cultured alone or in the presence of IL-7 (1 ng/ml) for 3 d. Results are presented as relative live cell counts to those of WT cells cultured without anti-CD3 and anti-CD28 Ab stimulation (means ± SD). Shown here is one representative result from at least four independent experiments. (**C**) FACS-sorted naive WT and ACC1ΔT CD8$^+$ T cells (CD44^{low}CD62L^{high}CD25^{neg}) were cultured in the presence of anti-CD3 and anti-CD28 Abs along with IL-2 for 24 h. Cells cultured without anti-CD3 and anti-CD28 Abs were supplemented with IL-7 instead. Graph shows number of live cells (means ± SD, n = 4). (**D**) Histograms show dilution of cellular proliferation dye of WT (grayed area) and ACC1ΔT CD8$^+$ (black line) T cells 72 h postactivation with anti-CD3 and anti-CD28 Abs. Results are presented as live cell counts relative to those of WT cells cultured without anti-CD3 and anti-CD28 Ab stimulation (means ± SD). One representative result shown from at least three independent experiments. ***$p < 0.0001$.

CD8$^+$ T cells was restored to WT levels when supplemented with exogenous FA, as evidenced by increased cell numbers and dilution of cellular proliferation dye (Fig. 6A). A 1:1 mixture of palmitic and oleic acids more dramatically rescued ACC1ΔT CD8$^+$ T cell expansion than addition of each FA alone (data not shown).

We further characterized the contribution of exogenous FAs in ACC1ΔT CD8$^+$ T cells during activation before cell division. Analysis of forward scatter (FSC; an assessment of cell size) and side scatter (SSC; an assessment of granularity) of cells at 24 h postactivation showed that although exogenous FA did not affect SSC of WT CD8$^+$ T cells, it significantly increased the FSC and SSC of ACC1ΔT CD8$^+$ T cells (Fig. 6B), suggesting that FA synthesis is an essential prerequisite for blastogenesis. Despite both cellular atrophy and defective CD44 upregulation (Supplemental Fig. 2) observed in ACC1ΔT CD8$^+$ T cells, they appeared to be capable of processing mitogenic signals normally at some level, as evidenced by upregulation of CD69, CD25, CD71, and CD98. Together, these data suggest that de novo lipogenesis is a limiting factor for proper cell growth and sustained proliferation of CD8$^+$ T cells upon activation.

Discussion

The importance of lipogenic enzymes in regulating the proliferative capacity and survival of cancer cells has previously been described (25, 34, 35). However, the role of ACC1 in the survival and proliferation of primary T cells has remained poorly understood. In this study, we demonstrated the importance of de novo lipogenesis to optimal T cell function under both homeostatic and inflammatory conditions. We have found that FA synthesis throughout the life span of T cells is required for regulating viability and proliferation.

ACC1 in quiescent T cells

Ag-inexperienced naive T cells circulate through the blood and peripheral lymphoid organs, are small in size, and have low metabolic activity. Their survival depends on TCR interactions with self-peptide:MHC and the availability of IL-7, and is shaped by growth factors and nutrients related to metabolic fitness (36). We were interested in determining whether these processes are actively influenced by de novo FA synthesis. Deletion of ACC1 in the T cell compartment resulted in diminished T lymphocyte cellularity in naive mice (Fig. 2A–D) and shorter life span of ACC1ΔT

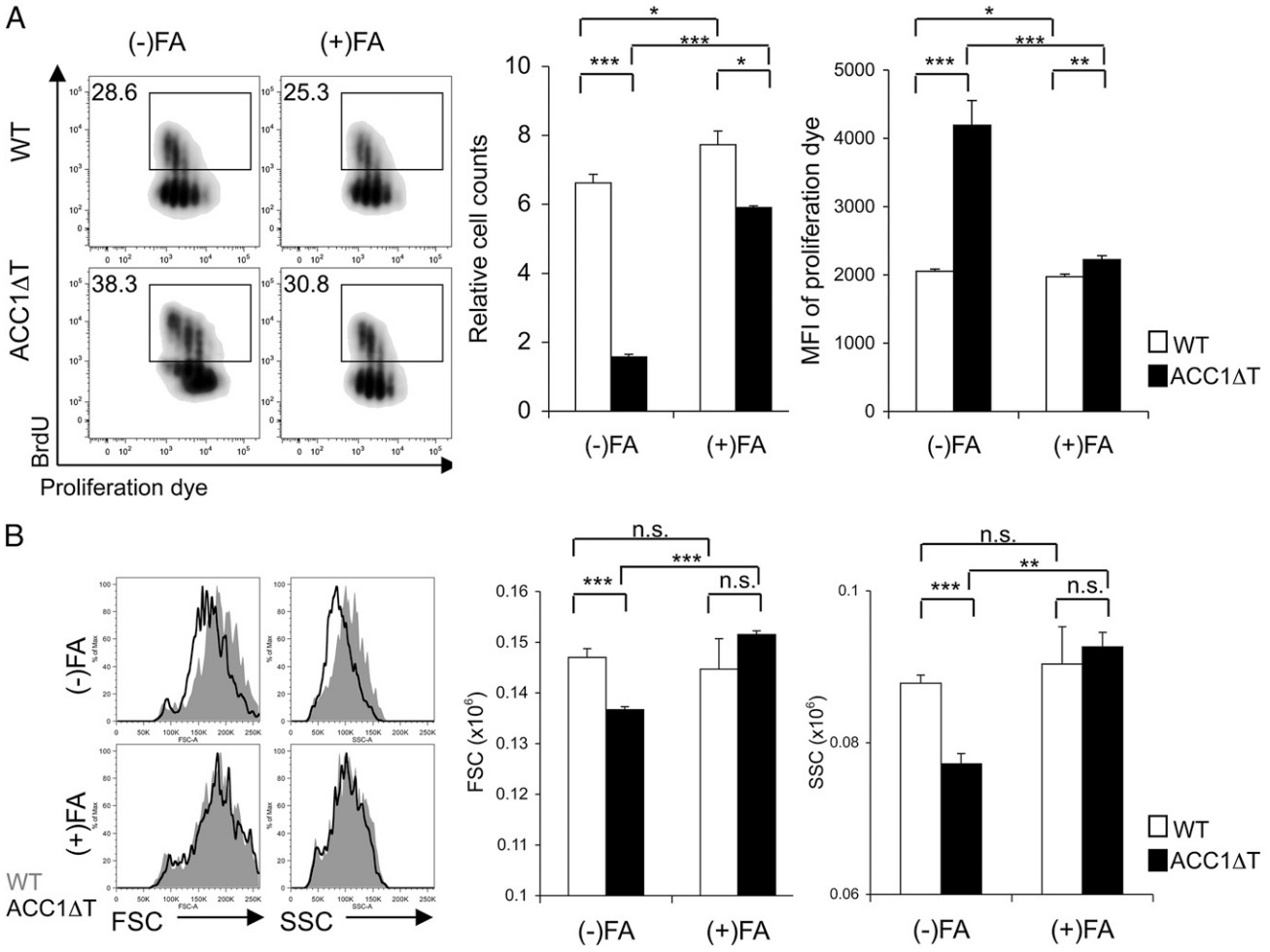

FIGURE 6. Exogenous FA rescue survival and proliferation of ACC1ΔT CD8$^+$ T cells under mitogenic conditions. (**A**) FACS-sorted naive WT or ACC1ΔT CD8$^+$ T cells were labeled with proliferation dye and cultured with anti-CD3 and anti-CD28 Abs alone or with 25 μM FA supplement for 60 h in the presence of IL-2 (100 U/ml), and were then pulsed with BrdU for 1 h, harvested, and stained for BrdU incorporation. Dot plots show dilution of proliferation dye and BrdU incorporating cells. Numbers in dot plots indicate percentage of BrdU incorporating cells in each group. Shown here is one representative result out of three independent experiments. (**B**) Analysis of cell enlargement by FACS 24 h postactivation with anti-CD3 and anti-CD28 Abs alone or FA supplement. Cells were gated on live events (TO-PRO-3^{neg}). Histograms show FSC and SSC of live WT (grayed area) and ACC1ΔT CD8$^+$ (black line) T cells. Graphs summarize changes in FSC and SSC on FA supplement. Shown is one representative result of three independent experiments. $*p < 0.05$, $**p < 0.001$, $***p < 0.0001$.

CD8$^+$ T cells transferred into naive WT mice (Fig. 3A), whereas ACC1ΔT CD8$^+$ T cell homing/distribution, cell size, surface marker expressions (Fig. 2A–C), and responsiveness to IL-7 in vitro (Fig. 5B) were not affected. T cells undergo proliferation in the periphery in response to self-peptide:MHC in addition to IL-7 and/or IL-15 under noninflammatory conditions. Cell divisions are observed in both naive and activated/memory compartments of T cells in WT mice (37), although the turnover of naive cells is much slower. Reduced proportions of CD44^{high} ACC1ΔT cells along with a defect in lymphopenia-induced proliferation suggest a possible perturbation of homeostatic proliferation of T cells by a loss of ACC1 function. However, in our experimental models, homeostatic proliferation reflects both proliferation and survival of naive CD8$^+$ T cells, rather than identifying a single mechanism. Recently, Kidani et al. (29) showed that deletion of *scap*, a SREBP-cleavage–activating protein that regulates the processing and transcriptional activity of SREBP1 and SREBP2 in CD8$^+$ T cells, influences neither cellularity nor homeostatic proliferation, although resulting in significant reduction in cellular quantity of cholesterol and long-chain FAs. As one of the transcription factors upstream of ACC1 expression, SREBP broadly impacts FA and cholesterol biosynthesis through interactions with other pathways, such as LXRα, Akt, and mTOR, to integrate metabolic signals (38–40). However, because ACC1 is an upstream molecular regulator of long-chain FA synthesis, its deletion has a more specific effect on lipid metabolism. Therefore, the different phenotypes observed in scapΔT and ACC1ΔT mice might be results of subtle differences in lipid composition and the microarchitecture of membrane, or a compensatory alteration in signaling pathways involved in T cell homeostasis.

ACC1 in proliferating T cells

Proliferating T cells need considerable energy and metabolites for biosynthetic pathways to support increased requirements for structural membrane and signaling molecules during cell-cycle progression (2, 4). For example, proliferating T cells use highly active FA synthesis pathways and FA incorporation into complex lipids, and imbalances in synthesis or turnover of lipids affect cell growth and viability (4). Accordingly, we observed that Ag-specific ACC1ΔT CD8$^+$ T cell accumulation is impaired during responses to bacterial infection despite normal progression to S phase of the cell cycle. This result shows synthesis of FAs per se is a limiting

factor in survival of proliferating $CD8^+$ T cells (Figs. 4, 5). Newly synthesized FAs in the form of phospholipids tend to partition into detergent-resistant membrane microdomains or rafts (41). The regulatory role of specific lipid clusters in the membrane has been implicated in a number of processes, including signal transduction, cell–cell interactions, and cell division. Previously, Emoto et al. (42) showed that localized production of the phospholipid, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), is required for proper completion of cytokinesis, possibly because formation of a unique lipid domain in the cleavage furrow membrane is necessary to coordinate contractile rearrangement. Therefore, it seems reasonable to speculate that progression toward cytokinesis may be blocked in ACC1ΔT $CD8^+$ T cells because of lack of biomass molecules and subsequent membrane remodeling. Also, our in vitro analysis showed a loss of ACC1 rendered $CD8^+$ T cells incapable of blasting, and subsequently resulted in lower proliferative capacity and viability (Fig. 5D), suggesting defects in earlier activation pathways, which could be rescued by FA provision.

As shown with defective ACC1ΔT CD44 expression, some signaling pathways reflective of cellular activation and proliferation remained defective in ACC1ΔT $CD8^+$ T cells even with provision of supplemental FA. Further studies of the role of de novo FA synthesis in the dynamics of membrane lipid clustering and remodeling during early blastogenesis will help us further elucidate the regulatory role of lipids in initiating T cell responses.

In addition, our data show that ACC1ΔT $CD8^+$ T cells are not defective in expressing T-bet, IFN-γ, and granzyme B upon Lm infection (Fig. 4B, 4C), suggesting there are de novo lipogenesis-independent pathways involved in acquiring effector T cell functions that are distinct from regulation of blastogenesis and viability. Metabolic requirements for acquiring or maintaining T cell function are of interest in understanding the mechanisms regulating immune responses, and some studies have implicated metabolic reprogramming in this context (29, 43, 44). Our data suggest that de novo lipogenesis per se is not a prerequisite of effector $CD8^+$ T cell differentiation, but rather supports accumulation of cells already committed. Further studies on lipid metabolism in the context of other metabolic processes involved in anabolic and catabolic metabolism throughout T cell life span will help us delineate intertwined mechanisms in $CD8^+$ T cell metabolism and differentiation.

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Disclosures

The authors have no financial conflicts of interest.

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