GSBS Student Publications | Graduate School of Biomedical Sciences

Regulation of tissue-dependent differences in CD8+ T cell apoptosis during viral infection

Varun N. Kapoor
University of Massachusetts Medical School

Et al.

Let us know how access to this document benefits you.

Repository Citation

Regulation of Tissue-Dependent Differences in CD8$^+$ T Cell Apoptosis during Viral Infection

Varun N. Kapoor, Hyun Mu Shin, Ok Hyun Cho, Leslie J. Berg, Joonsoo Kang, Raymond M. Welsh

Department of Pathology, Program in Immunology and Virology, University of Massachusetts Medical School, Worcester, Massachusetts, USA

ABSTRACT

Virus-specific CD8$^+$ T cells in the lymphoid organs contract at the resolution of virus infections by apoptosis or by dissemination into peripheral tissues, and those residing in nonlymphoid organs, including the peritoneal cavity and fat pads, are more resistant to apoptosis than those in the spleen and lymph nodes. This stability of memory T cells in the nonlymphoid tissues may enhance protection to secondary challenges. Here, we show that lymphocytic choriomeningitis virus (LCMV)-specific CD8$^+$ T cells in nonlymphoid tissues were enriched for memory precursors (expressing high levels of interleukin-7 receptor and low levels of killer cell lectin-like receptor G1 [IL-7R^{hi} KLRG1^{lo}]) and had higher expression of CD27, CXCR3, and T cell factor-1 (TCF-1), each a marker that is individually correlated with decreased apoptosis. CD8$^+$ T cells in the peritoneal cavity of TCF-1-deficient mice had decreased survival, suggesting a role for TCF-1 in promoting survival in the nonlymphoid tissues. CXCR3$^+$ CD8$^+$ T cells resisted apoptosis and accumulated in the lymph nodes of mice treated with FTY720, which blocks the export of lymph node cells into peripheral tissue. The peritoneal exudate cells (PEC) expressed increased amounts of CXCR3 ligands, CXCL9 and CXCL10, which may normally recruit these nonapoptotic cells from the lymph nodes. In addition, adoptive transfer of splenic CD8$^+$ T cells into PEC or spleen environments showed that the peritoneal environment promoted survival of CD8$^+$ T cells. Thus, intrinsic stability of T cells which are present in the nonlymphoid tissues along with preferential migration of apoptosis-resistant CD8$^+$ T cells into peripheral sites and the availability of tissue-specific factors that enhance memory cell survival may collectively account for the tissue-dependent apoptotic differences.

IMPORTANCE

Most infections are initiated at nonlymphoid tissue sites, and the presence of memory T cells in nonlymphoid tissues is critical for protective immunity in various viral infection models. Virus-specific CD8$^+$ T cells in the nonlymphoid tissues are more resistant to apoptosis than those in lymphoid organs during the resolution and memory phase of the immune response to acute LCMV infection. Here, we investigated the mechanisms promoting stability of T cells in the nonlymphoid tissues. This increased resistance to apoptosis of virus-specific CD8$^+$ T cells in nonlymphoid tissues was due to several factors. Nonlymphoid tissues were enriched in memory phenotype CD8$^+$ T cells, which were intrinsically resistant to apoptosis irrespective of the tissue environment. Furthermore, apoptosis-resistant CD8$^+$ T cells preferentially migrated into the nonlymphoid tissues, where the availability of tissue-specific factors may enhance memory cell survival. Our findings are relevant for the generation of long-lasting vaccines providing protection at peripheral infection sites.

Programmed cell death, mostly in the form of apoptosis, is critical for regulating viral pathogenesis and the host immune response during viral infections. Several viruses can first modulate the apoptotic machinery to promote viral replication within cells by inhibiting apoptosis and then promote dissemination of virus by triggering apoptosis (1). The immune response to virus infections is also regulated by apoptotic events. Interferon (IFN)-driven apoptosis of memory T cells during early stages of lymphocytic choriomeningitis virus (LCMV) infection opens up space in the immune system and allows for generation of a diverse T cell response (2, 3), whereas apoptosis of virus-specific effector T cells after the peak of the immune response is essential for curtailing the response and restoring immune homeostasis upon clearance of the viral antigens (4, 5). At this later time, a small population of virus-specific T cells escapes apoptosis and forms memory cells that provide long-lived immunity. Our laboratory has previously shown that during this transition from the acute to the memory phase of the immune response, LCMV-specific CD8$^+$ T cells in the peripheral nonlymphoid tissues, including peritoneal cavity, fat pads, and lungs, are more resistant to apoptosis than those in the spleen and lymph nodes, and these differences persist for sev-

eral months thereafter (6). Infections by a number of viruses are initiated at nonlymphoid tissue sites, and tissue-resident memory T cells have been shown to be important in mediating protection against secondary virus challenges (7–10). Therefore, this resistance to apoptosis may provide a mechanism by which protective memory CD8$^+$ T cells could persist in nonlymphoid organs.

CD8$^+$ T cells generated during the course of an immune response are heterogeneous and express phenotypic markers, such as interleukin-7 receptor (IL-7R), killer cell lectin-like receptor G1 (KLRG1), CD27, and CXCR3 that characterize their activation state and portend their conversion into memory cells. CD8$^+$ T cells that express high levels of IL-7R (IL-7R^{hi}) and low levels of

KLRG1 (KLRG1^{lo}) are defined as memory precursor effector cells (MPEC), are capable of self-renewal, and can provide long-term protection against reinfection (11–13). CD27 belongs to the tumor necrosis factor receptor (TNFR) family and has also been used as a marker to identify memory precursor CD8$^+$ T cells (12). Expression of CD27 on virus-specific CD8$^+$ T cells promotes survival, induces IL-7R expression, and protects against Fas-dependent apoptosis (14–17). CD27-CD70 interactions have been shown to induce autocrine IL-2 production in CD8$^+$ T cells, thereby promoting their survival (18). Activated T cells may also express CXCR3, a chemokine receptor required for T cell chemotaxis to the site of antigen (19, 20). Moreover, individual expression of IL-7R, CD27, and CXCR3 has been used to identify memory CD8$^+$ T cell populations with an efficient recall response (21). Whether expression of these phenotypic markers correlates with tissue-dependent differences in apoptosis during resolution of the immune response has not been clarified.

Differences in CD8$^+$ T cell transcriptional regulation may contribute to differential survival of CD8$^+$ T cells in tissue environments, but few transcription factors regulating the fate of CD8$^+$ T cell effector-memory generation have been identified. T cell factor-1 (TCF-1; encoded by *Tcf7*) is a transcription factor acting downstream in the Wnt signaling pathway (22). Apart from its role in thymocyte differentiation (23), TCF-1 was shown to be important for promoting generation and maintenance of memory CD8$^+$ T cells (24, 25) as TCF-1-deficient CD8$^+$ T cells had impaired expansion after a secondary antigen challenge (24–26). TCF-1 deficiency was associated with decreased expression of another transcription factor, eomesodermin (Eomes), and the loss of proliferation driven by IL-15, whose receptor is regulated by the transcription factor Eomes (25). However, the contribution of TCF-1 in the resistance of CD8$^+$ T cells to apoptosis in different tissue environments during resolution of the immune response has not been tested.

T cells are initially activated in the spleen and lymph nodes, after which they migrate to nonlymphoid tissues to eliminate foreign antigens (27). Activated T cells express chemokine receptors that guide their migration to sites of antigen. CXCR3 is a chemokine receptor expressed on activated Th1 CD4$^+$ T cells and on effector CD8$^+$ T cells (19), and it promotes migration of activated CD8$^+$ T cells into nonlymphoid tissue infection sites under the influence of its chemokine ligands CXCL9 and CXCL10 (28, 29).

Our present study examines the mechanisms by which CD8$^+$ T cells preferentially survive in the nonlymphoid tissues. We show here that nonlymphoid tissues were enriched in memory phenotype CD8$^+$ T cells intrinsically resistant to apoptosis, irrespective of the tissue environment. Furthermore, we show that apoptosis-resistant CD8$^+$ T cells may preferentially migrate into the nonlymphoid tissues where the availability of tissue-specific factors may enhance memory cell survival.

MATERIALS AND METHODS

Mice. C57BL/6J male mice were purchased from the Jackson Laboratory (Bar Harbor, ME). P14 transgenic mice were bred onto CD90.1 (Thy1.1) and CD45.1 (Ly5.1) C57BL/6 backgrounds to distinguish the transgenic T cells from wild-type (WT) cells in the C57BL/6 (CD90.2$^+$ [Thy1.2] CD45.2$^+$ [Ly5.2]) mice. *Tcf7*$^{-/-}$ mice on the C57BL/6 background have been described previously (30) and were bred at the University of Massachusetts Medical School (UMMS). *Tcf7*$^{+/+}$ littermate mice were used as WT controls. The LCMV glycoprotein GP61-80-specific CD4 transgenic SMARTA CD45.1$^+$ mice (31) were bred at UMMS. Mice were bred and housed under specific-pathogen-free conditions at the UMMS in accordance with the guidelines of the Institutional Animal Care and Use Committee of UMMS (IACUC).

Preparation of leukocytes. Mice were sacrificed by cervical dislocation. Peritoneal exudate cells (PEC) were collected by lavage with 10 ml of cold RPMI 1640 medium (Gibco). Spleen, lymph nodes, and fat pads were ground between glass microscope slides to prepare single-cell suspensions. To remove contaminating erythrocytes, leukocyte preparations were treated with 0.84% ammonium chloride (NH$_4$Cl).

Virus. LCMV, strain Armstrong, was propagated in BHK cells as previously described (32). Mice were inoculated intraperitoneally (i.p.) with 0.1 ml containing 5×10^4 PFU in phosphate-buffered saline (PBS).

Antibodies, flow cytometry, and intracellular cytokine staining. Leukocytes were stained for CD8α (53-6.7; BD Pharmingen), CD8β (YTS156.7.7; Biolegend), CD4 (RM4-5; BD Pharmingen), CD44 (IM7; eBiosciences), Thy1.1/CD90.1 (HIS51; eBiosciences), Ly5.1/CD45.1 (A20; Biolegend), KLRG1 (2F1; eBiosciences), CD127 (A7R34; eBiosciences), CD27 (LG.310; BD Pharmingen), CXCR3 (CXCR3-173; Biolegend), CXCL9 (MIG-2F5.5; eBiosciences), TCF-1 (C63D9; Cell Signaling), Eomes (Dan11mag; eBiosciences), Bcl-2 (3F11; BD Pharmingen), CD122 (TM.BETA-1; BD Pharmingen), and CD70 (FR70; Biolegend). For TCF-1 intracellular staining Alexa Fluor 647-conjugated goat anti-rabbit (Invitrogen) secondary antibody was used. To stain for intracellular antigens a FOXP3/transcription factor staining buffer kit (eBiosciences) was used. For intracellular cytokine secretion assays, leukocytes were incubated for 5 h *ex vivo* with a 1 μM concentration of an LCMV-specific nucleoprotein peptide comprised of residues 396 to 404 (NP$_{396-404}$), GP peptide comprised of residues 33 to 41 (GP$_{33-41}$), or GP$_{61-80}$ peptide in the presence of Golgi Plug (BD Biosciences). Cells were then permeabilized using a Cytofix/Cytoperm kit (BD Biosciences), followed by intracellular staining for IFN-γ (XMG1.2; eBiosciences), IL-2 (JES65H4; BD Pharmingen), and TNF (MP6-XT22; Biolegend). Fluorescence-activated cell sorting (FACS) plots were gated on 7-amino-actinomycin D-negative (7-AAD$^-$) cells versus forward scatter area (FSC-A; live), on forward scatter height (FSC-H) versus FSC-A (singlets), on FSC-A versus side scatter area (SSC-A) (lymphocytes), and then on the population of interest. Samples were analyzed on an LSRII flow cytometer (Becton, Dickinson), and data were further analyzed using FlowJo software (Tree Star).

Annexin V, TUNEL, and caspase-3 assay. Leukocytes were stained for Annexin V (eBiosciences), and samples were analyzed live according to the manufacturer's protocol. 7-AAD (BD Pharmingen) was used to gate out dead and late-apoptotic populations. For terminal deoxynucleotidyl-transferase-mediated dUTP-biotin nick end labeling (TUNEL) and active caspase-3 staining, leukocytes were incubated at 37°C directly *ex vivo* for 5 h in culture medium. The TUNEL assay (Apo-Direct Kit; BD Pharmingen) and active caspase-3 (C92-605; BD Pharmingen) staining were carried out according to the manufacturer's protocol. For active caspase-3 and TCF-1 costaining, after samples were stained with rabbit TCF-1 primary and goat anti-rabbit secondary antibody, they were incubated with unlabeled rabbit IgG (Southern Biotech) to saturate the goat anti-rabbit secondary antibody. Cells were then incubated with rabbit active caspase-3 antibody. For inhibiting caspase-dependent apoptosis, leukocytes were cultured for 5 h *ex vivo* in the presence of 100 μM Z-VAD-FMK (carboxybenzyl-Val-Ala-Asp-fluoromethylketone; Enzo Life Sciences).

Adoptive transfer studies and cell labeling. Unless otherwise noted, virus-specific CD8$^+$ T cell responses were tracked by transferring 2×10^5 P14 splenocytes (CD90.1 or CD45.1) into congenic C57BL/6 hosts (CD90.2$^+$ CD45.2$^+$). Splenocytes from day 7 LCMV-infected mice were labeled with 2 μM carboxyfluorescein succinimidyl ester (CFSE) or 0.5 μM DDAO [7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one)], washed, and adoptively transferred into an infection-matched C57BL/6 host as shown in Fig. 6A.

Apyrase and FTY720 treatment. Day 7 LCMV-infected mice were i.p. injected with 5 U of apyrase (New England BioLabs) or succinate buffer
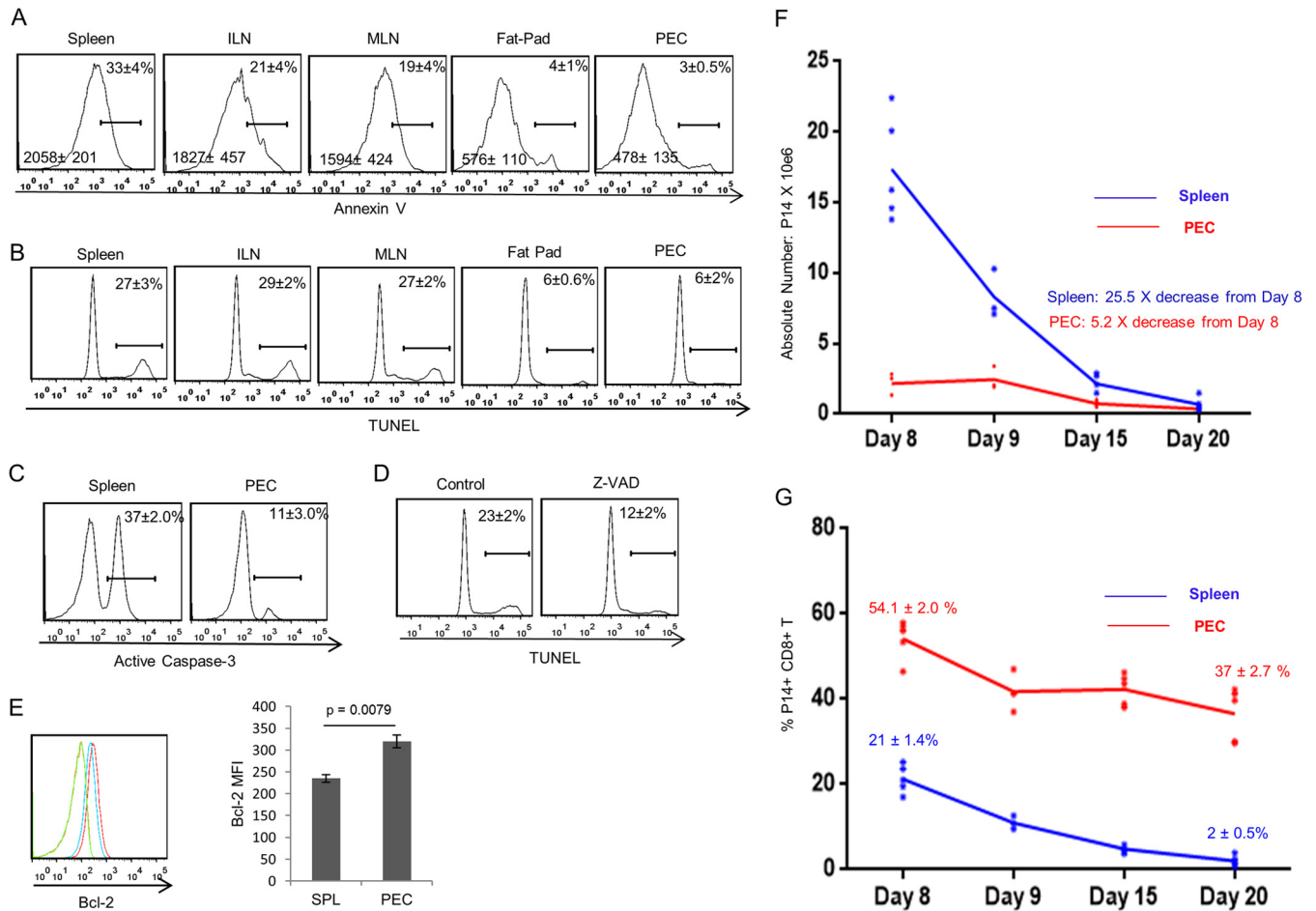
FIG 1 Virus-specific CD8$^+$ T cells in the nonlymphoid tissues are resistant to apoptosis. (A) Annexin V reactivity of viable 7-AAD$^-$ P14$^+$ CD8$^+$ T cells in lymphoid (spleen, inguinal lymph nodes [ILN], mesenteric lymph nodes [MLN]) and nonlymphoid (PEC and fat pad) tissues at day 9 post-LCMV infection. Five mice were used for the experiment, and the experiment was done three times. Mean fluorescence intensity (MFI) for annexin V reactivity of P14$^+$ CD8$^+$ T cells is depicted in each histogram. Annexin V reactivity of spleen, inguinal lymph nodes, and mesenteric lymph nodes was significantly different from that in the PEC or fat pads ($P = 0.0001$). (B) Lymphocytes from lymphoid (spleen, inguinal lymph nodes, and mesenteric lymph nodes) and nonlymphoid (PEC and fat pad) tissues were isolated at day 9 post-LCMV infection and cultured *ex vivo* for 5 h postisolation. TUNEL reactivity of P14$^+$ CD8$^+$ T cells is represented in the histograms. Five mice were used for the experiment, and the experiment was done three times. TUNEL reactivity of spleen, inguinal lymph nodes, and mesenteric lymph nodes was significantly different from that in the PEC or fat pads ($P = 0.0001$). (C) Lymphocytes from spleen and PEC were isolated at day 9 post-LCMV infection and cultured *ex vivo* for 5 h postisolation. Values in histograms are percentages ± SD of P14$^+$ CD8$^+$ T cells reactive with active caspase-3. The experiment was done three times with 3 mice in each experiment. (D) Splenocytes isolated at day 9 postinfection were cultured for 5 h *ex vivo* in presence or absence of Z-VAD FMK. Values in histograms are percentages ± SD of P14$^+$ CD8$^+$ T cells reactive in the TUNEL assay. The experiment was done two times with 3 mice in each experiment. (E) Overlay of Bcl-2 expression (left) in P14$^+$ CD8$^+$ T cells in the PEC (red), spleen (blue) or isotype control (green, PEC isotype; orange, spleen isotype) at day 9 post-LCMV infection. MFIs of Bcl-2 expression in P14$^+$ CD8$^+$ T cells in PEC and spleen (SPL) are shown in the histogram (right). The experiment was done two times with 3 mice in each experiment. (F and G) Absolute number and percentage of P14$^+$ CD8$^+$ T cells at day 8, 9, 15, and 20 post-LCMV infection. Data are from 5 mice for days 8, 15, and 20 and from 3 mice for day 9, and the experiment was done two times. Percentages and error bars are represented ± SD.

control and were sacrificed after 6 h. FTY720 (Cayman Chemical Company) at 1 mg/kg was given intravenously (i.v.) (days 5 and 6) and i.p. (day 7) post-LCMV infection as shown in Fig. 5A.

RT-PCR. Total RNA was isolated using an RNeasy minikit (Qiagen), converted to cDNA (Quantitect reverse transcription kit; Qiagen), and analyzed by real-time quantitative PCR amplification on a Bio-Rad iCycler by using a Quanti-Fast RT-PCR SYBR green kit (Qiagen), according to the manufacturer's protocol. Primers for *Cxcl9*, *Cxcl10*, and β-*actin* were purchased from Qiagen.

Statistical analysis. Data significance (*P* values) was calculated using an unpaired Student's *t* test. All error bars in the manuscript represent standard deviations (SD) from the means.

RESULTS

CD8$^+$ T cells in the nonlymphoid tissues are resistant to apoptosis.
LCMV-specific CD8$^+$ T cells in the nonlymphoid tissues (liver [otherwise considered a graveyard for apoptotic T cells], lung, fat pad, and peritoneal cavity) were previously shown to react less with the preapoptotic marker annexin V (6, 33, 34) than those in spleen and lymphoid organs, and splenocytes that reacted with annexin V failed to develop into memory cells (35). However, because annexin V can also bind at some level to activated and proliferating T cells (36, 37), we reassessed bona fide end-stage apoptotic cells within cells from representative organs using the

TUNEL assay to detect fragmented DNA in addition to the annexin V assay to detect membrane changes (Fig. 1A and B). Splenocytes from P14 transgenic mice (harboring CD8$^+$ T cells specific for the LCMV GP$_{33-41}$ epitope) were adoptively transferred into a congenic C57BL/6 host, followed by infection with LCMV strain Armstrong. At day 9 post-LCMV infection, lymphoid (spleen, inguinal lymph nodes, and mesenteric lymph nodes) and nonlymphoid (fat pad and PEC) tissues were harvested. Detection of end-stage apoptotic cells directly *ex vivo* is difficult as most of them are efficiently cleared *in vivo* by phagocytes (38). We therefore cultured cells directly *ex vivo* for 5 h to detect TUNEL-positive (TUNEL$^+$) end-stage apoptotic cells. In accordance with our previous observations, we found that at day 9 post-LCMV infection, virus-specific CD8$^+$ T cells in the nonlymphoid tissues, including the peritoneal cavity and fat pad, were more resistant to apoptosis and less reactive with TUNEL than those in the lymphoid organs, including spleen, inguinal lymph nodes, and mesenteric lymph nodes (Fig. 1B).

These tissue-dependent apoptotic differences persisted up to at least 4 months post-LCMV infection, as detected by annexin V assays (data not shown).

Levels of activated caspase-3 increase in apoptotic T cells (39), and T cells from caspase-3-deficient mice have reduced susceptibility to activation-induced cell death (AICD) driven by CD3 cross-linking and Fas ligands (40). To test whether increased apoptosis of virus-specific CD8$^+$ T cells in lymphoid organs correlated with increased caspase-3 activation, virus-specific CD8$^+$ T cells from day 9 LCMV-infected PEC or spleen were cultured for 5 h directly *ex vivo* and stained for active caspase-3. Consistent with the increased apoptosis, virus-specific CD8$^+$ T cells in spleen also had higher levels of active caspase-3 than PEC (Fig. 1C). To confirm that the CD8$^+$ T cell apoptosis was caspase dependent, day 9 splenocytes from LCMV-infected mice were incubated in the presence of the pan-caspase inhibitor ZVAD-FMK. The virus-specific CD8$^+$ T cells from the spleen were less TUNEL reactive when incubated in the presence of ZVAD-FMK, suggesting that the CD8$^+$ T cell apoptosis required caspases (Fig. 1D).

Cellular apoptosis results from the interplay between pro- and antiapoptotic factors, and higher levels of the antiapoptotic Bcl-2 in effector CD8$^+$ T cells have been shown to be important in counteracting the effects of proapoptotic Bim, thereby allowing survival of cells during contraction of the immune response (41). Activated virus-specific CD8$^+$ T cells that become memory cells also express higher levels of Bcl-2 (42). Virus-specific CD8$^+$ T cells in the PEC expressed higher levels of Bcl-2 than those from the spleen during the resolution of the immune response (Fig. 1E). These results collectively show by several criteria that virus-specific CD8$^+$ T cells in the peripheral tissues are more resistant to apoptosis than those in the lymphoid organs.

We hypothesized that the decreased apoptosis in the peritoneum would lead to increased survival and stability of virus-specific CD8$^+$ T cells during contraction of the immune response. To test this hypothesis, the kinetics of the virus-specific T cell response were analyzed in the spleen and PEC at days 8, 9, 15, and 20 post-LCMV infection. Consistent with their resistance to apoptosis, by day 20 virus-specific CD8$^+$ T cells in the peritoneum had reduced contraction (5.2-fold decrease from the peak at day 8) compared to T cell populations in the spleen (25.5-fold decrease from the peak at day 8) (Fig. 1F). Furthermore, the peritoneal cavity maintained a higher proportion of virus-specific CD8$^+$ T

cells than the spleen through day 20 post-LCMV infection (Fig. 1G). These results show that decreased T cell apoptosis leads to increased T cell stability in the peritoneal environment, allowing the generation of memory T cell populations which may be able to productively respond to a secondary challenge.

Nonlymphoid tissues are enriched for apoptosis-resistant memory phenotype CD8$^+$ T cells. Antigenic characterizations were employed to determine why CD8$^+$ T cells in the peripheral tissues were more resistant to apoptosis. Higher proportions of virus-specific CD8$^+$ T cells with an MPEC phenotype (IL-7R^{hi} KLRG1^{lo}) were present in the nonlymphoid tissues than in lymphoid organs at day 8 post-LCMV infection (Fig. 2A), and this pattern still persisted at day 40 post-LCMV infection (day 40, 62% IL-7R^{hi} KLRG1^{lo} in PEC versus 37% IL-7R^{hi} KLRG1^{lo} in spleen [data representative of pooled samples from five mice]). Cells expressing large amounts of IL-7-R have been shown to express more Bcl-2 and to be relatively resistant to apoptosis (11, 35). Stimulation of CD27 on CD8$^+$ T cells *in vitro* has been shown to promote survival (16, 17), and *in vivo* blockade of CD27 promotes apoptosis of virus-specific CD8$^+$ T cells (15). Correspondingly, higher proportions of virus-specific CD8$^+$ T cells in the PEC expressed CD27 than in the spleen (Fig. 2B). To confirm that CD27 expression on CD8$^+$ T cells promotes survival, we compared apoptotic profiles of CD27^{hi} and CD27^{lo} CD8α^+ T cells. Levels of TUNEL and active caspase-3 reactivity revealed that at day 8 after LCMV infection virus-specific CD27^{hi} CD8$^+$ T cells were less apoptotic than the CD27^{lo} populations in both the spleen and PEC (Fig. 2B). CXCR3 is another marker preferentially expressed on memory phenotype CD8$^+$ T cells. At day 8 post-LCMV infection, a higher proportion of CXCR3-expressing virus-specific CD8$^+$ T cells were present in PEC than in the spleen (Fig. 2C). Levels of TUNEL and active caspase-3 reactivity revealed that virus-specific CXCR3^{hi} CD8$^+$ T cells were less apoptotic than the CXCR3^{lo} populations in both spleen and PEC (Fig. 2C). Therefore, by several criteria, memory T cells resistant to apoptosis were present at a higher frequency in PEC than in spleen. Moreover, during resolution of the immune response, CD27 and CXCR3 expression on CD8$^+$ T cells correlated with decreased apoptosis.

The ability of virus-specific CD8$^+$ T cells to produce multiple cytokines, such as IFN-γ, TNF, and IL-2, simultaneously during viral infection is a primary indicator of their functional capacity, and IL-2-producing CD8$^+$ T cells are more likely to become memory cells than those that do not make IL-2 (43). We thus examined the cytokine repertoire of virus-specific CD8$^+$ T cells isolated from PEC and spleen. On stimulation with LCMV-specific GP$_{33-41}$ and NP$_{396-404}$ peptide epitopes, virus-specific CD8$^+$ T cells from PEC were enriched in IFN-γ, TNF, and IL-2 triple producers compared to the spleen (Fig. 2D). Moreover, CD27- and CXCR3-expressing virus-specific CD8$^+$ T cells were enriched in IL-2 producers, suggesting that expression of these markers represented CD8$^+$ T cells that could become memory cells (data not shown). Overall, these results indicated that the nonlymphoid tissues are enriched in memory precursor T cells that are intrinsically resistant to apoptosis.

TCF-1 promotes survival of CD8$^+$ T cells in the peritoneum during resolution of the immune response. Transcription factors in addition to cell surface antigens were assessed as correlates of resistance to apoptosis. TCF-1 is a transcription factor which promotes generation and maintenance of functional memory CD8$^+$ T cells (24, 25, 26). Since the nonlymphoid tissue environment
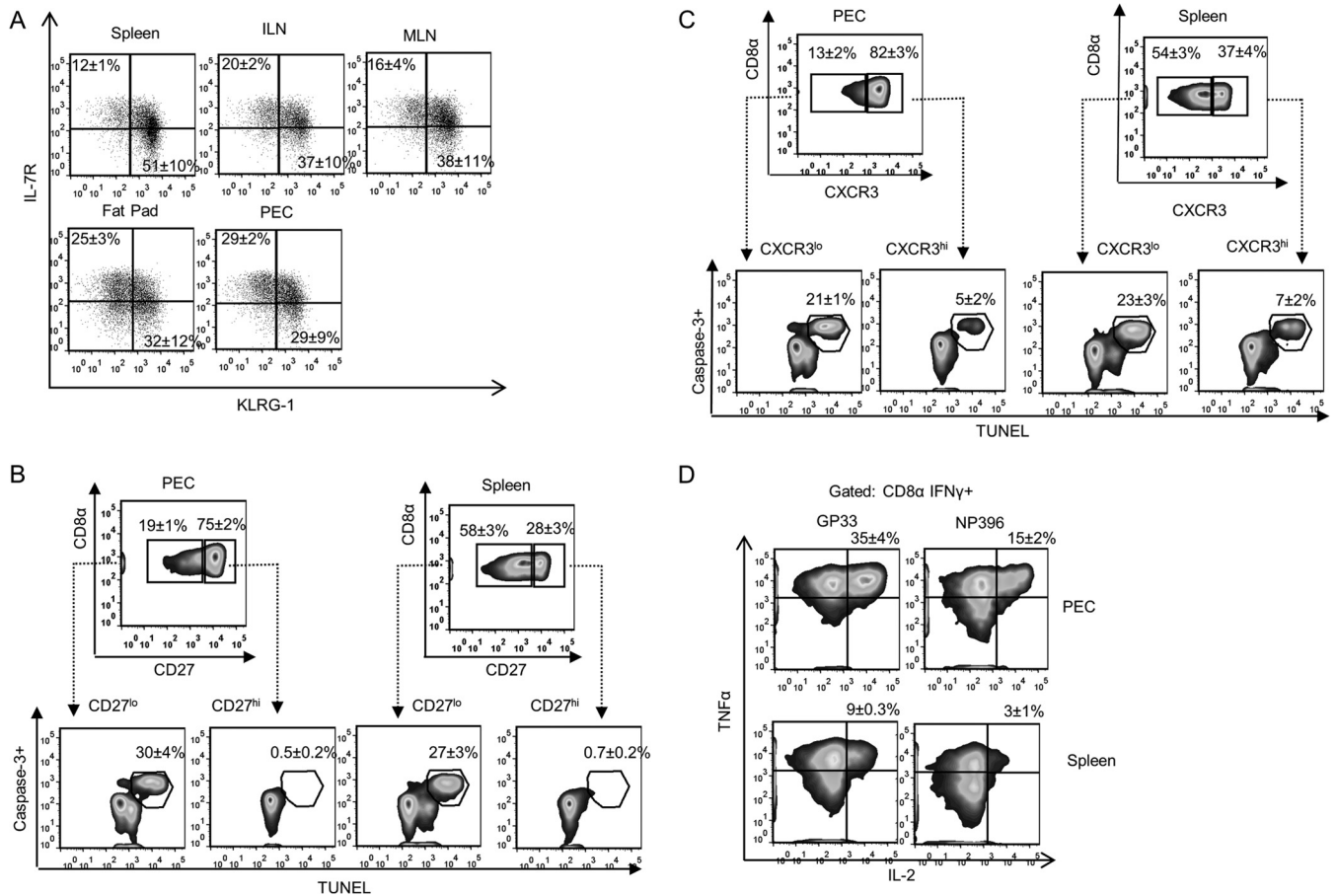
FIG 2 Higher percentage of MPEC and CD27- and CXCR3-expressing cells in the PEC and their resistance to apoptosis. (A) P14$^+$ CD8$^+$ T cells in lymphoid (spleen, inguinal lymph nodes [ILN], mesenteric lymph nodes [MLN]) and nonlymphoid (PEC and fat pad) tissues at day 8 post-LCMV infection were stained for IL-7R and KLRG1. Leukocytes from 5 mice were pooled for the experiment, and the data are a compilation of the results from three independent experiments. The percentages of memory precursor effector cells (MPEC, top left) or short-lived effector cells (bottom right) are depicted. (B and C) Percentages of CD27-expressing (B, top) or CXCR3-expressing (C, top) P14$^+$ CD8$^+$ T cells in PEC or spleen at day 8 post-LCMV infection. At day 8 post-LCMV infection lymphocytes from PEC and spleen were cultured for 5 h directly *ex vivo*, followed by TUNEL and active caspase-3 staining. TUNEL and active caspase-3 reactivity of CD27^{hi} or CD27^{lo} (B, bottom) and CXCR3^{hi} or CXCR3^{lo} (C, bottom) P14$^+$ CD8$^+$ T cells in PEC or spleen are represented. Graphs are representative of three experiments with 3 mice in each experiment. (D) Day 8 LCMV-infected lymphocytes from PEC and spleen were cultured for 5 h in the presence of LCMV-specific GP33 and NP396 peptides. Plots are gated on virus-specific CD8$^+$ IFN-γ^+ T cells. Percentages of TNF$^+$ IL-2$^+$ virus-specific CD8$^+$ T cells are depicted at the top right. Graphs are representative of three experiments with 3 mice in each experiment. Percentages and error bars are represented ± SD.

was enriched in memory phenotype CD8$^+$ T cells, we determined TCF-1 expression in the T cells from PEC as representative of nonlymphoid tissues and from spleen as a representative of lymphoid organs. At day 9 post-LCMV infection, a higher proportion of virus-specific and activated CD44^{hi} CD8$^+$ T cells expressed TCF-1 in the PEC than in the spleen (Fig. 3A). The expression level of TCF-1 was also higher in virus-specific and activated CD44^{hi} CD8$^+$ T cells in PEC than in spleen, as shown by the mean fluorescence intensity (MFI) (Fig. 3A). Virus-specific CD8$^+$ T cells in PEC continued to have higher expression of TCF-1 at day 30 post-LCMV infection than in spleen (Fig. 3A). TCF-1 deficiency has been shown to limit the generation of CD44^{hi} CD62L^{hi} (central memory) CD8$^+$ T cells (25). Virus-specific CD8$^+$ T cells in the spleen and PEC expressing large amounts of TCF-1 were enriched in MPEC, with most expressing CD27 and CXCR3 (Fig. 3B and C). Cells expressing high levels of TCF-1 (TCF-1^{hi}) were also less apoptotic, as revealed by low levels of active caspase-3 staining (Fig. 3B and C). Overall, these results demonstrate that the PEC

environment was enriched in TCF-1^{hi} CD8$^+$ T cells and that this correlated with increased memory and increased survival. TCF-1 has been shown to be necessary and sufficient for expression of Eomes, a transcription factor associated with memory CD8$^+$ T cells (25). Correspondingly, there was a higher expression of Eomes in virus-specific CD8$^+$ T cells in the PEC than in spleen at day 9 post-LCMV infection (MFI of Eomes on P14$^+$ CD8$^+$ T cells of 362 ± 7.5 for spleen versus 524 ± 21 for PEC).

Since a higher proportion of virus-specific CD8$^+$ T cells in the PEC expressed TCF-1, we next sought more direct evidence to identify whether TCF-1 was promoting survival of CD8$^+$ T cells in the nonlymphoid tissues. In accordance with previous reports (24, 25), TCF-1-deficient mice at day 9 postinfection had a decreased proportion and absolute number of activated CD44^{hi} CD8$^+$ T cells in both PEC and spleen (data not shown). Interestingly, the proportion of MPEC in the CD44^{hi} CD8$^+$ T cell pool was reduced to far lower levels in the PEC than in the spleen in the TCF-1-deficient mice at day 9 postinfection (Fig. 4A). To test whether
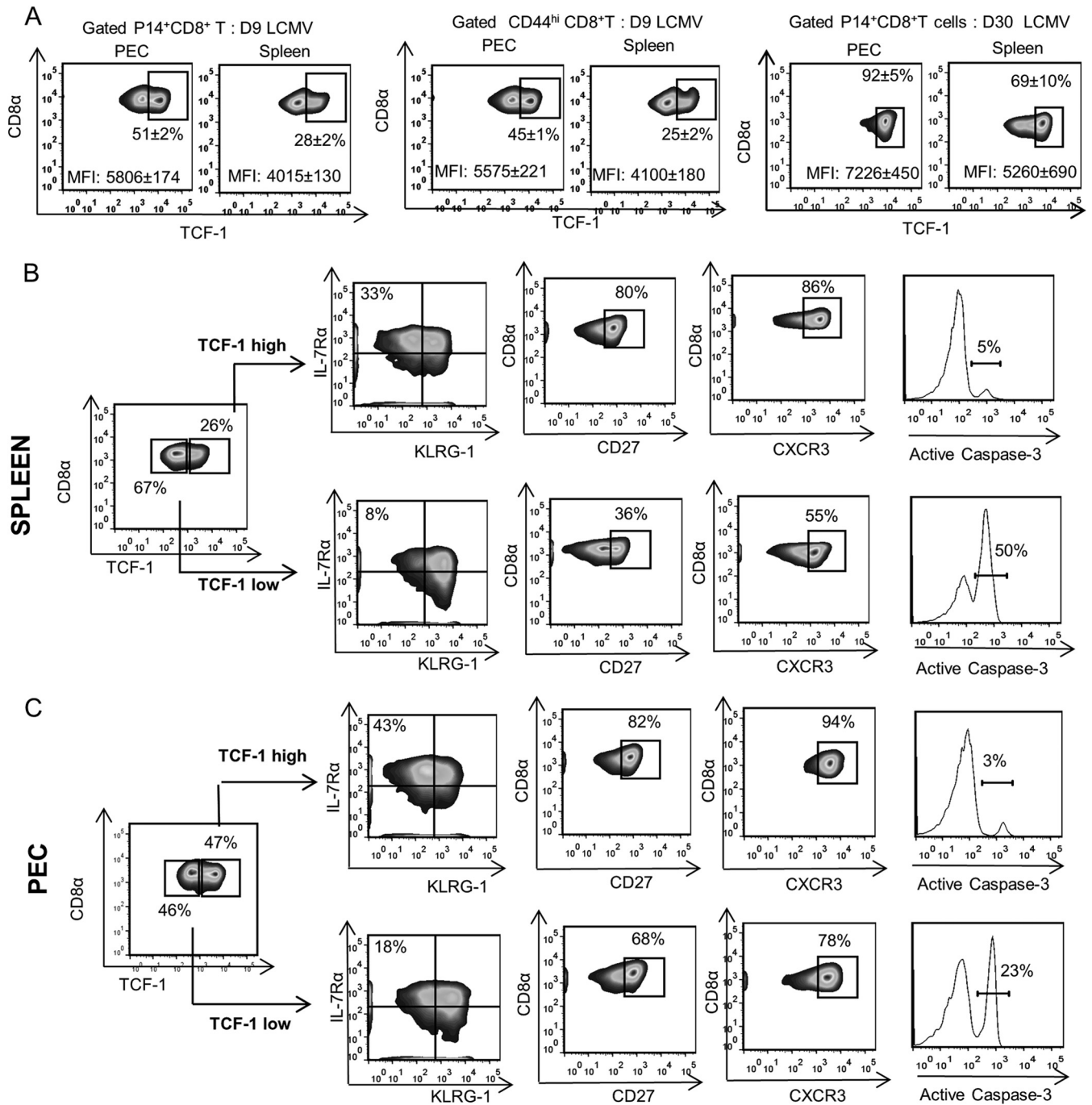
FIG 3 Higher percentage of TCF-1-expressing cells in the PEC and their resistance to apoptosis. (A) Percentages ± SD of TCF-1-expressing P14$^+$ CD8$^+$ T cells (left) or CD44^{hi} CD8$^+$ T cells (middle) at day 9 (D9) and of P14$^+$ CD8$^+$ T cells (right) at day 30 post-LCMV infection in PEC and spleen. The MFIs for TCF-1 expression in total day 9 P14$^+$ CD8$^+$ T cells (left) or CD44^{hi} CD8$^+$ T cells (middle) and in day 30 P14$^+$ CD8$^+$ T cells (right) are depicted in histograms. Plots are representative of two experiments with 3 mice in each experiment. (B and C) TCF-1 staining of P14$^+$ CD8$^+$ T cells from spleen and PEC at day 9 postinfection. Representative IL-7R, KLRG1, CD27, CXCR3, and active caspase-3 staining in TCF-1^{hi} and TCF-1^{lo} subsets from spleen or PEC is depicted. For panels B and C, data are representative of two independent experiments, and 5 mice were pooled for each experiment.

maintenance of TCF-1 expression promotes survival of CD8$^+$ T cells in the nonlymphoid tissues, WT or TCF-1 knockout (KO) cells were isolated from spleen and PEC of day 9 LCMV-infected mice and cultured *ex vivo* for 5 h, followed by TUNEL staining. Activated CD44^{hi} CD8$^+$ T cells from PEC of TCF-1 KO mice were more apoptotic than those from WT littermates, with about twice

the number of TUNEL$^+$ cells (Fig. 4B), and similar results were also observed by active caspase-3 staining (data not shown). Supporting a previous observation (25), we found no statistically significant difference in apoptosis of CD44^{hi} CD8$^+$ T cells from spleen between the TCF-1 KO and WT mice (Fig. 4B). Although overall numbers and proportions of CD44^{hi} CD8$^+$ T cells in
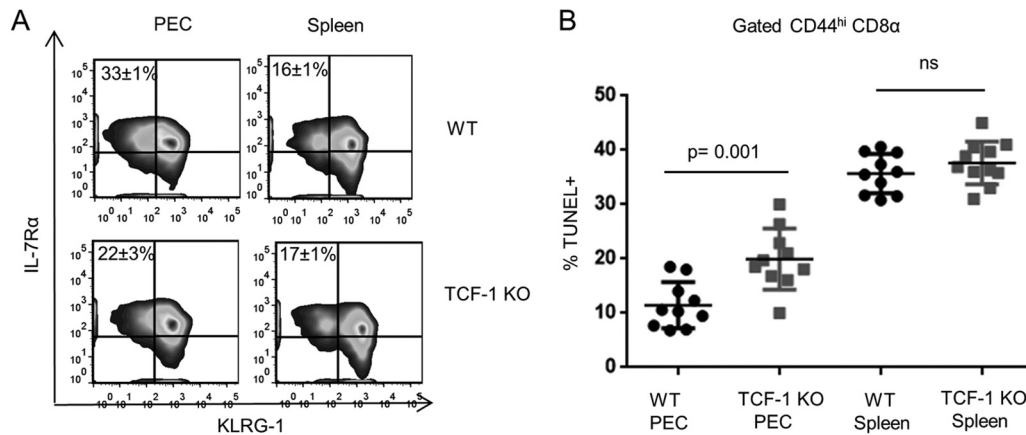
FIG 4 TCF-1 promotes survival of CD8$^+$ T cells in nonlymphoid tissues. (A) Percentage of IL-7R- and KLRG1-expressing CD44^{hi} CD8$^+$ T cells in PEC and spleen of WT or TCF-1 KO mice at day 9 post-LCMV infection. Numbers represent percentages ± SD of IL-7R^{hi} KLRG1^{lo} CD8$^+$ T cells. Graphs are representative of four experiments with at least 3 mice in each experiment. (B) Lymphocytes from spleen and PEC were isolated at day 9 post-LCMV infection from WT and TCF-1 KO mice and cultured *ex vivo* for 5 h postisolation. TUNEL reactivity of CD44^{hi} CD8$^+$ T cells is represented in the histograms. Data in the graph are a compilation of four experiments.

TCF-1 KO mice were reduced in both PEC and spleen, TCF-1 appeared to be more critical for the survival of CD8$^+$ T cells in the nonlymphoid tissues during resolution of the immune response. Importantly, CD44^{hi} CD8$^+$ T cells in PEC of TCF-1-deficient mice were still less apoptotic than those in the spleen, suggesting that additional mechanisms might regulate tissue-dependent differences in CD8$^+$ T cell apoptosis.

Accumulation of nonapoptotic CXCR3$^+$ CD8$^+$ T cells in the lymph nodes on FTY720 treatment. Experiments were designed to evaluate whether tissue-dependent differences in CD8$^+$ T cell apoptosis were associated with preferential migration of nonapoptotic CD8$^+$ T cells to the nonlymphoid tissues. FTY720 is an immunosuppressive drug which can modulate trafficking of lymphocytes and prevent emigration of T cells from the lymph nodes into the periphery (44–46). Transgenic P14$^+$ CD8$^+$ T cells were adoptively transferred into a congenic host, which was then infected with LCMV. Mice were treated with FTY720 at 1 mg/kg according to the treatment regimen shown in Fig. 5A. FTY720 treatment was more efficacious in sequestering the T cells in the lymph nodes than in the spleen; therefore, focus was placed on lymph nodes for further analysis. There was an increase in absolute number and proportion of virus-specific CD8$^+$ T cells in the inguinal lymph nodes and mesenteric lymph nodes of treated mice (Fig. 5B and data not shown) and a decrease in absolute number and proportion of virus-specific CD8$^+$ T cells in the PEC (Fig. 5B). We reasoned that if nonapoptotic CD8$^+$ T cells preferentially leave the lymph nodes, blocking their egress should lead to accumulation of nonapoptotic CD8$^+$ T cells in those lymph nodes. Lymphocytes were thus isolated from PEC, inguinal lymph nodes, and mesenteric lymph nodes of day 8 LCMV-infected control or FTY720-treated mice and cultured *ex vivo* for 5 h, followed by TUNEL staining. A lower percentage of virus-specific CD8$^+$ T cells in the inguinal and mesenteric lymph nodes were apoptotic in the FTY720-treated group than in the control group (Fig. 5C), and similar results were obtained with active caspase-3 staining (data not shown). Virus-specific CD8$^+$ T cells in the PEC trended more apoptotic in the FTY720-treated mice than in the control mice (Fig. 5C). Thus, these data are consistent with the hypothesis

that nonapoptotic CD8$^+$ T cells preferentially migrate into nonlymphoid tissues, and when migration is stopped, these apoptosis-resistant T cells accumulate in the lymph nodes.

CXCR3 has been shown to be important for migration of antigen-specific CD8$^+$ T cells into tissue effector sites (19, 28), and it has been previously reported that the peritoneal cavity is dominated by CXCR3$^+$ Th1 CD4$^+$ T cells (47). We here have shown that a higher proportion of virus-specific CD8$^+$ T cells expressed CXCR3 in the PEC and that these were less apoptotic (Fig. 2C). Figure 5D shows increased proportions of CXCR3$^+$ CD8$^+$ T cells accumulating in the inguinal lymph nodes on FTY720 treatment, suggesting that CXCR3$^+$ CD8$^+$ T cells were normally preferentially migrating out of the lymph nodes into the nonlymphoid tissues. CXCR3$^+$ T cells migrate under the influence of the IFN-γ-inducible chemokine ligands CXCL9 and CXCL10 (19, 20). At day 8 post-LCMV infection, there was a higher proportion of total lymphocytes expressing CXCL9 in the PEC environment than in the spleen (Fig. 5E). Further, mRNA for CXCL9 and CXCL10 was upregulated 4.7- and 2.6-fold, respectively, in the total PEC lymphocytes compared to total splenocytes at day 7 post-LCMV infection (Fig. 5F). Thus, the PEC environment had a higher expression of CXCR3 ligands that could promote preferential migration of CXCR3$^+$CD8$^+$ T cells. Alternatively, it is also possible that CXCR3 expression might promote preferential retention of apoptosis-resistant T cells in the peritoneal environment. Overall, these results are consistent with the hypothesis that nonapoptotic CXCR3$^+$ CD8$^+$ T cells may preferentially migrate out of secondary lymphoid organs into the nonlymphoid tissues under the influence of chemokines CXCL9 and CXCL10. This phenomenon may provide an additional mechanism by which healthy nonapoptotic memory CD8$^+$ T cells populate nonlymphoid tissues to provide protection to secondary challenge.

In vivo **PEC environment promotes survival of CD8$^+$ T cells.** To test whether the *in vivo* PEC environment itself had any effect on promoting survival of CD8$^+$ T cells, LCMV-specific P14$^+$ CD8$^+$ T cells were adoptively transferred into congenic hosts, followed by LCMV infection. At day 7 postinfection, splenocytes were isolated and labeled with tracking dyes CFSE or DDAO and
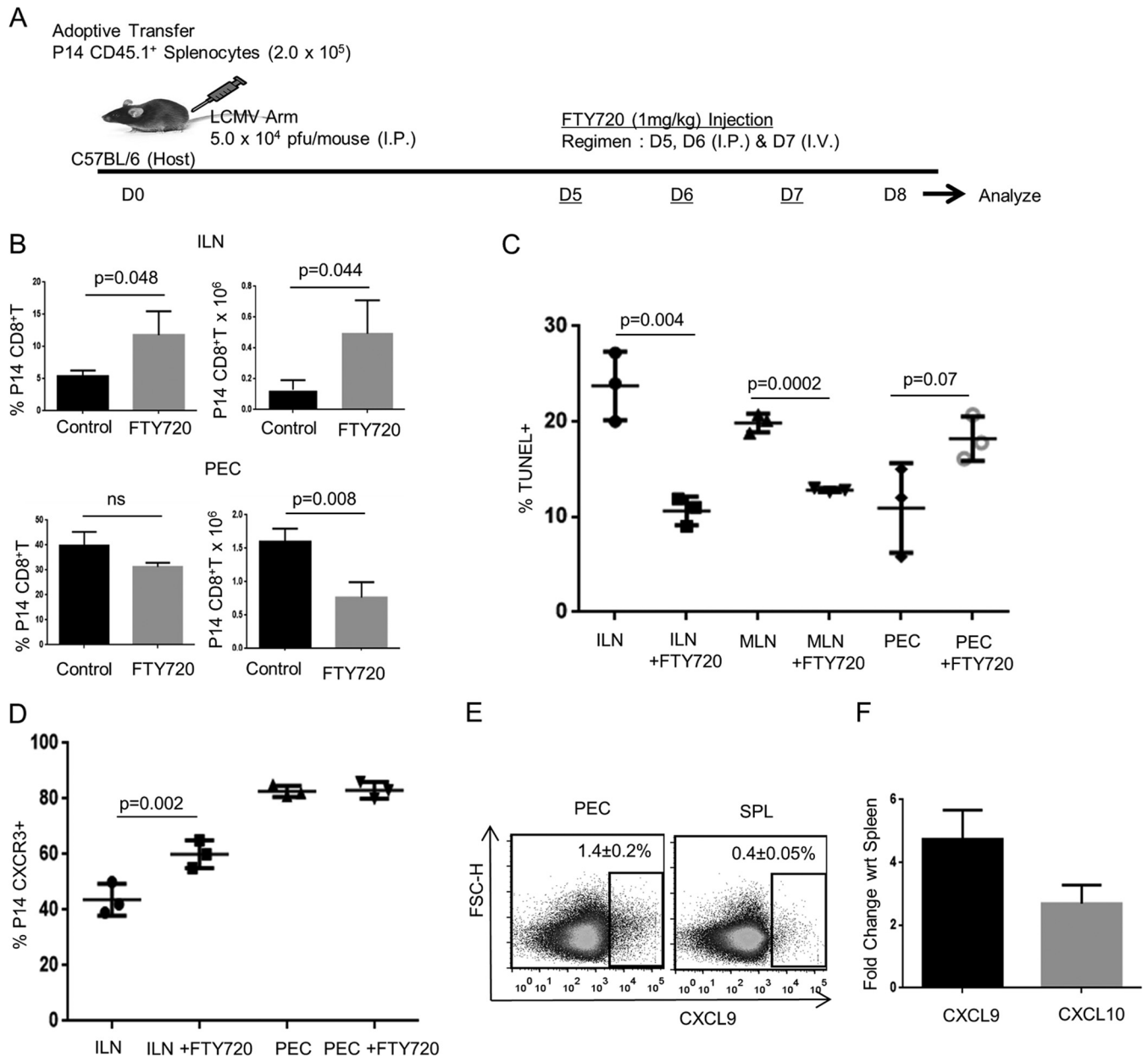
FIG 5 Accumulation of CXCR3+ CD8+ T cells in the inguinal lymph nodes on FTY720 treatment. (A) FTY720 treatment regimen. (B) Percentage and absolute number of P14+ CD8+ T cells in inguinal lymph nodes (ILN) and PEC of FTY720- or control-treated mice at day 8 post-LCMV infection. (C) Percentage of TUNEL+ P14+ CD8+ T cells in FTY720- and control-treated mice in inguinal lymph nodes (ILN), mesenteric lymph nodes (MLN), and PEC at day 8 post-LCMV infection. (D) Percentage of CXCR3+ P14+ CD8+ T cells in FTY720- and control-treated mice in inguinal lymph nodes (ILN) and PEC at day 8 post-LCMV infection. (E) Percentage of CXCL9-expressing lymphocytes in spleen or PEC at day 8 post-LCMV infection. Plots were gated based on isotype controls. (F) Fold change in CXCL9 and CXCL10 mRNAs in the total PEC leukocytes with respect to spleen leukocytes at day 7 post-LCMV infection. Data are representative of two experiments with 3 mice in each experimental group. Percentages and error bars are represented ± SD.

transferred back into an infection-matched host i.p. or i.v. (Fig. 6A). Splenocytes and PEC were isolated from the infection-matched host mice at 36 h posttransfer. Thus, day 7 infected splenocytes (which are highly apoptotic) were labeled and introduced into the spleen or the PEC environment of a similarly infected host to address whether the tissue environment contributed to CD8+ T cell survival. Donor P14+ CD8+ T splenocytes introduced directly into the PEC environment via the i.p. route were less apoptotic than the P14 CD8+ T cells, which were present in the spleen (Fig. 6B). A small number of donor P14+ CD8+ T splenocytes inoculated intravenously were found in the PEC, and those also were less apoptotic than the donor CD8+ T cells which populated the spleen (data not shown). Moreover, transferred P14 CD8+ T cells which were present in the PEC environment had higher expression of CD27 than P14 CD8+ T cells present in the spleen (Fig. 6B). Thus, the *in vivo* PEC environment was not only promoting survival of CD8+ T cells but also selectively retaining cells expressing memory markers.
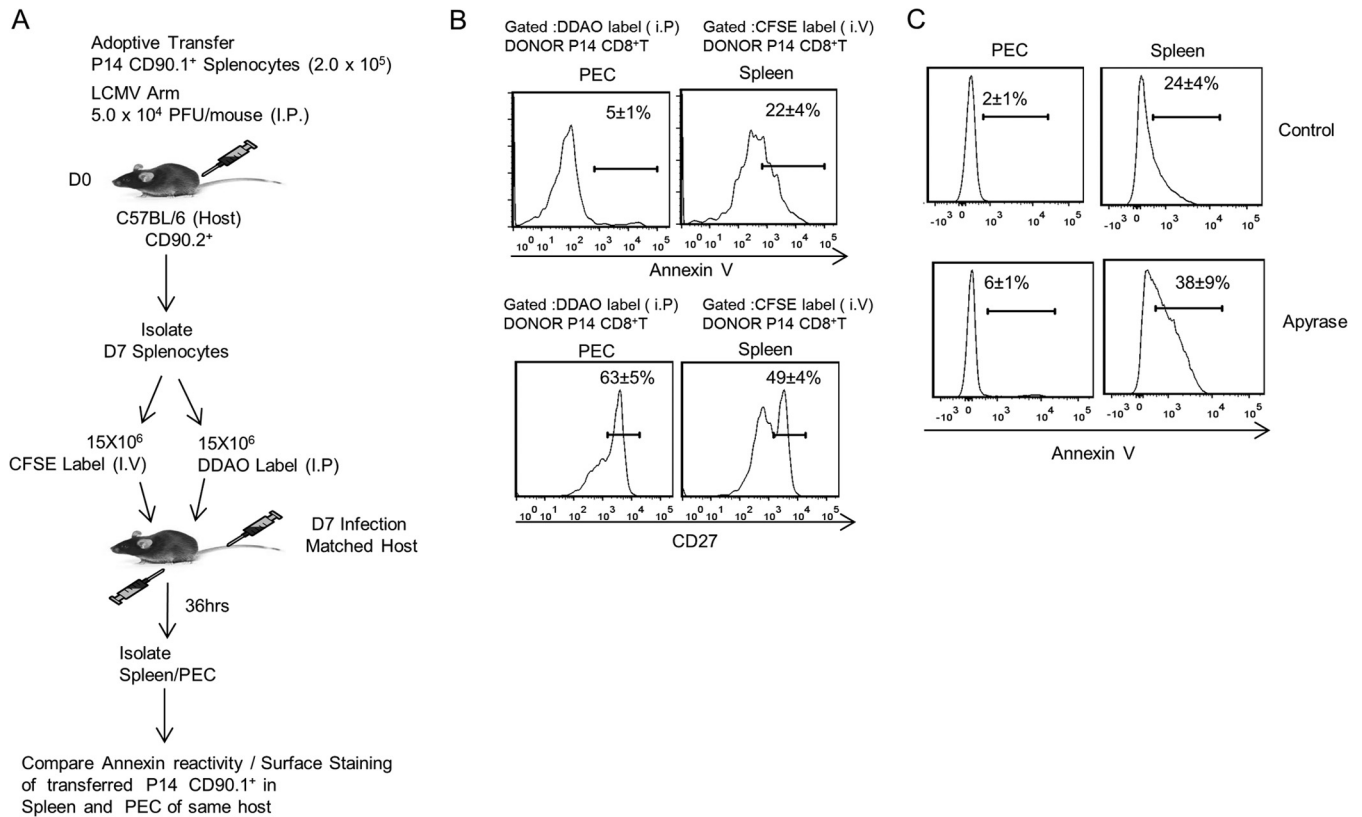
FIG 6 *In vivo* PEC environment promotes CD8$^+$ T cell survival. (A) Schematic of experimental setup to test effect of *in vivo* tissue environment in promoting survival of CD8$^+$ T cells. (B) Annexin V reactivity (top) and CD27 expression (bottom) on P14$^+$ CD8$^+$ T cells which were labeled and adoptively transferred from a day 7 LCMV-infected donor to an infection-matched host as shown in the schematic in panel A. (C) Annexin V reactivity of P14$^+$ CD8$^+$ T cells in PEC and spleen from day 8 LCMV-infected mice which were treated with apyrase or a buffer control for 6 h. Data are representative of two experiments with 3 mice in each experiment. Percentages are represented ± SD.

A potential complicating factor in these analyses was the possibility that apoptotic cells were more rapidly cleared in the peripheral tissues than in the spleen and lymph nodes. To test this possibility, P14 CD8$^+$ T cells were adoptively transferred into congenic hosts which were subsequently infected with LCMV. Nucleotides released by apoptotic cells can be recognized by phagocytic scavenger cells and promote clearance of apoptotic cells (48). Apyrase, an enzyme which hydrolyzes nucleoside triphosphates, can be used to prevent phagocytic clearance of apoptotic cells (48). At day 8 postinfection mice were treated with apyrase or a succinate buffer control for 6 h, following which splenocytes and PEC were isolated. Virus-specific CD8$^+$ T cells in the PEC and spleen of apyrase-treated mice were, as expected, more apoptotic than those of the control groups (Fig. 6C), thus providing evidence that apyrase inhibited phagocytic clearance. However, virus-specific, apoptosis-resistant CD8$^+$ T cells were still enriched in the PEC compared to spleen in the apyrase-treated group (Fig. 6C). This suggests that enhanced phagocytic clearance was not a major contributor to tissue-dependent differences in CD8$^+$ T cell apoptosis.

Increased frequency of apoptosis-resistant IL-2-producing CD4$^+$ T cells in the nonlymphoid tissues. Survival and proliferation of CD8$^+$ T cells can also be influenced by other cell types present in the tissue environment. CD4$^+$ T cell help has been shown to be important for survival, maintenance, and generation of functional memory CD8$^+$ T cells (49–52), and the absence of CD4$^+$ T cells decreases the proportion and survival of CD8$^+$ T cells at tissue effector sites (53, 54). We questioned whether CD4$^+$ T cells in the nonlymphoid tissues were also resistant to apoptosis during resolution of the immune response. At day 8 post-LCMV infection, lymphoid (spleen, inguinal lymph nodes, and mesenteric lymph nodes) and nonlymphoid (fat pad and PEC) tissues were harvested and stained directly *ex vivo* for annexin V. CD44^{hi} CD4$^+$ T cells in the nonlymphoid tissues reacted much less with the apoptotic marker annexin V than those in the lymphoid organs (Fig. 7A). Day 8 LCMV-infected CD44^{hi} CD4$^+$ T cells from nonlymphoid tissues were also less TUNEL reactive than those in lymphoid organs (Fig. 7B), further confirming the stability of CD4$^+$ T cells in the nonlymphoid tissues. Therefore, similar to CD8$^+$ T cells, CD4$^+$ T cells were also less apoptotic in the nonlymphoid tissues.

We explored the possibility of CD4$^+$ T cells promoting CD8$^+$ T cell survival in the PEC. Transgenic LCMV GP$_{61}$ epitope-specific CD4$^+$ T cells (SMARTA) were adoptively transferred into a C57BL/6 congenic host, which was subsequently infected with LCMV. At day 9 and day 15 post-LCMV infection, a higher percentage of IFN-γ-producing virus-specific CD4$^+$ T cells was present in the PEC than in the spleen (Fig. 7C). When stimulated with cognate peptide, virus-specific CD4$^+$ T cells in the PEC made much more IL-2 than those in the spleen (Fig. 7C). IL-2 is a T cell
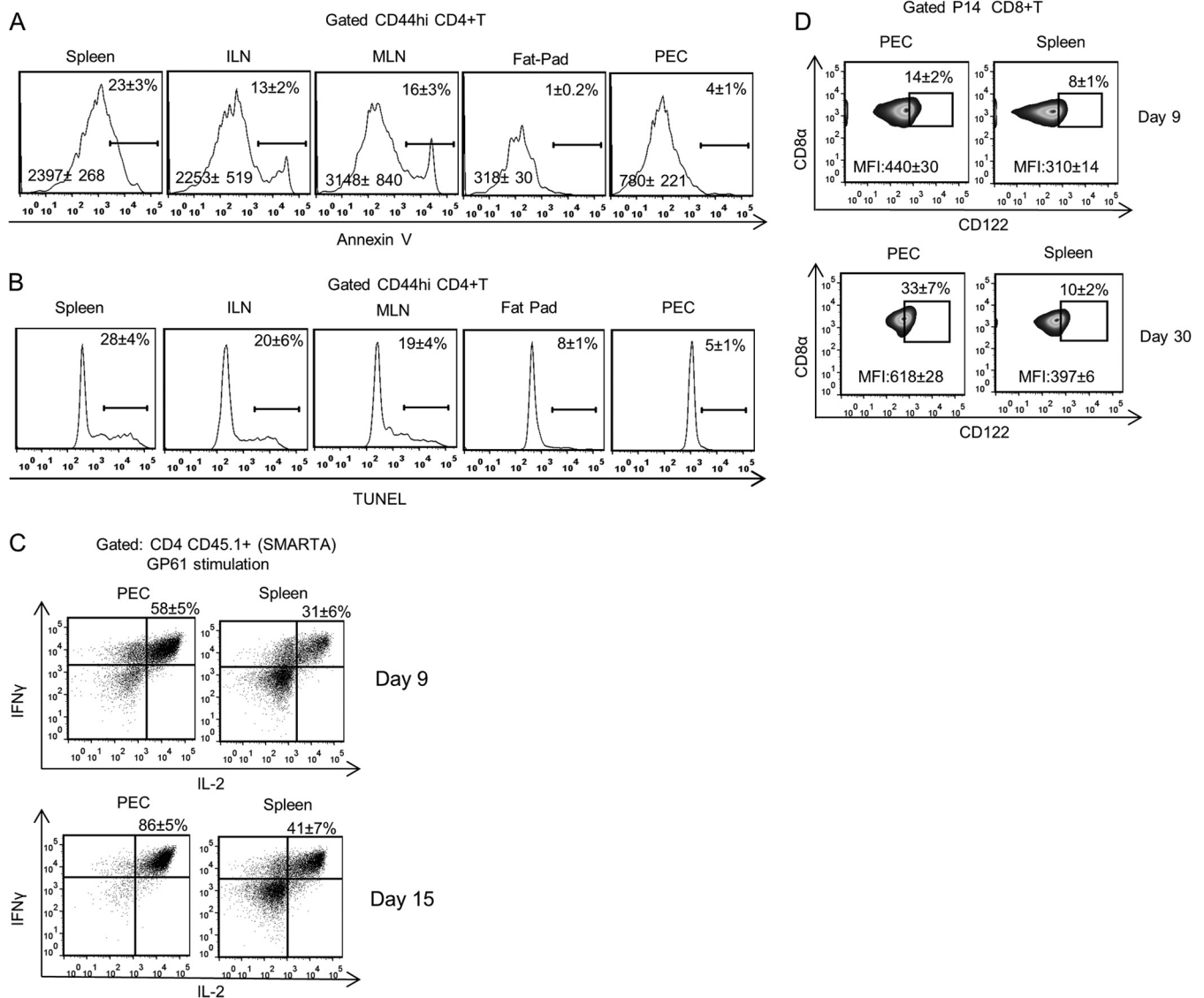
FIG 7 Increased frequency of apoptosis-resistant IL-2-producing CD4^{+} T cells in the nonlymphoid tissues. (A) Annexin V reactivity of CD44^{hi} CD4^{+} T cells in lymphoid (spleen, inguinal lymph nodes [ILN], mesenteric lymph nodes [MLN]) and nonlymphoid (PEC and fat pad) tissues at day 8 post-LCMV infection. Five mice were used for the experiment, and the experiment was done two times. Mean fluorescence intensity (MFI) for Annexin V reactivity on CD44^{hi} CD4^{+} T cells is listed in each histogram. (B) TUNEL reactivity of CD44^{hi} CD4^{+} T cells in lymphoid (spleen, inguinal lymph nodes, and mesenteric lymph nodes) and nonlymphoid (PEC and fat pad) tissues at day 8 post-LCMV infection. Five mice were used for the experiment, and the experiment was done two times. (C) Percentage of IL-2^{+} IFN-γ^{+} double cytokine producers at day 9 and day 15 post-LCMV infection responding to GP61 stimulation in the PEC and spleen. Plots are gated on CD4^{+} TCR transgenic SMARTA T cells. (C) CD122 expression on P14^{+} CD8^{+} T cells in PEC and spleen at day 9 and day 30 post-LCMV infection. Data are representative of two independent experiments with 3 mice in each experiment. Plots were gated based on isotype control, and percentages are represented ± SD.

growth factor, and while it can promote AICD of T cells under some circumstances (4), IL-2 therapy during contraction of the immune response has been shown to increase CD8^{+} T cell proliferation and survival (55). Therefore, a higher proportion of virus-specific CD4^{+} T cells and CD8^{+} T cells in the PEC are capable of making IL-2, and low-level secretion of IL-2 could contribute to the enhanced survival of CD8^{+} T cells seen in the PEC environment. However, we were unable to show the presence of IL-2 in the PEC environment directly *ex vivo* by ELISAs at day 7 post-LCMV infection. We questioned if depleting CD4^{+} T cells would preferentially affect the survival of CD8^{+} T cells in the PEC. A

modest effect was observed, in that CD8^{+} T cell apoptosis in the PEC trended higher at day 8 post-LCMV infection in mice depleted of CD4^{+} T cells at day 6 postinfection (18.1% ± 5.7% TUNEL^{+} in control mice versus 25.2% ± 3.8% TUNEL^{+} in CD4-depleted mice), but no change was observed in the spleen (34.3% ± 1.2% TUNEL[+] in control mice versus 35.1% ± 2.4% TUNEL^{+} in CD4-depleted mice). This effect was quite modest but may indicate that the increased proportion of IL-2-producing CD4^{+} T cells in the PEC environment could account for some of the increased resistance to apoptosis of virus-specific CD8^{+} T cells in the PEC. IL-2 signals are transduced by the high-affinity IL-2R, which is

comprised of CD25 (IL-2Rα), CD122 (IL-2Rβ), and CD132 (common γ chain). CD25 expression is uniformly downregulated in virus-specific CD8$^+$ T cells during resolution of the immune response (56). However, intermediate-affinity IL-2R comprised of CD122 and CD132 is sufficient for responsiveness to IL-2 and IL-15 (57). We found a modest increase in virus-specific CD8$^+$ T cells expressing CD122 in the PEC at day 9 post-LCMV infection compared to spleen, and this difference was enhanced at day 30 post-LCMV infection (Fig. 7D). This might allow IL-2- and IL-15-mediated CD8$^+$ T cell survival in the PEC environment if trace levels of these cytokines were made. Overall, these results indicate that decreased CD4$^+$ T cell apoptosis and their enhanced ability to produce IL-2 in the nonlymphoid tissues might be another factor promoting stability of CD8$^+$ T cells in the peripheral tissue environment.

DISCUSSION

T cells persist at high frequencies in nonlymphoid tissues after resolution of the immune response, and these T cells are uniquely geared to produce effector cytokines (58–62). The presence of memory T cells at nonlymphoid tissue sites is critical for protection against secondary virus challenge (8–10, 61). We previously showed that during and after silencing of the immune response, LCMV-specific CD8$^+$ T cells in nonlymphoid tissues are more resistant to apoptosis than those in the lymphoid organs (6), likely explaining their abilities to survive in the periphery. Here, we demonstrated this stability and the factors that contributed to this resistance to cell death. It was first noted that cells in the periphery were enriched for those expressing an antigenic signature (IL-7R^{hi}, KLRG1^{lo}, CD27^{hi}, and CXCR3^{hi}) that correlated with resistance to apoptosis, regardless of whether the cells were isolated from lymphoid organs or peripheral sites. Second, cells in the periphery had enhanced expression of the transcription factor TCF-1, which regulates Eomes and other genes leading to long-term memory. Third, the chemokine receptor CXCR3, which guides cells in their migration from lymph nodes to peripheral sites, were markedly resistant to apoptosis, indicating a preferential entry of apoptosis-resistant cells into peripheral sites encoding ligands for CXCR3. Fourth, adoptive transfer studies indicated that the peripheral environment has a protective effect on transferred cells by an as yet undefined mechanism. The differences in apoptotic cells between lymphoid and peripheral tissues, however, could not be explained simply by differences in the rates of uptake of these cells by phagocytes, as shown by apyrase blocking studies. Thus, apoptosis-resistant CD8$^+$ T cells seem to leave the spleen and lymph nodes and get into the peripheral sites, where they encounter an environment that sustains their survival, enabling them to be present to respond to a secondary challenge. Possible factors promoting survival in the peritoneal environment include IL-2-producing CD4$^+$ T cells, which are similarly resistant to apoptosis.

The mechanisms underlying tissue-dependent differences in CD8$^+$ T cell apoptosis were addressed in this study. Higher proportions of virus-specific CD8$^+$ T cells in the nonlymphoid tissues expressed surface markers that correlated with an apoptosis-resistant phenotype. CD8$^+$ T cells which are IL-7R^{hi} and KLRG1^{lo} are thought to preferentially become memory cells (11–13), and these were found in higher proportions in nonlymphoid tissues (Fig. 2A). IL-7 therapy during contraction of the immune response enhances the generation of memory CD8$^+$ T cells (63). IL-7 sig-

naling induces STAT-5 phosphorylation, which is known to be associated with survival of effector and memory CD8$^+$ T cells (64, 65). We found that a higher proportion of virus-specific CD8$^+$ T cells in the PEC exhibited STAT-5 phosphorylation in response to IL-7 directly *ex vivo* than the spleen counterparts (49% in the PEC versus 31% in the spleen), so constitutively produced IL-7 may contribute to the survival of virus-specific CD8$^+$ T cells in the PEC. CD27 is another molecule that sustains survival of virus-specific CD8$^+$ T cells by inducing autocrine IL-2 production (17). A higher proportion of virus-specific CD8$^+$ T cells in the PEC expressed CD27, and these were also less apoptotic (Fig. 2B). Stimulation through CD27 requires the presence of its costimulatory ligand CD70, which is expressed on dendritic cells (DCs), B cells, and activated CD4 and CD8 T cells. A study by another group showed that blocking CD70-CD27 costimulation during acute LCMV infection decreased the CD8$^+$ T cell response and IL-7Rα expression on virus-specific CD8$^+$ T cells (66). We found that at day 9 post-LCMV infection, total PEC leukocytes had a higher expression of CD70 than spleen leukocytes (data not shown). Thus, sustained CD27 stimulation due to continued presence of CD70$^+$ cells in the PEC environment might promote survival of virus-specific CD8$^+$ T cells. T cell apoptosis in lung and liver sites has been studied by other groups (33, 34, 67). The liver is problematic to analyze because it is thought to be a graveyard for apoptotic cells. T cells in the lung, however, are very resistant to apoptosis, and the collagen binding α1β1 integrin VLA-1 has been shown to be important in maintenance and survival of influenza A virus-specific CD8$^+$ T cells in lungs (67). However, we did not find VLA-1 to be highly expressed on LCMV-specific CD8$^+$ T cells in the PEC or spleen, suggesting that different mechanisms might regulate tissue-dependent survival in different tissues and during different viral infections.

Initially we performed experiments with mice immunized with serum-containing LCMV stocks. We found that in *in vitro* assays, CD4$^+$ T cells from the PEC but not spleen responded strongly to serum, thereby inducing IL-2 production that contributed to CD8$^+$ T cell survival in the PEC but not the spleen. Serum-free LCMV stocks were thus used for the experiments in the current study. Since CD4$^+$ T cells in the PEC had a dramatic response to serum antigen, we can speculate that a similar response to limited amounts of viral antigen in the PEC might promote CD4$^+$ T cell-dependent CD8$^+$ T cell survival in the PEC. Influenza virus-derived antigens are known to be presented for a prolonged period in the lung after apparent resolution of infection (68), and whether similar persistence of LCMV antigen contributes to T cell survival is not clear. However, we found that tissue-dependent differences in CD8$^+$ T cell apoptosis persisted even if the peritoneal cavity was not the initial site for antigen delivery. In mice challenged with LCMV via the i.v. route, the influx of activated CD8$^+$ T cells in the PEC was decreased, but CD8$^+$ T cells in the PEC were still more resistant to apoptosis than those in the spleen (data not shown).

Transcription factors can regulate CD8$^+$ T cell effector-memory fate decisions. We show that a higher proportion of CD8$^+$ T cells in the PEC express large amounts of TCF-1, which positively correlated with T cell memory potential and survival (Fig. 3A, B, and C). By using Tcf7$^{-/-}$ mice, we showed that TCF-1 was relatively more important in promoting survival of CD8$^+$ T cells in the peritoneum than in the spleen during resolution of the immune response. Our results indicate that loss of TCF-1 does not have any additive effect on T cell apoptosis in the spleen (since

they are contracting due to cytokine deprivation and apoptotic signals), but TCF-1 expression is relatively more important in promoting T cell survival in the PEC during resolution of the immune response. We observed that by day 30 post-LCMV, infection nearly 93% of virus-specific CD8^{+} T cells in the PEC were TCF-1 positive (Fig. 3A) and 62% of these were IL-7R^{hi} KLRG1^{lo} (MPEC) (data not shown), suggesting that increased survival in nonlymphoid tissues was not simply due to the presence of a higher proportion of memory-precursor effector cells. Expression of TCF-1 can be modulated by Wnt ligands and by T cell receptor (TCR) and cytokine signals (25, 69, 70). It has been suggested that Wnt proteins might induce the TCF-1–Eomes–IL-2Rb axis to enhance cytokine responsiveness in memory CD8^{+} T cells (25). How do virus-specific CD8^{+} T cells maintain higher TCF-1 expression in the PEC? The PEC environment is enriched in macrophages and dendritic cells, which *in vitro* are known to produce Wnt5a, which induces the noncanonical Wnt pathway (71). Alternatively, it is possible that TCF-1-expressing cells might preferentially populate the peritoneal cavity. Future studies will need to define the underlying mechanism for the biased recruitment and/or maintenance of TCF-1^{hi} cells in nonlymphoid tissues.

T cells migrate into tissue effector sites postactivation to eliminate foreign antigens, and we provide evidence that nonapoptotic CXCR3^{+} CD8^{+} T cells accumulated in the lymph nodes if their egress was blocked by FTY720 treatment. Studies done by other groups have shown that CXCR3-deficient CD8^{+} T cells undergo decreased contraction and form better memory populations (20, 72). The explanation for this may be complex, but because CXCR3-deficient T cells do not efficiently localize to sites of antigen and inflammation, they may undergo abnormal differentiation that renders them less sensitive to apoptosis. An alternate explanation consistent with our results could be that apoptosis-resistant T cells that lack CXCR3 accumulate in the lymphoid organs, and the accumulation of those apoptosis-resistant T cells retards the contraction effect. CXCR3^{+}CD8^{+} T cells are known to migrate in response to IFN-γ-inducible chemokines CXCL9 and CXCL10. CXCL9 and CXCL10 are produced by activated macrophages and DCs, and we found that the PEC environment expressed these chemokines. Thus, during resolution of the immune response, nonapoptotic memory phenotype CD8^{+} T cells likely migrate into nonlymphoid tissues. This trafficking pattern, in conjunction with tissue-intrinsic differences in promoting cell survival, provides an attractive mechanism for promoting long-lived immunity at nonlymphoid tissues.

Thus, multiple mechanisms seem to account for tissue-dependent differences in CD8^{+} T cell apoptosis seen during resolution of the immune response. These mechanisms would work in conjunction to promote survival and overall maintenance of CD8^{+} T cell populations in nonlymphoid tissues. The presence of functional long-lived memory CD8^{+} T cells at nonlymphoid tissue effector sites would ensure a productive response upon an encounter with the antigen providing protection against secondary virus challenge.

ACKNOWLEDGMENTS

REFERENCES

1. **Roulston A, Marcellus RC, Branton PE.** 1999. Viruses and apoptosis. Annu. Rev. Microbiol. **53**:577–628. http://dx.doi.org/10.1146/annurev.micro.53.1.577.
2. **Bahl K, Kim S, Calcagno C, Ghersi D, Puzone R, Celada F, Selin LK, Welsh RM.** 2006. IFN-induced attrition of CD8 T cells in the presence or absence of cognate antigen during the early stages of viral infections. J. Immunol. **176**:4284–4295. http://dx.doi.org/10.4049/jimmunol.176.7.4284.
3. **McNally J, Zarozinski C, Lin M, Brehm M, Chen H, Welsh R.** 2001. Attrition of bystander CD8 T cells during virus-induced T-cell and interferon responses. J. Virol. **75**:5965–5976. http://dx.doi.org/10.1128/JVI.75.13.5965-5976.2001.
4. **Razvi ES, Welsh RM.** 1993. Programmed cell death of T lymphocytes during acute viral infection: a mechanism for virus-induced immune deficiency. J. Virol. **67**:5754–5765.
5. **Ahmed R, Gray D.** 1996. Immunological memory and protective immunity: understanding their relation. Science **272**:54–60. http://dx.doi.org/10.1126/science.272.5258.54.
6. **Wang XZ, Stepp SE, Brehm MA, Chen HD, Selin LK, Welsh RM.** 2003. Virus-specific CD8 T cells in peripheral tissues are more resistant to apoptosis than those in lymphoid organs. Immunity **18**:631–642. http://dx.doi.org/10.1016/S1074-7613(03)00116-X.
7. **Schenkel JM, Fraser KA, Vezys V, Masopust D.** 2013. Sensing and alarm function of resident memory CD8^{+} T cells. Nat. Immunol. **14**:509–513. http://dx.doi.org/10.1038/ni.2568.
8. **Gebhardt T, Wakim L, Eidsmo L, Reading P, Heath W, Carbone F.** 2009. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. Nat. Immunol. **10**:524–530. http://dx.doi.org/10.1038/ni.1718.
9. **Hogan RJ, Zhong W, Usherwood EJ, Cookenham T, Roberts AD, Woodland DL.** 2001. Protection from respiratory virus infections can be mediated by antigen-specific CD4^{+} T cells that persist in the lungs. J. Exp. Med. **193**:981–986. http://dx.doi.org/10.1084/jem.193.8.981.
10. **Teijaro J, Turner D, Pham Q, Wherry E, Lefrancois L, Farber D.** 2011. Cutting edge: tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection. J. Immunol. **187**:5510–5514. http://dx.doi.org/10.4049/jimmunol.1102243.
11. **Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R.** 2003. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat. Immunol. **4**:1191–1198. http://dx.doi.org/10.1038/ni1009.
12. **Joshi NS, Kaech SM.** 2008. Effector CD8 T cell development: a balancing act between memory cell potential and terminal differentiation. J. Immunol. **180**:1309–1315. http://dx.doi.org/10.4049/jimmunol.180.3.1309.
13. **Sarkar S, Kalia V, Haining WN, Konieczny BT, Subramaniam S, Ahmed R.** 2008. Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. J. Exp. Med. **205**:625–640. http://dx.doi.org/10.1084/jem.20071641.
14. **Dong H, Franklin NA, Roberts DJ, Yagita H, Glennie MJ, Bullock TN.** 2012. CD27 stimulation promotes the frequency of IL-7 receptor-expressing memory precursors and prevents IL-12-mediated loss of CD8^{+} T cell memory in the absence of CD4^{+} T cell help. J. Immunol. **188**:3829–3838. http://dx.doi.org/10.4049/jimmunol.1103329.
15. **Dolfi DV, Boesteanu AC, Petrovas C, Xia D, Butz EA, Katsikis PD.** 2008. Late signals from CD27 prevent Fas-dependent apoptosis of primary CD8^{+} T cells. J. Immunol. **180**:2912–2921. http://dx.doi.org/10.4049/jimmunol.180.5.2912.
16. **Ochsenbein AF, Riddell SR, Brown M, Corey L, Baerlocher GM, Lansdorp PM, Greenberg PD.** 2004. CD27 expression promotes long-term survival of functional effector-memory CD8^{+} cytotoxic T lymphocytes in HIV-infected patients. J. Exp. Med. **200**:1407–1417. http://dx.doi.org/10.1084/jem.20040717.
17. **Hendriks J, Xiao Y, Borst J.** 2003. CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the ef-

fector T cell pool. J. Exp. Med. **198:**1369–1380. http://dx.doi.org/10.1084/jem.20030916.

18. **Peperzak V, Xiao Y, Veraar EA, Borst J.** 2010. CD27 sustains survival of CTLs in virus-infected nonlymphoid tissue in mice by inducing autocrine IL-2 production. J. Clin. Invest. **120:**168–178. http://dx.doi.org/10.1172/JCI40178.

19. **Groom JR, Luster AD.** 2011. CXCR3 in T cell function. Exp. Cell Res. **317:**620–631. http://dx.doi.org/10.1016/j.yexcr.2010.12.017.

20. **Hu JK, Kagari T, Clingan JM, Matloubian M.** 2011. Expression of chemokine receptor CXCR3 on T cells affects the balance between effector and memory CD8 T-cell generation. Proc. Natl. Acad. Sci. U. S. A. **108:**E118–E127. http://dx.doi.org/10.1073/pnas.1101881108.

21. **Hikono H, Kohlmeier JE, Takamura S, Wittmer ST, Roberts AD, Woodland DL.** 2007. Activation phenotype, rather than central- or effector-memory phenotype, predicts the recall efficacy of memory CD8$^+$ T cells. J. Exp. Med. **204:**1625–1636. http://dx.doi.org/10.1084/jem.20070322.

22. **Xue H, Zhao D.** 2012. Regulation of mature T cell responses by the Wnt signaling pathway. Ann. N. Y. Acad. Sci. **1247:**16–33. http://dx.doi.org/10.1111/j.1749-6632.2011.06302.x.

23. **Verbeek S, Izon D, Hofhuis F, Robanus-Maandag E, Riele HT, van de Wetering M, Oosterwegel M, Wilson A, MacDonald HR, Clevers H.** 1995. An HMG-box-containing T-cell factor required for thymocyte differentiation. Nature **374:**70–74. http://dx.doi.org/10.1038/374070a0.

24. **Zhou X, Xue H.** 2012. Cutting edge: generation of memory precursors and functional memory CD8$^+$ T cells depends on T cell factor-1 and lymphoid enhancer-binding factor-1. J. Immunol. **189:**2722–2726. http://dx.doi.org/10.4049/jimmunol.1201150.

25. **Zhou X, Yu S, Zhao D, Harty JT, Badovinac VP, Xue H.** 2010. Differentiation and persistence of memory CD8$^+$ T cells depend on T cell factor 1. Immunity **33:**229–240. http://dx.doi.org/10.1016/j.immuni.2010.08.002.

26. **Jeannet G, Boudousquie C, Gardiol N, Kang J, Huelsken J, Held W.** 2010. Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory. Proc. Natl. Acad. Sci. U. S. A. **107:**9777–9782. http://dx.doi.org/10.1073/pnas.0914127107.

27. **McLachlan JB, Jenkins MK.** 2007. Migration and accumulation of effector CD4$^+$ T cells in nonlymphoid tissues. Proc. Am. Thorac. Soc. **4:**439–442. http://dx.doi.org/10.1513/pats.200606-137MS.

28. **Hokeness K, Hokeness K, Deweerd E, Deweerd E, Munks M, Munks M, Lewis C, Lewis C, Gladue R, Salazar-Mather T.** 2007. CXCR3-dependent recruitment of antigen-specific T lymphocytes to the liver during murine cytomegalovirus infection. J. Virol. **81:**1241–1250. http://dx.doi.org/10.1128/JVI.01937-06.

29. **Campanella G, Tager A, Khoury J, Thomas S, Abrazinski T, Manice L, Colvin R, Luster A.** 2008. Chemokine receptor CXCR3 and its ligands CXCL9 and CXCL10 are required for the development of murine cerebral malaria. Proc. Natl. Acad. Sci. U. S. A. **105:**4814–4819. http://dx.doi.org/10.1073/pnas.0801544105.

30. **Ioannidis V, Beermann F, Clevers H, Held W.** 2001. The beta-catenin–TCF-1 pathway ensures CD4$^+$ CD8$^+$ thymocyte survival. Nat. Immunol. **2:**691–697. http://dx.doi.org/10.1038/90623.

31. **Oxenius A, Bachmann MF, Zinkernagel RM, Hengartner H.** 1998. Virus-specific MHC-class II-restricted TCR-transgenic mice: effects on humoral and cellular immune responses after viral infection. Eur. J. Immunol. **28:**390–400. http://dx.doi.org/10.1002/(SICI)1521-4141(199801)28:01<390::AID-IMMU390>3.0.CO;2-O.

32. **Welsh RM, Seedhom MO.** 2008. Lymphocytic choriomeningitis virus (LCMV): propagation, quantitation, and storage. Curr. Protoc. Microbiol. Chapter 15:Unit 15A.1. http://dx.doi.org/10.1002/9780471729259.mc15a01s8.

33. **Liu ZX, Govindarajan S, Okamoto S, Dennert G.** 2001. Fas-mediated apoptosis causes elimination of virus-specific cytotoxic T cells in the virus-infected liver. J. Immunol. **166:**3035–3041. http://dx.doi.org/10.4049/jimmunol.166.5.3035.

34. **Huang L, Soldevila G, Leeker M, Flavell R, Crispe IN.** 1994. The liver eliminates T cells undergoing antigen-triggered apoptosis in vivo. Immunity **1:**741–749. http://dx.doi.org/10.1016/S1074-7613(94)80016-2.

35. **Wang X, Brehm M, Welsh R.** 2004. Preapoptotic phenotype of viral epitope-specific CD8 T cells precludes memory development and is an intrinsic property of the epitope. J. Immunol. **173:**5138–5147. http://dx.doi.org/10.4049/jimmunol.173.8.5138.

36. **Dillon SR, Mancini M, Rosen A, Schlissel MS.** 2000. Annexin V binds to viable B cells and colocalizes with a marker of lipid rafts upon B cell

receptor activation. J. Immunol. **164:**1322–1332. http://dx.doi.org/10.4049/jimmunol.164.3.1322.

37. **Span LF, Pennings AH, Vierwinden G, Boezeman JB, Raymakers RA, de Witte T.** 2002. The dynamic process of apoptosis analyzed by flow cytometry using annexin-V/propidium iodide and a modified in situ end labeling technique. Cytometry **47:**24–31. http://dx.doi.org/10.1002/cyto.10028.

38. **van Engeland M, Nieland LJ, Ramaekers FC, Schutte B, Reutelingsperger CP.** 1998. Annexin V-affinity assay: a review on an apoptosis detection system based on phosphatidylserine exposure. Cytometry **31:**1–9. http://dx.doi.org/10.1002/(SICI)1097-0320(19980101)31:1<1::AID-CYTO1>3.0.CO;2-R.

39. **Sabbagh L, Kaech SM, Bourbonnière M, Woo M, Cohen LY, Haddad EK, Labrecque N, Ahmed R, Sékaly R.** 2004. The selective increase in caspase-3 expression in effector but not memory T cells allows susceptibility to apoptosis. J. Immunol. **173:**5425–5433. http://dx.doi.org/10.4049/jimmunol.173.9.5425.

40. **Woo M, Hakem R, Soengas MS, Duncan GS, Shahinian A, Kägi D, Hakem A, McCurrach M, Khoo W, Kaufman SA, Senaldi G, Howard T, Lowe SW, Mak TW.** 1998. Essential contribution of caspase 3/CPP32 to apoptosis and its associated nuclear changes. Genes Dev. **12:**806–819. http://dx.doi.org/10.1101/gad.12.6.806.

41. **Kurtulus S, Tripathi P, Moreno-Fernandez ME, Sholl A, Katz JD, Grimes HL, Hildeman DA.** 2011. Bcl-2 allows effector and memory CD8$^+$ T cells to tolerate higher expression of Bim. J. Immunol. **186:**5729–5737. http://dx.doi.org/10.4049/jimmunol.1100102.

42. **Grayson JM, Zajac AJ, Altman JD, Ahmed R.** 2000. Cutting edge: increased expression of Bcl-2 in antigen-specific memory CD8$^+$ T cells. J. Immunol. **164:**3950–3954. http://dx.doi.org/10.4049/jimmunol.164.8.3950.

43. **Kristensen NN, Christensen JP, Thomsen AR.** 2002. High numbers of IL-2-producing CD8$^+$ T cells during viral infection: correlation with stable memory development. J. Gen. Virol. **83:**2123–2133.

44. **Pinschewer DD, Ochsenbein AF, Odermatt B, Brinkmann V, Hengartner H, Zinkernagel RM.** 2000. FTY720 immunosuppression impairs effector T cell peripheral homing without affecting induction, expansion, and memory. J. Immunol. **164:**5761–5770. http://dx.doi.org/10.4049/jimmunol.164.11.5761.

45. **Vora K.** 2005. Sphingosine 1-phosphate receptor agonist FTY720-phosphate causes marginal zone B cell displacement. J. Leukoc. Biol. **78:**471–480. http://dx.doi.org/10.1189/jlb.0904487.

46. **Yanagawa Y, Sugahara K, Kataoka H, Kawaguchi T, Masubuchi Y, Chiba K.** 1998. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. II. FTY720 prolongs skin allograft survival by decreasing T cell infiltration into grafts but not cytokine production in vivo. J. Immunol. **160:**5493–5499.

47. **Zygmunt B, Groebe L, Guzman C, Braunschweig G.** 2011. Peritoneal cavity is dominated by IFN-γ-secreting CXCR3$^+$ Th1 cells. PLoS One **6:**e18032. http://dx.doi.org/10.1371/journal.pone.0018032.

48. **Elliott MR, Chekeni FB, Trampont PC, Lazarowski ER, Kadl A, Walk SF, Park D, Woodson RI, Ostankovich M, Sharma P, Lysiak JJ, Harden TK, Leitinger N, Ravichandran KS.** 2009. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature **461:**282–286. http://dx.doi.org/10.1038/nature08296.

49. **Shedlock DJ, Shen H.** 2003. Requirement for CD4 T cell help in generating functional CD8 T cell memory. Science **300:**337–339. http://dx.doi.org/10.1126/science.1082305.

50. **Sun JC, Williams MA, Bevan MJ.** 2004. CD4$^+$ T cells are required for the maintenance, not programming, of memory CD8$^+$ T cells after acute infection. Nat. Immunol. **5:**927–933. http://dx.doi.org/10.1038/ni1105.

51. **Sun JC, Bevan MJ.** 2003. Defective CD8 T cell memory following acute infection without CD4 T cell help. Science **300:**339–342. http://dx.doi.org/10.1126/science.1083317.

52. **Novy P, Quigley M, Huang X, Yang Y.** 2007. CD4 T cells are required for CD8 T cell survival during both primary and memory recall responses. J. Immunol. **179:**8243–8251. http://dx.doi.org/10.4049/jimmunol.179.12.8243.

53. **Overstreet MG, Chen Y, Cockburn IA, Tse S, Zavala F.** 2011. CD4$^+$ T cells modulate expansion and survival but not functional properties of effector and memory CD8$^+$ T cells induced by malaria sporozoites. PLoS One **6:**e15948. http://dx.doi.org/10.1371/journal.pone.0015948.

54. **Phares TW, Stohlman SA, Hwang M, Min B, Hinton DR, Bergmann CC.** 2012. CD4 T cells promote CD8 T cell immunity at the priming and effector site during viral encephalitis. J. Virol. **86:**2416–2427. http://dx.doi.org/10.1128/JVI.06797-11.

55. **Blattman JN, Grayson JM, Wherry EJ, Kaech SM, Smith KA, Ahmed R.** 2003. Therapeutic use of IL-2 to enhance antiviral T-cell responses in vivo. Nat. Med. **9:**540–547. http://dx.doi.org/10.1038/nm866.

56. **Shin HM, Kapoor VN, Guan T, Kaech SM, Welsh RM, Berg LJ.** 2013. Epigenetic modifications induced by Blimp-1 regulate CD8$^+$ T cell memory progression during acute virus infection. Immunity **39:**661–675. http://dx.doi.org/10.1016/j.immuni.2013.08.032.

57. **Liao W, Lin J, Leonard W.** 2011. IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. Curr. Opin. Immunol. **23:**598–604. http://dx.doi.org/10.1016/j.coi.2011.08.003.

58. **Reinhardt RL, Khoruts A, Merica R, Zell T, Jenkins MK.** 2001. Visualizing the generation of memory CD4 T cells in the whole body. Nature **410:**101–105. http://dx.doi.org/10.1038/35065111.

59. **Hogan RJ, Usherwood EJ, Zhong W, Roberts AD, Dutton RW, Harmsen AG, Woodland DL.** 2001. Activated antigen-specific CD8$^+$ T cells persist in the lungs following recovery from respiratory virus infections. J. Immunol. **166:**1813–1822. http://dx.doi.org/10.4049/jimmunol.166.3.1813.

60. **Chen HD, Fraire AE, Joris I, Brehm MA, Welsh RM, Selin LK.** 2001. Memory CD8$^+$ T cells in heterologous antiviral immunity and immunopathology in the lung. Nat. Immunol. **2:**1067–1076. http://dx.doi.org/10.1038/ni727.

61. **Schenkel J, Fraser K, Vezys V, Masopust D.** 2013. Sensing and alarm function of resident memory CD8$^+$ T cells. Nat. Immunol. **14:**509–513. http://dx.doi.org/10.1038/ni.2568.

62. **Masopust D, Vezys V, Marzo AL, Lefrançois L.** 2001. Preferential localization of effector memory cells in nonlymphoid tissue. Science **291:**2413–2417. http://dx.doi.org/10.1126/science.1058867.

63. **Nanjappa S, Walent J, Morre M, Suresh M.** 2008. Effects of IL-7 on memory CD8$^+$ T cell homeostasis are influenced by the timing of therapy in mice. J. Clin. Invest. **118:**1027–1039. http://dx.doi.org/10.1172/JCI32020.

64. **Hand TW, Cui W, Jung YW, Sefik E, Joshi NS, Chandele A, Liu Y, Kaech SM.** 2010. Differential effects of STAT5 and PI3K/AKT signaling on effector and memory CD8 T-cell survival. Proc. Natl. Acad. Sci. U. S. A. **107:**16601–16606. http://dx.doi.org/10.1073/pnas.1003457107.

65. **Tripathi P, Kurtulus S, Wojciechowski S, Sholl A, Hoebe K, Morris SC, Finkelman FD, Grimes HL, Hildeman DA.** 2010. STAT5 is critical to maintain effector CD8$^+$ T cell responses. J. Immunol. **185:**2116–2124. http://dx.doi.org/10.4049/jimmunol.1000842.

66. **Penaloza-MacMaster P, Rasheed A, Iyer S, Yagita H, Blazar B, Ahmed R.** 2011. Opposing effects of CD70 costimulation during acute and chronic lymphocytic choriomeningitis virus infection of mice. J. Virol. **85:**6168–6174. http://dx.doi.org/10.1128/JVI.02205-10.

67. **Ray SJ, Franki SN, Pierce RH, Dimitrova S, Koteliansky V, Sprague AG, Doherty PC, de Fougerolles AR, Topham DJ.** 2004. The collagen binding α1β1 integrin VLA-1 regulates CD8 T cell-mediated immune protection against heterologous influenza infection. Immunity **20:**167–179. http://dx.doi.org/10.1016/S1074-7613(04)00021-4.

68. **Jelley-Gibbs DM, Brown DM, Dibble JP, Haynes L, Eaton SM, Swain SL.** 2005. Unexpected prolonged presentation of influenza antigens promotes CD4 T cell memory generation. J. Exp. Med. **202:**697–706. http://dx.doi.org/10.1084/jem.20050227.

69. **Staal F, Luis T, Tiemessen M.** 2008. WNT signalling in the immune system: WNT is spreading its wings. Nat. Rev. Immunol. **8:**581–593. http://dx.doi.org/10.1038/nri2360.

70. **Zhao D, Yu S, Zhou X, Haring J, Held W, Badovinac P, Harty J, Xue H.** 2010. Constitutive activation of Wnt signaling favors generation of memory CD8 T cells. J. Immunol. **184:**1191–1199. http://dx.doi.org/10.4049/jimmunol.0901199.

71. **Lehtonen A, Ahlfors H, Veckman V, Miettinen M, Lahesmaa R, Julkunen I.** 2007. Gene expression profiling during differentiation of human monocytes to macrophages or dendritic cells. J. Leukoc. Biol. **82:**710–720. http://dx.doi.org/10.1189/jlb.0307194.

72. **Kohlmeier JE, Reiley WW, Perona-Wright G, Freeman ML, Yager EJ, Connor LM, Brincks EL, Cookenham T, Roberts AD, Burkum CE, Sell S, Winslow GM, Blackman MA, Mohrs M, Woodland DL.** 2011. Inflammatory chemokine receptors regulate CD8$^+$ T cell contraction and memory generation following infection. J. Exp. Med. **208:**1621–1634. http://dx.doi.org/10.1084/jem.20102110.