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

Varun N. Kapoor  
*University of Massachusetts Medical School*

HyunMu Shin  
*University of Massachusetts Medical School*

Ok Hyun Cho  
*University of Massachusetts Medical School Worcester*

*See next page for additional authors*

Follow this and additional works at: [http://escholarship.umassmed.edu/gsbs_sp](http://escholarship.umassmed.edu/gsbs_sp)

Part of the [Allergy and Immunology Commons](http://escholarship.umassmed.edu/gsbs_sp), [Immunology of Infectious Disease Commons](http://escholarship.umassmed.edu/gsbs_sp), [Immunoprophylaxis and Therapy Commons](http://escholarship.umassmed.edu/gsbs_sp), [Infectious Disease Commons](http://escholarship.umassmed.edu/gsbs_sp), [Virology Commons](http://escholarship.umassmed.edu/gsbs_sp), and the [Virus Diseases Commons](http://escholarship.umassmed.edu/gsbs_sp)

Repository Citation

Kapoor, Varun N.; Shin, HyunMu; Cho, Ok Hyun; Berg, Leslie J.; Kang, Joonsoo; and Welsh, Raymond M., "Regulation of tissue-dependent differences in CD8+ T cell apoptosis during viral infection" (2014). GSBS Student Publications. 1936.

[http://escholarship.umassmed.edu/gsbs_sp/1936](http://escholarship.umassmed.edu/gsbs_sp/1936)

This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in GSBS Student Publications by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.
Regulation of tissue-dependent differences in CD8+ T cell apoptosis during viral infection

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

Comments
Copyright © 2014, American Society for Microbiology. All Rights Reserved. Publisher PDF posted as allowed by the publisher's author rights policy at http://journals.asm.org/site/misc/ASM_Author_Statement.xhtml.

Rights and Permissions

This article is available at eScholarship@UMMS: http://escholarship.umassmed.edu/gsbs_sp/1936
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-7Rhi KLRG1lo]) 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 TTY720, 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 several 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-7Rhi) and low levels of
Expression of the immune response has not been clarified. 

Pairing CD8+ T cells promotes survival, induces IL-7R expression, and protects against fate-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, comodermin (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 phenotypic 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 C90.1 (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 (NH4Cl).

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 × 10^6 PFU in phosphate-buffered saline (PBS).

Antibodies, flow cytomtery, and intracellular cytokine staining. Leukocytes were stained for CD8α (53-6.7; BD Pharmingen), CD8β (YTS156.7.7; Biologend), CD4 (RM4-5; BD Pharmingen), CD44 (IM7; eBiosciences), Thy1.1/CD90.1 (H1851; eBiosciences), Ly5.1/CD45.1 (A20; Biologend), KLRG1 (2F1; eBiosciences), CD127 (A7R34; eBiosciences), CD27 (LG.310; BD Pharmingen), CXCR3 (CXCR3-173; Biologend), CXCL19 (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; Biologend). For TCF-1 intracellular staining Alexa Fluor 647-conjugated goat anti-rabbit (Invitrogen) secondary antibody was used. To stain for intracellular antigens a FOXP3/transition 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 (NP396-404), GP peptide comprised of residues 33 to 41 (GP33-41), or GP61-80 peptide in the presence of Golgi Plug (Biologend). Cells were then permeabilized using a Cytofix/Cytoperm kit (BD Biosciences), followed by intracellular staining for IFN-γ (XMGL1.2; eBiosciences), IL-2 (JES65H4; BD Pharmingen), and TNF (MP6-XT22; Biologend). Fluorescence-activated cell sorting (FACS) plots were gated on 7-amoxytaminocyn D-negative (7-ADD-) cells versus forward scatter area (FSC-A; live), on forward scatter height (FSC-H) 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 deoxynucleotidyltransferase-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 inhabiting 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
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

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. MFI 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.
assays (data not shown). In effector CD8 T cells specific for the LCMV GP33–41 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 effect 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-7Rhi KLRG1lo) 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-7Rhi KLRG1lo in PEC versus 37% IL-7Rhi KLRG1lo 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 in than in the spleen (Fig. 2B). To confirm that CD27 expression on CD8 T cells promotes survival, we compared apoptotic profiles of CD27hi and CD27lo CD8 T cells. Levels of TUNEL and active caspase-3 reactivity revealed that at day 8 after LCMV infection virus-specific CD27hi CD8 T cells were less apoptotic than the CD27lo 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 CXCR3hi CD8 T cells were less apoptotic than the CXCR3lo 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 GP33–41 and NP396–404 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...
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 CD44hi 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 CD44hi 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 CD44hi CD62Lhi (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-1hi) 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-1hi 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 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 CD44hi CD8⁺ T cells in both PEC and spleen (data not shown). Interestingly, the proportion of MPEC in the CD44hi 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
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 \textit{ex vivo} for 5 h, followed by TUNEL staining. Activated CD44\textsuperscript{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\textsuperscript{hi} CD8\(^+\) T cells from spleen between the TCF-1 KO and WT mice (Fig. 4B).

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\textsuperscript{+} CD8\(^+\) T cells (left) or CD44\textsuperscript{hi} CD8\(^+\) T cells (middle) at day 9 (D9) and of P14\textsuperscript{+} CD8\(^+\) T cells (right) at day 30 post-LCMV infection in PEC and spleen. The MFI\(\text{ s}\) for TCF-1 expression in total day 9 P14\textsuperscript{+} CD8\(^+\) T cells (left) or CD44\textsuperscript{hi} CD8\(^+\) T cells (middle) and in day 30 P14\textsuperscript{+} 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\textsuperscript{+} 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\textsuperscript{hi} and TCF-1\textsuperscript{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.
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 node 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 have here 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-\(\gamma\)-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...
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.
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+/H11001 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+/H11001 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+/H11001 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+/H11001 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. CD44hi CD4+/H11001 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 CD44hi CD4+/H11001 T cells from nonlymphoid tissues were also less TUNEL reactive than those in lymphoid organs (Fig. 7B), further confirming the stability of CD4+/H11001 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 GP61 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...
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

**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-\(\gamma\) 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.
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\(^\alpha\) and KLRG1\(^\beta\) 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 signaling 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-7R\(\alpha\) 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 \textit{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 Tcf-1\(^{-/-}\) 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-\(\gamma\)-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.

The views are those of the authors and do not necessarily reflect the views of the NIH.

We thank Keith Daniels for technical assistance and helpful discussions. We thank R. Rohatgi for helpful discussion and critical reading of the manuscript.

We have no financial conflicts of interest.

REFERENCES


Acknowledgments

This study was supported by U.S. National Institutes of Health research grant AI017672 (R.M.W.), AI106833 (L.I.B.), and AI101301 (J.K.). This study was also supported by UMASS Diabetes Endocrinology Research Center (DERC) grant DK32520.
In summary, the liver plays a crucial role in the recruitment of antigen-specific T lymphocytes to the liver during murine infection. The liver, as a highly vascularized organ, facilitates the migration of T cells into the liver sinusoids. This process is mediated by the chemokines CXCL9 and CXCL10, which are critical for the development of murine cerebral toxoplasmosis. The recruitment of T cells to the liver is not only important for the control of infection but also for the regulation of the immune response. The liver’s ability to recruit and sustain T cells is essential for the establishment of effective immune responses against viral infections. This process involves the interaction of chemokines, chemokine receptors, and T cell subsets, highlighting the complexity of immune cell trafficking in the liver.


