The Salivary Gland Acts as a Sink for Tissue-Resident Memory CD8+ T Cells, Facilitating Protection from Local Cytomegalovirus Infection

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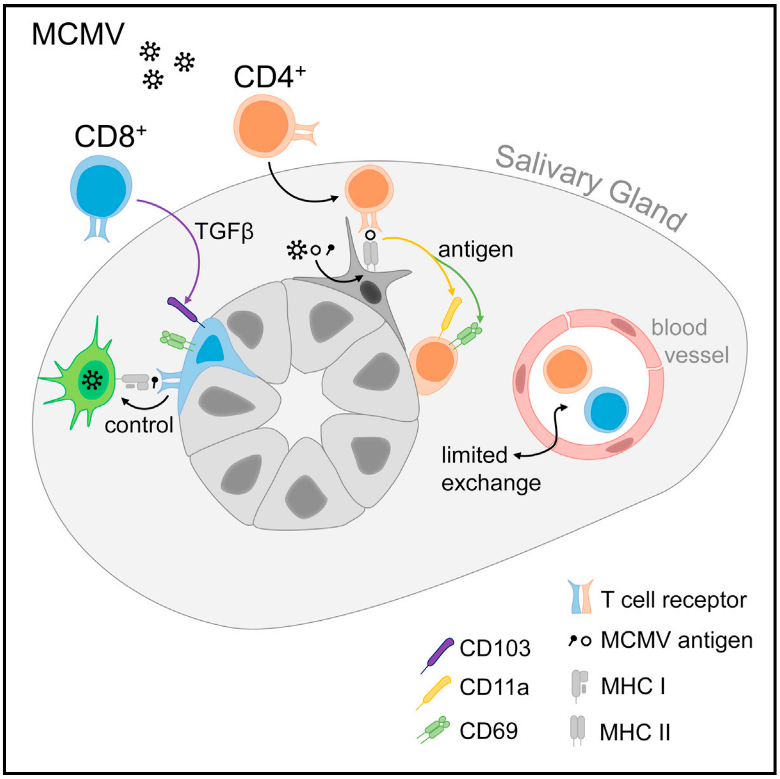
Graphical Abstract



Highlights

- MCMV infection leads to effective generation of CD8$^+$ and CD4$^+$ T_{RM} cells in the SG
- T_{RM} generation in the SG depends on cognate antigen for CD4$^+$, but not CD8$^+$, T cells
- Memory CD8$^+$ T cells protect from localized MCMV challenge infection
- Early target cells of intraglandular infection resist MHC I downregulation

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In Brief

Tissue-resident memory T (T_{RM}) cells provide efficient protection toward mucosal infection. Thom et al. show that murine cytomegalovirus (MCMV) is a potent inducer of CD8$^+$ and CD4$^+$ T_{RM} cells in the salivary glands, an important site of CMV transmission. These findings offer implications for CMV-based vaccines against mucosa-invading pathogens.

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The Salivary Gland Acts as a Sink for Tissue-Resident Memory CD8$^+$ T Cells, Facilitating Protection from Local Cytomegalovirus Infection

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SUMMARY

Tissue-resident memory T cells (T_{RM}) reside in barrier tissues and provide local immediate protective immunity. Here, we show that the salivary gland (SG) most-effectively induces CD8$^+$ and CD4$^+$ T_{RM} cells against murine cytomegalovirus (MCMV), which persists in and spreads from this organ. T_{RM} generation depended on local antigen for CD4$^+$, but not CD8$^+$, T_{RM} cells, highlighting major differences in T cell subset-specific demands for T_{RM} development. CMV-specific CD8$^+$ T cells fail to control virus replication upon primary infection in the SG due to CMV-induced MHC I downregulation in glandular epithelial cells. Using intraglandular infection, we challenge this notion and demonstrate that memory CD8$^+$ T cells confer immediate protection against locally introduced MCMV despite active viral immune evasion, owing to early viral tropism to cells that largely withstand MHC I downregulation. Thus, we unravel a yet-unappreciated role for memory CD8$^+$ T cells in protecting mucosal tissues against CMV infection.

INTRODUCTION

Memory T cells are classically divided into central memory T cells (T_{CM}) and effector memory T cells (T_{EM}) (Sallusto et al., 1999). T_{CM} cells express CD62L and CCR7, localize mainly to lymphoid organs, and rapidly expand upon antigen re-encounter. T_{EM} cells lack CD62L and CCR7 but express homing molecules that enable them to enter inflamed peripheral sites and exert immediate effector functions. Whereas T_{EM} cells can provide protection against systemic infection, their ability to contain peripheral infections is surprisingly limited (Bachmann et al., 2005; Jiang et al., 2012; Mackay et al., 2012), owing to progressive contraction and loss of tissue-homing molecules.

Early blood-based studies have established the concept of circulatory immune surveillance in which memory T cells are in constant exchange with either lymphoid or non-lymphoid tissues via the circulation. Recent advances claim for a revision of this paradigm, as a significant proportion of memory T cells resides in the periphery without recirculating (Gebhardt et al., 2009; Hawke et al., 1998; Steinert et al., 2015). These tissue-resident memory T cells (T_{RM}) are positioned at barrier tissues prone to pathogenic invasion and are superior to circulating T_{EM} cells in protecting against local secondary infections.

A large proportion of CD8$^+$ and CD4$^+$ T_{RM} cells expresses specific signatures: CD4$^+$ T_{RM} cells identified in the lung co-express CD11a and CD69 (Teijaro et al., 2011), whereas CD8$^+$ T_{RM} cells co-expressing CD103 and CD69 have been found in many tissues such as the skin, the brain, sensory ganglia, the gut, and secretory glands (Gebhardt et al., 2009; Kim et al., 1997; Klonowski et al., 2004; Pircher et al., 1990; Schön et al., 1999; Wakim et al., 2010). Secretory glands are an attractive target tissue for viruses to persist in and exploit mucosal secretions for dissemination. Human cytomegalovirus (HCMV) is prevalent in 60%–90% of the world population and is transmitted by prolonged shedding from infected mucosae such as the salivary glands (SGs). Experimental infection of mice with murine CMV (MCMV) has shown that CD8$^+$ T cells are able to control lytic virus infection in all organs with the striking exception of the SG, owing to most-efficient virally mediated MHC I downregulation in infected acinar glandular epithelial cells (Lu et al., 2006). Instead, CD4$^+$ T cells are uniquely required to cease virus replication in the SG (Lucin et al., 1992; Walton et al., 2011). This paradigm was based on studies during primary CMV infection and suggests that CD8$^+$ T cells may also be incapable of curtailing viral reactivation or superinfection events during latency. Yet, the capacities of CD8$^+$ and CD4$^+$ T cells to protect the SG from re-infection and viral reactivation have not been evaluated.

Here, we address these questions by scrutinizing MCMV-specific CD8$^+$ and CD4$^+$ memory T cells in the SG for their migratory potential, their maintenance, and their protective capacity upon localized pathogen encounter. We found an exquisite ability of the SG to induce both CD4$^+$ and CD8$^+$ T_{RM} populations, which are largely excluded from the circulation. Whereas CD8$^+$ T_{RM} induction was independent of local cognate antigen, CD4$^+$ T_{RM} generation was strictly antigen dependent. CD103 expression in CD8$^+$ T cells depended on TGFβ, coincided with localization of CD8$^+$ T cells within glandular ducts, and supported the establishment of a SG-resident CD8$^+$ T cell population but was

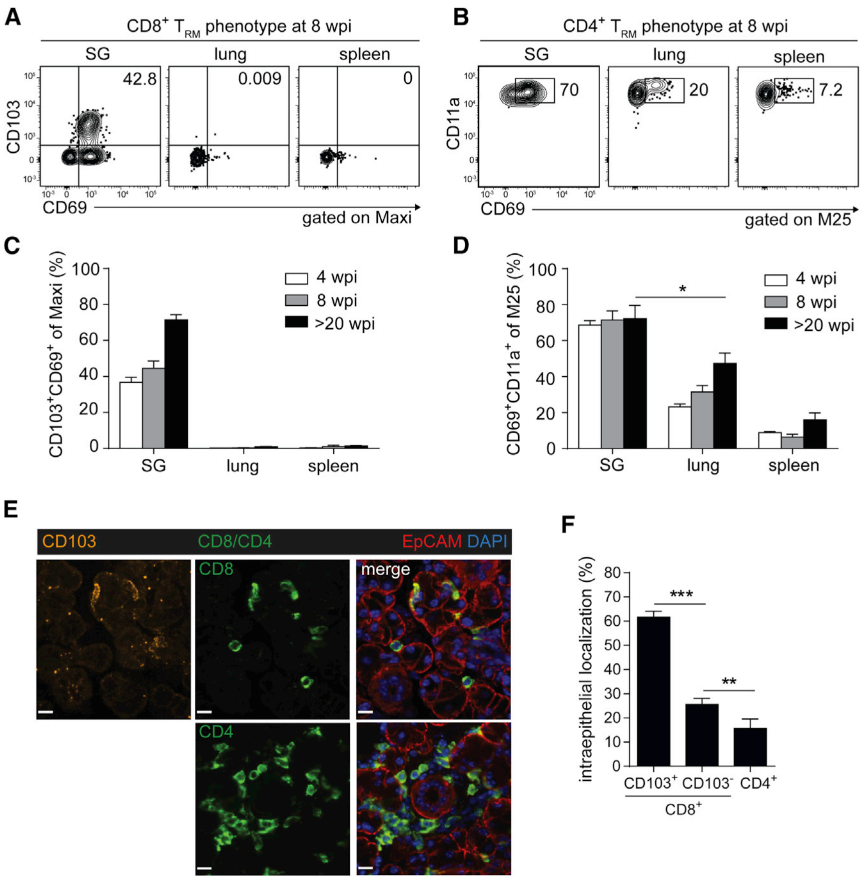

Figure 1. CD8⁺ and CD4⁺ T Cells in the SG Express T_{RM} Signatures upon MCMV Infection

Naive Maxi CD8⁺ T cells and M25 CD4⁺ T cells were adoptively transferred into naive recipients followed by i.v. infection with 5×10^6 pfu MCMV.

(A and B) Representative flow cytometry contour plots of Maxi (A) and M25 (B) T_{RM} formation in the SG, lung, and spleen at 8 wpi are shown.

(C and D) The percentage of Maxi (C) and M25 (D) cells with a T_{RM} phenotype at 4, 8, and >20 wpi is shown as mean + SEM of at least n = 10 mice per time point pooled from at least three independent experiments. *$p < 0.05$ (unpaired two-tailed t test).

(E) Sections of SG tissue at 4 wpi were stained for CD103 (orange), CD8/CD4 (green), EpCAM (red), and DAPI (blue). The scale bars represent 20 μm.

(F) Statistical analysis of CD4⁺ and CD8⁺ T cell localization in relation to EpCAM⁺ ducts. Data are shown as mean + SEM of n = 4 mice from three independent experiments. In total, the location of 638 CD8⁺ T cells and 1,069 CD4⁺ T cells was determined. **$p < 0.01$; ***$p < 0.001$ (unpaired two-tailed t test).

See also Figure S1.

not required for its long-term maintenance. Functionally, using intraglandular infection, we demonstrate that MCMV-specific memory CD8⁺ T cells provide local protection, owing to initial virus replication in non-epithelial cells that largely withstand MCMV-mediated MHC I downregulation. Our findings establish a role for MCMV-specific CD8⁺ T cells in the control of localized SG infection.

RESULTS

CD8⁺ and CD4⁺ T Cells in the SG Express T_{RM} Signatures upon MCMV Infection

T_{RM} cells afford protection against local re-infection in a number of infection models, prompting us to examine the SG's potential to form and maintain T_{RM} cells specific for CMV, a virus that persists in and spreads from this organ (Britt, 2007; Jonjić et al., 1989).

To analyze the formation of T_{RM} cells in the SG, we employed an adoptive transfer approach using monoclonal Ly5.1⁺ TCR transgenic (TCRtg) CD8⁺ and CD4⁺ T cells, which are specific for the immunodominant MCMV epitopes $M38_{316–323}$ (Maxi CD8⁺ T cells; Torti et al., 2011) and $M25_{411–425}$ (M25 CD4⁺ T cells; Jeitziner et al., 2013). Following systemic MCMV infection, Maxi cells progressively upregulated CD69 and CD103, the signature markers of CD8⁺ T_{RM} cells, exclusively in the SG (Figures 1A and 1C). The majority of M25 cells in the SG as well as a minor fraction in lung and spleen co-expressed CD11a and CD69 (Figures 1B and 1D), the signature of CD4⁺ T_{RM} cells (Teijaro et al., 2011). Comparable patterns of CD103, CD69, and CD11a expression were also observed in endogenous, non-transgenic CD8⁺ and CD4⁺ T cells (Figure S1).

With CD103 acting as a molecular tether to E-cadherin-expressing epithelial cells (Cepek et al., 1994), we reasoned that CD103⁺ CD8⁺ T cells would be selectively sequestered to epithelia in the SG. We analyzed sections of infected SG tissue by fluorescence microscopy and classified T cells located within the borders of EpCAM-labeled SG ducts and acini as intraepithelial (Figure 1E). The percentage of CD103⁺ cells localizing within epithelial ducts was significantly higher compared to CD103⁻ cells and CD4⁺ T cells (Figure 1F), indicating a selective preference of CD103⁺ CD8⁺ T cells for intraepithelial localization.

CD103 Expression in CD8⁺ and CD69 Expression in CD4⁺ Memory T Cells Identify Populations with Restricted Circulatory Interchange

Disconnection from the circulation is a key feature of T_{RM} cells and clearly demarcates them from their circulating T_{EM} or T_{CM} counterparts (Gebhardt et al., 2011). To confirm that T cells with a T_{RM} phenotype in the SG have limited access to the circulation, we performed intravascular (i.v.) labeling using fluorochrome-coupled antibodies (Anderson et al., 2014; Teijaro et al., 2011). Whereas more than 97% of Maxi cells were stained i.v.⁺ in the lung and spleen, over 80% of Maxi

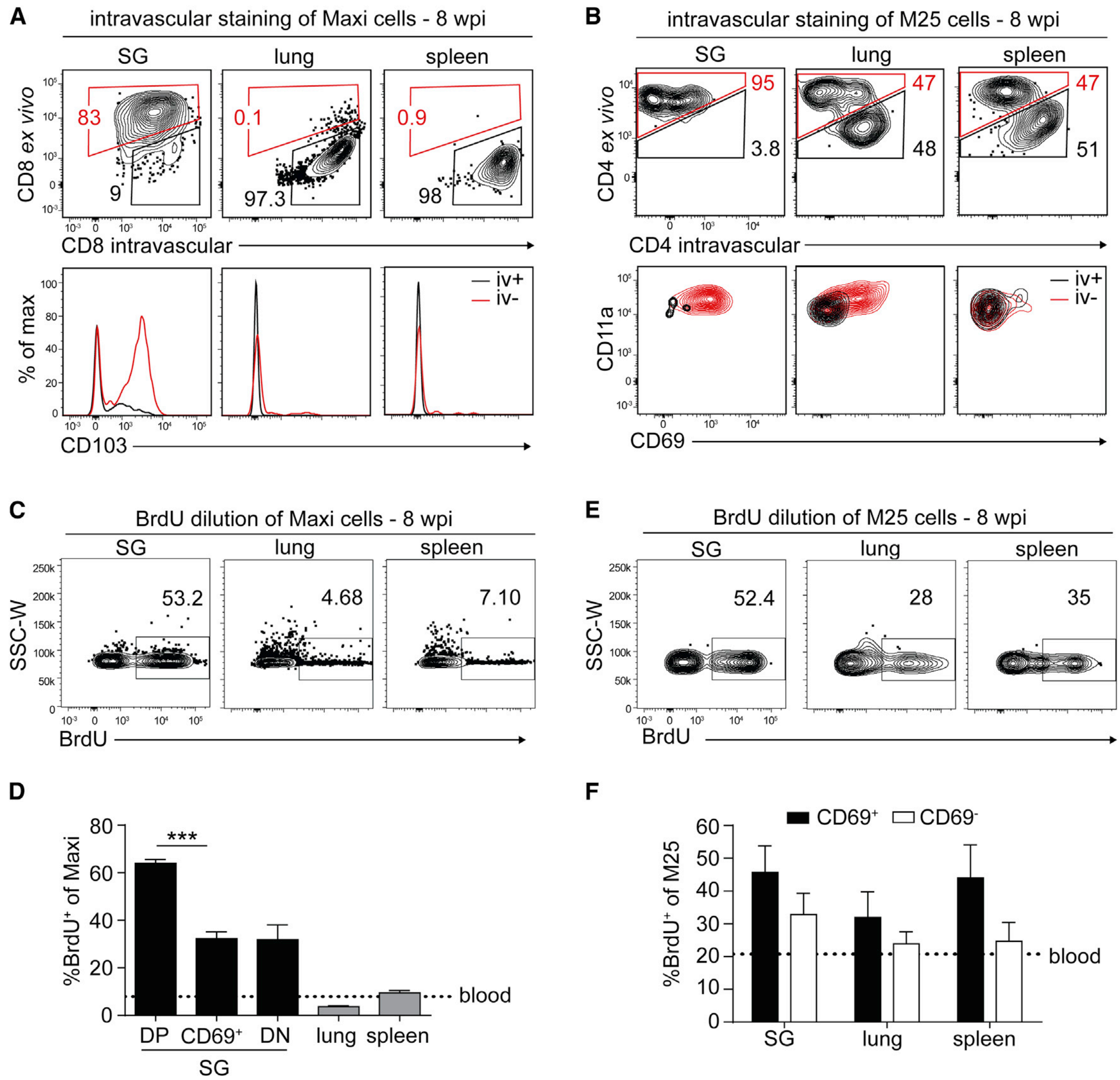

Figure 2. CD103 Expression in CD8$^+$ and CD69 Expression in CD4$^+$ Memory T Cells Indicate Restricted Circulatory Interchange

(A and B) Mice were injected i.v. with anti-CD8-PE or anti-CD4-PerCP 3 min before sacrifice. Representative contour plots of i.v. labeling and T_{RM} signature expression of Maxi (A) and M25 (B) cells are shown.

(C–F) Mice were treated with BrdU in drinking water for 2 weeks starting the day of infection. BrdU dilution was analyzed at 8 wpi. Representative contour plots of BrdU staining in Maxi (C) and M25 cells (E) are shown. Statistical analysis of %BrdU$^+$ Maxi (D) and M25 (F) cells is shown.

Data are shown as mean + SEM of n = 5 mice from one experiment. Dotted line indicates BrdU$^+$ fraction of Maxi or M25 in blood. DN, CD103 CD69 double negative; DP, CD103 CD69 double positive; ns, not significant; *$p < 0.05$; ***$p < 0.001$ (unpaired two-tailed t test). See also Figure S2.

cells in the SG were spared from i.v. labeling (i.v.$^-$; Figure 2A, top). The majority of SG-isolated i.v.$^-$ Maxi cells expressed CD103 (Figure 2A, bottom). Similarly, the majority of M25 cells was spared from i.v. labeling in the SG (Figure 2B, top), coinciding with pronounced expression of the CD4$^+$ T_{RM} signature (Figure 2B, bottom). These results were not due to insufficient antibody transfer to the SG, as i.v. labeling of CD31$^+$ endothelial cells was equally effective in the SG and the lung (Figure S2).

To gain further insight into the exchange of SG and circulating T cell compartments, we treated mice with BrdU for 2 weeks starting the day of infection, thus labeling all proliferating cells including T_{RM} cells that are seeded to the SG. A hallmark of CMV infection is the continuous proliferation of effector T cells with certain specificities (termed “memory inflation”; Karrer et al., 2003; Munks et al., 2006), yielding a growing population of circulating BrdU$^-$ CD8$^+$ T_{EM} cells, which will consequently dilute circulating BrdU$^+$ T cells after discontinuation of BrdU administration. We hypothesized that T_{RM} cells will maintain a high BrdU$^+$ fraction as a result of restricted circulatory exchange. Indeed, at 8 wpi, BrdU$^+$ Maxi cells were strongly diluted in blood, lung, and spleen (Figures 2C and 2D). In contrast, the majority of Maxi cells in the SG remained BrdU$^+$, indicating that their circulatory exchange

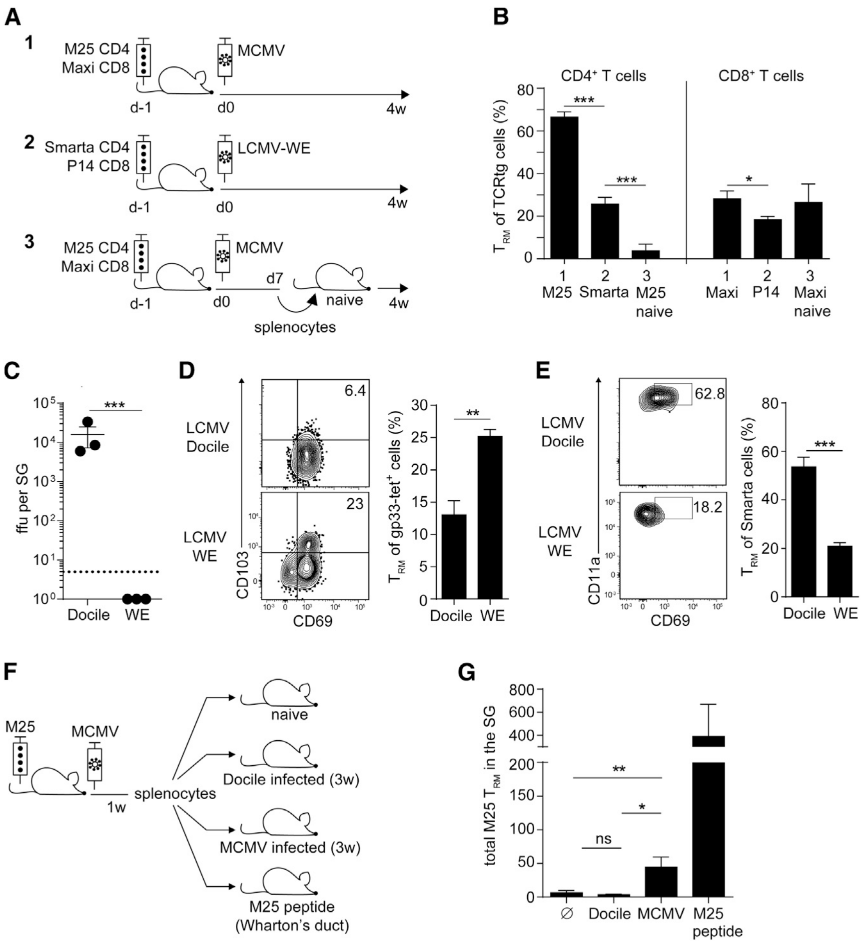

Figure 3. Local Cognate Antigen Is Required for CD4$^+$, but Not CD8$^+$, T_{RM} Formation and Maintenance

(A) Experimental setup for the generation of three different settings of antigen availability. (1) M25 and Maxi cells were adoptively co-transferred into C57BL/6 recipients with subsequent MCMV infection (5×10^6 pfu) to provide antigen in the SG and spleen. (2) Smarta and P14 cells were adoptively co-transferred into C57BL/6 mice with subsequent LCMV-WE infection (200 ffu) to provide antigen in the spleen, but not in the SG. (3) M25 and Maxi cells were adoptively co-transferred prior to MCMV infection. At 1 wpi, CD4$^+$ and CD8$^+$ splenocytes were isolated by MACS and transferred into naive recipients with no cognate antigen in the SG and spleen.

(B) CD4$^+$ and CD8$^+$ T_{RM} formation at 4 weeks post-cell transfer is shown as mean + SEM of at least n = 10 mice pooled from two independent experiments.

(C) Mice were infected i.v. with 10^6 ffu LCMV Docile or 200 ffu LCMV-WE. Viral titers in the SG were determined at 8 wpi.

(D and E) Representative contour plots (left) and statistical analysis (right) of gp33-tet$^+$ CD8$^+$ T cells 8 wpi (D) or Smarta CD4$^+$ T cells 4 wpi (E) with 200 ffu LCMV-WE or 10^6 ffu LCMV Docile are shown as mean + SEM of n = 5 mice from one experiment.

(F) Experimental setup: in-vivo-activated spleen-derived M25 cells were transferred into naive mice, mice infected for 3 weeks with LCMV Docile, mice infected for 3 weeks with MCMV, or naive mice that received the M25 peptide via the excretory duct of the SG at the day of transfer.

(G) Total M25 cells with a T_{RM} phenotype at 4 weeks posttransfer. Transfer to naive: n = 15 mice; transfer to Docile infected: n = 6 mice; transfer to MCMV infected: n = 8 mice; transfer to mice that received peptide: n = 2 mice.

In (B)–(G), *p < 0.05; **p < 0.01; ***p < 0.001 (unpaired two-tailed t test). See also Figure S3.

was significantly limited. Among Maxi cells in the SG, the CD103$^+$ CD69$^+$ subset retained the largest BrdU$^+$ fraction (Figure 2D), suggesting CD103 as a marker of CD8$^+$ T cells with restricted recirculation. M25 T cells do not inflate, resulting in higher BrdU$^+$ frequencies in circulating compartments (Figures 2E and 2F). Nevertheless, M25 cells in the SG retained significantly higher proportions of BrdU$^+$ cells compared to blood, and CD69$^+$ M25 cells in all compartments maintained a higher BrdU$^+$ fraction than their CD69$^-$ counterparts. As an alternative explanation, the BrdU disequilibrium might have been caused by differences in organ-specific cell division rates, i.e., T cells in the SG might divide less rapidly than in the lung, thus retaining a larger BrdU fraction. However, if circulatory exchange was possible, one would assume that the resulting discrepancy in BrdU levels would equilibrate between these compartments.

Taken together, these data indicate that MCMV infection results in substantial formation of MCMV-specific SG-resident CD8$^+$ and CD4$^+$ T_{RM} cell populations that express T_{RM} signatures, show restricted vascular accessibility, and are largely disconnected from circulatory interchange.

Local Cognate Antigen Is Required for CD4$^+$, but Not CD8$^+$, T_{RM} Formation and Maintenance

The presence of both CD8$^+$ and CD4$^+$ T_{RM} cells within the same organ allowed us to directly compare the requirements for their recruitment, retention, and T_{RM} phenotype induction. Previous studies indicate that the tissue of residence dictates whether local antigen is required for CD8$^+$ T_{RM} formation (reviewed in Gebhardt and Mackay, 2012). We assessed the requirement of cognate antigen for T_{RM} formation in the SG by three experimental setups with varying local and systemic, i.e., splenic, antigen availability (Figure 3A). To create a situation providing cognate antigen in the spleen and locally in the SG, we adoptively transferred M25 or Maxi T cells and subsequently infected mice with MCMV (Figure 3A, panel 1). To provide antigen in the spleen, but not in the SG, LCMV-specific TCRtg Smarta CD4$^+$ T cells and P14 CD8$^+$ T cells were transferred with subsequent low-dose LCMV-WE infection (Figure 3A, panel 2), which does not lead to SG infection (Hofmann and Pircher, 2011). To evaluate T_{RM} induction in absence of systemic and local SG antigen, we transferred purified, previously in vivo MCMV-activated Maxi and M25 cells into naive recipients (Figure 3A, panel 3).

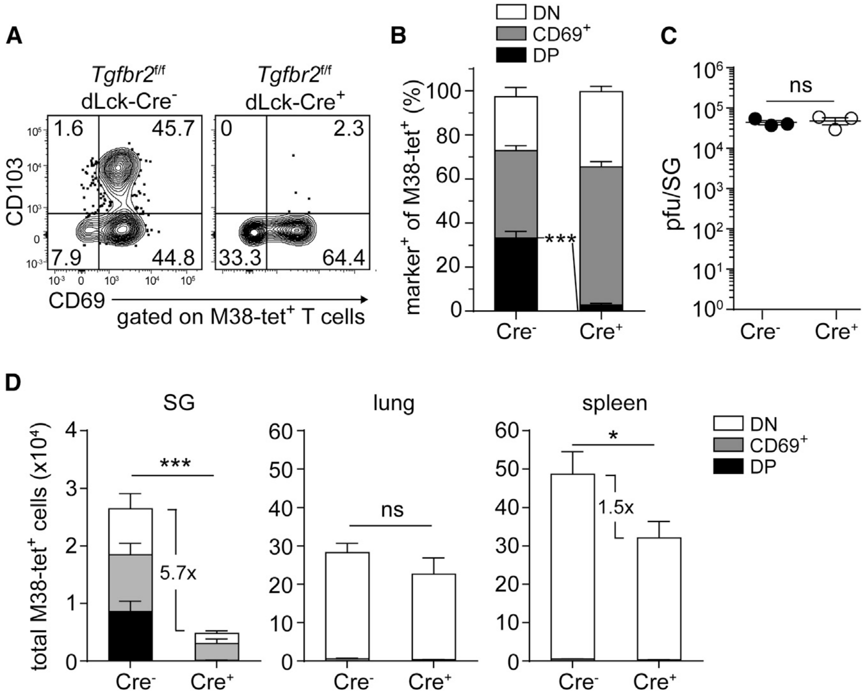

Figure 4. TGFβ Is Required for Induction of CD103, but Not CD69, Expression

Tgfbr2$^{f/f}$ dLck-Cre^{+} mice and *Tgfbr2*$^{f/f}$ dLck-Cre^{-} littermates were infected i.v. with 5 × 10^6 pfu MCMV, and M38-specific cells were tracked by tetramer staining at 4 wpi.

(A) Representative contour plots of M38-tet^{+} cells are shown.

(B) Percentages of M38-tet^{+} cells expressing CD103 and CD69 are shown as mean + SEM of n = 10 mice pooled from two independent experiments.

(C) Viral titers of SG tissue at 4 wpi.

(D) Total numbers of M38-specific CD8^{+} T cells are shown as mean + SEM of n = 10 mice pooled from two independent experiments.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (unpaired two-tailed t test). See also Figure S4.

Restriction of systemic and local antigen negatively affected T_{RM} formation in CD4^{+}, but not in CD8^{+}, TCRtg T cells at 4 weeks posttransfer (Figure 3B), correlating with total T cell numbers in the SG: whereas both CD4^{+} and CD8^{+} TCRtg T cells were detectable at 4 weeks posttransfer regardless of whether antigen was present, CD4^{+}, but not CD8^{+}, TCRtg T cells were strikingly reduced at 8 weeks posttransfer, and the magnitude of reduction correlated with the deprivation of antigen (Figure S3). Previously published data suggest that sustained antigen presence prevents CD8^{+} T_{RM} differentiation in the gut (Casey et al., 2012). We analyzed the phenotype of LCMV-specific CD8^{+} T cells in the SG after chronic infection with LCMV Docile, leading to pronounced LCMV replication in the SG (Figure 3C). We found that CD8^{+} T cells were significantly impaired in CD103 expression during chronic LCMV Docile compared to resolved LCMV-WE infection (Figure 3D), supporting a negative impact of persistent antigen on CD8^{+} T_{RM} formation in the SG. In line with our hypothesis that presence of cognate antigen enables CD4^{+} T_{RM} formation, T_{RM} signature expression in Smarta CD4^{+} T cells was significantly enhanced in chronically infected mice (Figure 3E).

Our data clearly indicate that CD8^{+} T_{RM} formation is independent of both antigen and inflammation. Next, we analyzed whether CD4^{+} T_{RM} formation relied on cognate antigen or whether unspecific inflammation was sufficient to induce CD4^{+} T_{RM} cells in the SG. We transferred in-vivo-activated, spleen-derived M25 CD4^{+} T cells into mice that had been infected for 3 weeks with LCMV Docile (virus replication in the SG and other organs; no cognate antigen) or mice that had been infected with MCMV for 3 weeks (virus replication only in the SG, but not in other organs) and naive mice that received the M25 peptide via the excretory duct of the SG at the day of transfer (Figure 3F). We found that the abundance of M25 cells with a T_{RM} phenotype was significantly elevated only when cognate antigen was present (Figure 3G).

Taken together, these data show that, whereas both CD8^{+} and CD4^{+} T cells can migrate to the SG independently of cognate antigen, only CD4^{+} T cells require local antigen for tissue maintenance and T_{RM} formation.

TGFβ Is Required for Induction of CD103, but Not CD69, Expression

TGFβ is a known inducer of CD103 in CD8^{+} T cells in various experimental systems (Casey et al., 2012; El-Asady et al., 2005; Lee et al., 2011). We analyzed the endogenous MCMV M38-specific CD8^{+} T cell response in mice exhibiting a T-cell-specific deletion of the TGFβRII (*Tgfbr2*$^{f/f}$ dLck-Cre mice; Zhang and Bevan, 2013). At 4 wpi, M38-specific CD8^{+} T cells in the SG were severely impaired in CD103 and CD69 co-expression in contrast to Cre^{-} littermates (Figures 4A and 4B) whereas viral titers were comparable in both mouse strains (Figure 4C). Whereas CD103 expression in CD8^{+} T cells was completely dependent on TGFβ, CD69 expression was not. *Tgfbr2*$^{f/f}$ dLck-Cre mice showed a significant reduction of the M38-specific CD8^{+} T cell pool in the SG, reaching a 5.7× reduction compared to Cre^{-} controls at 4 wpi (Figure 4D). This reduction could not only be explained by the absence of the CD103^{+} fraction, as the CD69^{+} and CD69^{-}CD103^{-} M38-specific CD8^{+} T cell populations were also strikingly reduced. Hence, besides being essential for CD103 induction in CD8^{+} T cells in the SG, TGFβ also promotes the size of the SG-resident CD8^{+} T cell population by additional, CD103-unrelated mechanisms. Consistent with the CD8^{+} T cells, CD4^{+} T cells in the SG of *Tgfbr2*$^{f/f}$ dLck-Cre mice were not restricted in CD69 expression; neither were they affected to express high levels of CD11a, and the size of the endogenous CD4^{+} T_{RM} population was unaffected (Figure S4A). The dependence of CD103 expression on TGFβ in the SG was confirmed using mice expressing a T-cell-specific dominant-negative variant of the TGFβRII (Gorelik and Flavell,

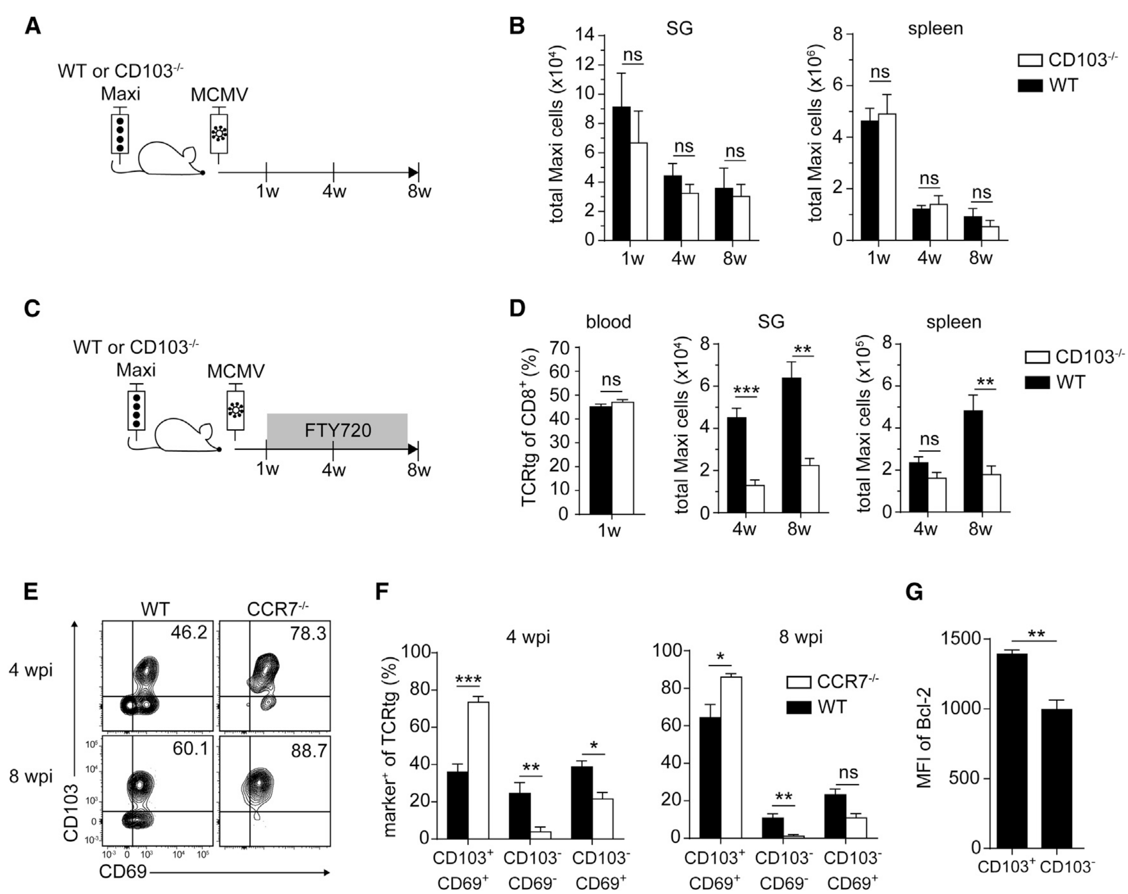

Figure 5. CD103 Promotes Initial Establishment, but Not Long-Term Maintenance, of CD8$^+$ T$_{RM}$ Cells

(A) Experimental setup. CD103$^{-/-}$ Maxi and WT Maxi cells were transferred separately into C57BL/6 hosts with subsequent MCMV infection.

(B) Total numbers of CD103$^{-/-}$ and WT Maxi cells are shown as mean + SEM of n = 10 mice pooled from two independent experiments.

(C) Experimental setup. CD103$^{-/-}$ Maxi and WT Maxi cells were transferred, and recipients were infected as in (A). Mice were treated with 5 μg/ml FTY720 in drinking water starting 1 wpi.

(D) Percentages of CD103$^{-/-}$ and WT Maxi in blood and total cell numbers in SG and spleen are shown as mean + SEM of n = 10 mice pooled from two independent experiments.

(E) Representative contour plots of CCR7$^{-/-}$ Mini and WT Mini cells in SGs of C57BL/6 recipients at 4 and 8 wpi are shown.

(F) Statistical analyses of T$_{RM}$ markers at 4 and 8 wpi in CCR7$^{-/-}$ Mini and WT Mini cells are shown as mean + SEM of n = 5 mice from one experiment.

(G) MFI of Bcl-2 expression in CD103$^+$ and CD103$^-$ Maxi cells in the SG at 8 wpi is shown as mean + SEM of n = 5 mice representative of three independent experiments.

In (B), (D), (F), and (G), *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (unpaired two-tailed t test).

2000; Figure S4B). IL-15 was previously identified as an inducer of CD103 in CD8$^+$ T$_{RM}$ cells in the skin (Mackay et al., 2013). By transferring in-vivo-activated Maxi cells into naive IL-15$^{-/-}$ recipients, we demonstrate that CD103 and CD69 co-expression was not compromised in the SG in absence of IL-15 (Figure S4C). Apart from TCR stimulation, IFNα induces CD69 expression in T cells (Shiow et al., 2006). We co-transferred wild-type (WT) P14 cells and P14 cells that lack the IFNα receptor (IFNAR$^{-/-}$ P14) into naive hosts followed by infection with recombinant Vaccinia virus expressing the glycoprotein of LCMV (VV-G2) and found that both IFNAR$^{-/-}$ P14 as well as WT P14 were not compromised in CD8$^+$ T$_{RM}$ formation (Figure S4D).

Taken together, these data indicate that CD8$^+$ T cells in the SG depend on TGFβ for CD103 expression, TGFβ additionally exerts a CD103-independent role in the establishment of a MCMV-specific CD8$^+$ T cell population, and IL-15 does not promote CD103 upregulation in the SG.

CD103 Promotes Initial Establishment, but Not Long-Term Maintenance, of CD8$^+$ T$_{RM}$ Cells

CD103 supports T cell retention in tissues by tethering CD8$^+$ T cells to epithelial cells via interaction with E-cadherin (Cepek et al., 1994) and upregulation of the anti-apoptotic molecule Bcl-2 (Mackay et al., 2013). To investigate whether CD103$^+$ CD8$^+$ T cells in the SG benefit from these mechanisms, we transferred CD103-deficient Maxi cells (CD103$^{-/-}$ Maxi) into C57BL/6 recipients and compared their abundance with WT Maxi in the SG following MCMV infection (Figure 5A). We found that both cell populations were equally well established and maintained in the SG and spleen (Figure 5B). MCMV-derived antigen presentation in lymph nodes (LNs) reactivates local CD8$^+$ T$_{CM}$ cells, which subsequently migrate to peripheral tissues to contribute to a constant effector CD8$^+$ T cell pool (Torti et al., 2011). A small proportion of these reactivated CD8$^+$ T cells also gains access to the SG, as demonstrated by the presence of BrdU$^-$ cells in the SG at 8 wpi (Figures 2C and 2D). We therefore investigated whether continuous recruitment of new effector CD8$^+$ T cells renders CD103 dispensable for the preservation of the CD8$^+$ T$_{RM}$ population in the SG. We restricted reseeding to the SG from LNs by abrogating LN egress of T cells via FTY720 treatment starting at 1 wpi (Figure 5C). Despite their ability to expand to equal levels in the blood at 1 wpi and to maintain their population in the spleen at 4 wpi, CD103$^{-/-}$ Maxi cells were markedly reduced compared to WT Maxi in the SG (Figure 5D). At 8 wpi, this deficit was still evident in the SG; however, total numbers of CD103$^{-/-}$ Maxi cells remained constant, indicating that CD103 is not required for long-term maintenance but can support the initial establishment of the SG-resident Maxi population. However, this supportive function of CD103 is dispensable when LN resupply is available.

The reduced numbers of CD103$^{-/-}$ CD8$^+$ T cells in the SG could either be due to a survival deficit or due to selective egress of CD103$^-$ CD8$^+$ T cells from the SG. To investigate the latter option, we transferred and tracked MCMV-specific CD8$^+$ T cells that lack CCR7, a receptor required for egress from peripheral tissues (Bromley et al., 2005; Debes et al., 2004) as well as for T cell entry into LNs (Förster et al., 1999). Being incapable of tissue emigration, CCR7$^{-/-}$ CD8$^+$ T cells are trapped in the respective organ. Furthermore, CCR7$^{-/-}$ CD8$^+$ T cells are incapable of entering LNs, thus their seeding to the periphery relies solely on the spleen.

CCR7$^{-/-}$ CD8$^+$ T cells in the SG were strongly skewed toward a T$_{RM}$ phenotype, with CD103$^-$ cells being essentially absent by 8 wpi (Figures 5E and 5F), indicating that the reduction of CD103$^-$ cells is not a result of selective tissue emigration but rather of a survival deficit. In line with this hypothesis, CD103$^+$ Maxi cells expressed significantly elevated levels of the anti-apoptotic molecule Bcl-2 compared to CD103$^-$ Maxi cells (Figure 5G). Taken together, these data suggest that CD103 can fulfill the previously described functions to support the maintenance of SG-resident CD8$^+$ T cells; however, its role becomes dispensable in the context of CMV infection due to continuous resupply of CD8$^+$ T cells.

CD8$^+$ T Cells Have Protective Capacity upon Local Infection

CD8$^+$ T cells are sufficient to control lytic MCMV replication in all organs except the SG, where MCMV-mediated MHC I downregulation in infected acinar glandular epithelial cells renders CD8$^+$ T cells inoperative and CD4$^+$ T cells are required for viral control (Jonjić et al., 1989; Walton et al., 2011). Whether CD4$^+$ T cells are also responsible to control viral reactivation or re-infection events is not known. To investigate this, we introduced virus via intraglandular (i.g.) injection (Pilgrim et al., 2007). Infection of one of the two submandibular SG lobes with 10^4 pfu MCMV resulted in confined virus replication to the injected lobe during the first 11 days postinjection (Figure 6A), validating this method to study the control of localized infection. We lodged in-vivo-activated Maxi or M25 cells in the SGs of naive recipients for 5 weeks and determined whether their presence affected virus titers at 4 days post-i.g. infection (Figure 6B, left). More than 70% of mice harboring T Maxi cells could completely control i.g.-administered virus, as opposed to mice that did not receive T cells via adoptive transfer or had received M25 cells (Figure 6B, right). Maxi cells located in the SG were readily capable to exert effector functions, as judged by the expression of IFNγ and the degranulation marker CD107a (Figures 6C and 6D). These data suggest that, despite their restricted potency to control primary systemic infection, memory CD8$^+$ T cells in the SG can control locally introduced MCMV.

Cells Targeted by Local Infection Withstand Complete CMV-Mediated MHC I Downregulation

CMV-mediated MHC I downregulation in the SG substantially contributes to the inability of CD8$^+$ T cells to control virus replication (Lu et al., 2006; Walton et al., 2011). To understand why memory Maxi T cells were able to control local infection, we characterized the early cell types targeted by i.g. injection. We visualized infected cells using a GFP expressing MCMV strain (MCMV-GFP). Cells infected by i.g. injection extended dendrites and were arranged in a network (Figures 7A and 7B), reminiscent of the previously described macrophage network in the SG (Thom et al., 2014). This morphology differed substantially from that of infected acinar glandular epithelial cells, the cell type of viral persistence (Figures 7C and 7D) at 2 wpi, suggesting that the virus initially replicates in cells of non-epithelial origin before occupying its niche of persistence. At 4 days post-i.g. infection, both hematopoietic and non-hematopoietic cells were infected and a fraction of the hematopoietic targets expressed CD11c, indicative of SG-resident APCs (Figures 7E and 7F). Infected non-hematopoietic cells were not EpCAM$^+$, confirming that epithelial cells are not the primary target of locally introduced MCMV (Figure 7G). Despite a detectable onset of MHC I downregulation, MHC I was abundant in infected non-hematopoietic (Figure 7G) and hematopoietic cells (Figure 7H), indicating that cells targeted early by i.g. infection largely resist virally mediated MHC I downregulation—at least up to 4 dpi. Thus, early local re-infection provides a window of opportunity for readily positioned CD8$^+$ T$_{RM}$ cells to recognize infected cells and control virus replication despite the presence of viral immune evasion genes. In contrast to the abundance of MHC I molecules, MHC II expression was rare and confined to a minority of CD11c$^+$ hematopoietic targets (Figure 7H), providing an explanation why CD8$^+$ T cells were superior in controlling i.g. infection compared to CD4$^+$ T cells.

DISCUSSION

CMV infection leads to protracted infection of the SG, fostering horizontal virus transmission in mice and humans. Our data highlight the SG's exquisite capacity to instruct and lodge CD8$^+$ and CD4$^+$ T$_{RM}$ cells following MCMV infection. Whereas both MCMV-specific CD4$^+$ and CD8$^+$ T$_{RM}$ cells were maintained long term in the SG after control of lytic replication, the requirements for their local induction, their micro-anatomical localization, and their protective capacities in localized infection events differed substantially.

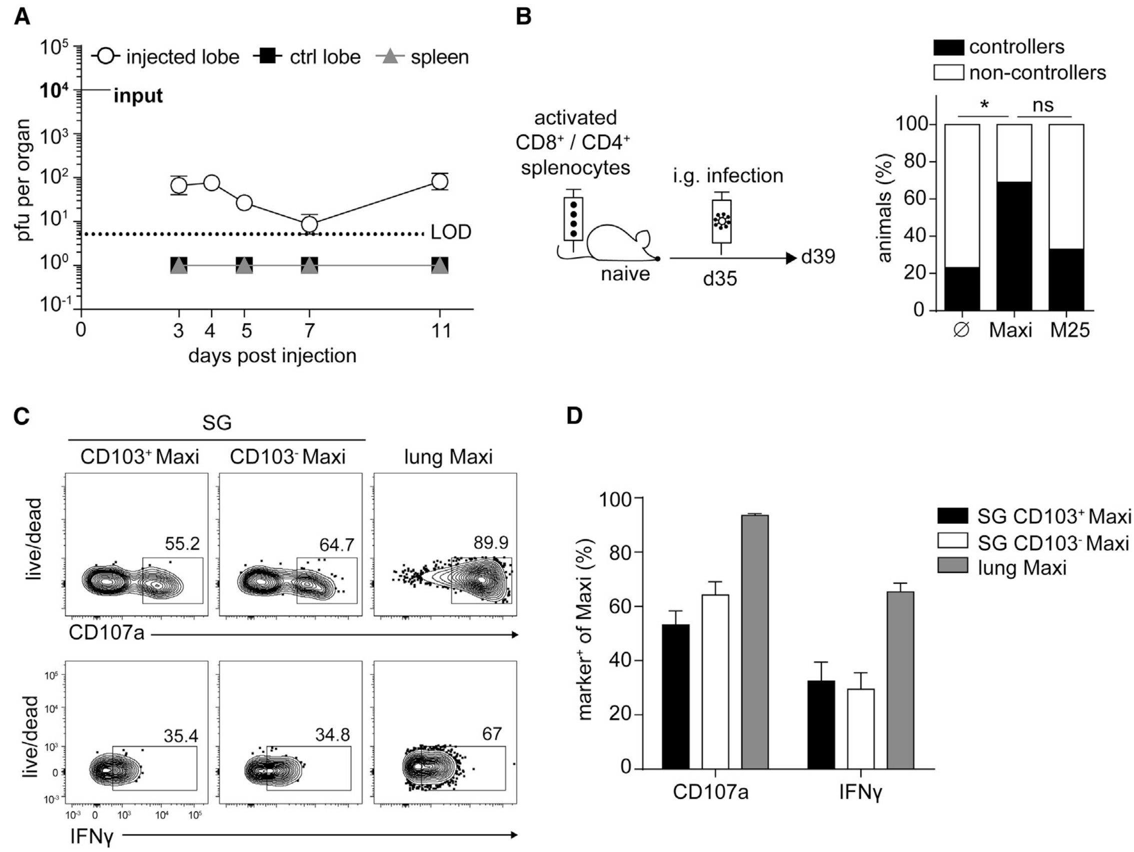

Figure 6. CD8$^+$ T Cells Have Protective Capacity upon Local Infection

(A) Virus kinetics after i.g. infection of one SG lobe with 10^4 pfu MCMV (input). Data are shown as mean ± SEM of n = 4 mice for each time point. LOD, limit of detection.

(B) Experimental design to assess the protective capacity of Maxi and M25 cells (left). Maxi and M25 cells were adoptively transferred into C57BL/6 recipients followed by MCMV infection. At 1 wpi, CD8$^+$ and CD4$^+$ T cells were isolated from spleens by MACS purification and injected into naive recipients. After 5 weeks, recipients were infected i.g. with 10^4 pfu MCMV to both SG lobes and virus titers were determined 4 days later. Percentage of controllers and non-controllers at 4 days after i.g. MCMV infection is shown (right). Results are pooled data from at least n = 10 mice per group from three independent experiments. *$p < 0.05$ (Fisher's exact test).

(C and D) T cells from SG and lung tissue were restimulated with M38 peptide. (C) Representative contour plots gated on Maxi cells showing IFNγ and CD107a staining are shown. (D) Statistical analysis of IFNγ and CD107a staining upon restimulation is shown as mean + SEM of n = 5 mice, representative of two independent experiments.

Previous studies indicate that the necessity of local antigen for T_{RM} formation is largely dictated by the organ of residence. Whereas antigen-independent CD8$^+$ T_{RM} induction is supported in the gut, the skin, the female reproductive tract, as well as in the SG upon LCMV infection (Casey et al., 2012; Hofmann and Pircher, 2011; Mackay et al., 2012), CD8$^+$ T_{RM} cell formation in the brain requires local antigen (Wakim et al., 2010). We show here that, in contrast to CD4$^+$ T cells, local antigen is dispensable for T_{RM} induction in MCMV-specific CD8$^+$ T cells in the SG, indicating that T cell subset-specific demands are different and outweigh organ-specific effects. Previous data indicate that lung-resident CD4$^+$ T cells acquire T_{RM} signatures independently of local cognate antigen (Teijaro et al., 2011), suggesting that the necessity of antigen for CD4$^+$ T_{RM} development depends on the tissue of residence.

Cytokines play a critical role in T_{RM} development. The expression of CD103 and CD69 phenotypically characterizes a major proportion of CD8$^+$ T_{RM} cells, and TGFβ is an instructive signal for CD103 expression. We confirm that this is also the case for MCMV-specific CD8$^+$ T_{RM} cells forming after MCMV infection in the SG. In line with our results, MCMV-specific T cells upregulated CD103 in response to TGFβ stimulation in vitro and MCMV infection did not increase levels of TGFβ protein in the SG, providing an explanation why the naive SG supports CD103 upregulation equally well as the MCMV-infected SG (Smith et al., 2015, this issue of *Cell Reports*). As IL-15 was recently shown to induce CD103 expression in HSV-specific CD8$^+$ T cells in the skin (Mackay et al., 2013), we assessed its role for CD8$^+$ T_{RM} induction in the SG but found no evidence for an involvement. The mechanisms leading to constitutive expression of CD69 on T_{RM} cells remain unclear. The reduced CD69 expression in CD8$^+$ T_{RM} cells in the gut of *Tgfbr2$^{f/f}$* dLck-Cre mice suggested a role for TGFβ in CD69 upregulation (Zhang and Bevan, 2013). Our data show that neither MCMV-specific CD8$^+$ nor

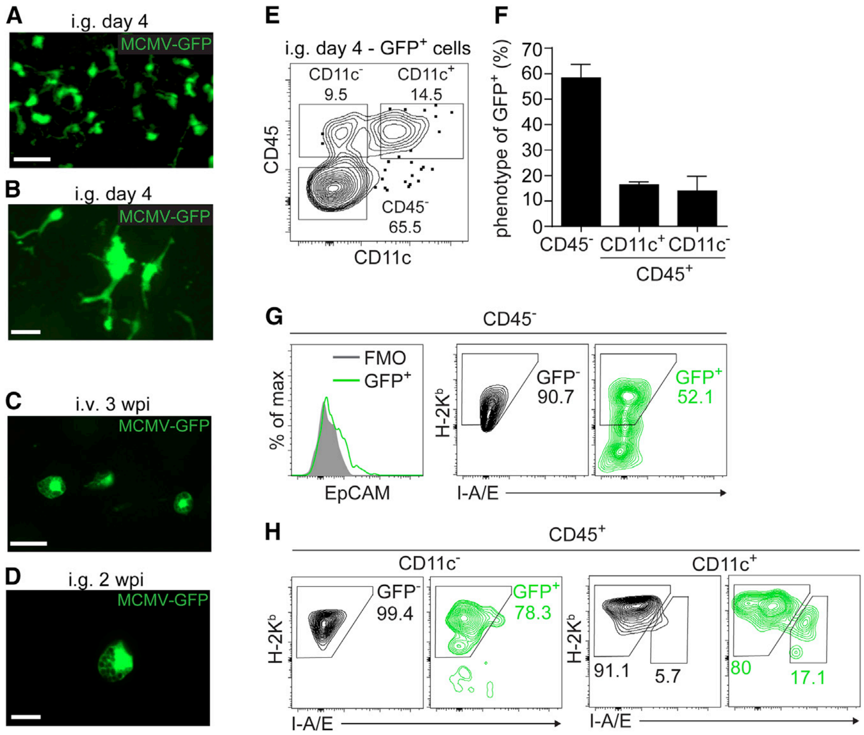

Figure 7. Cells Targeted by Local Infection Withstand to Complete CMV-Mediated MHC I Downregulation

(A and B) Sections of SG tissue at 4 days post-i.g. infection with 5×10^5 pfu MCMV-GFP representative of n = 3 mice. The scale bar represents 50 μm (A) and 20 μm (B).

(C) Section of SG tissue at 3 weeks post-i.v. infection with 5×10^6 pfu MCMV-GFP representative of n = 3 mice. The scale bar represents 50 μm.

(D) Section of SG tissue at 2 weeks post-i.g. infection with 5×10^5 pfu MCMV-GFP representative of n = 3 mice. The scale bar represents 20 μm.

(E–H) Flow cytometry analysis of SG isolated cells targeted by MCMV-GFP at 4 days post-i.g. infection. Infected cells were distinguished from non-infected cells by GFP expression. Results are representative of three independent experiments.

(E) Representative contour plot of GFP^+ cells showing CD45 and CD11c expression is shown.

(F) Statistical analysis of cell types infected after i.g. MCMV-GFP infection is shown. Shown are means + SEM of n = 3 mice from one experiment.

(G) Representative histogram and contour plot of EpCAM, MHC I, and MHC II expression of non-hematopoietic ($CD45^-$) GFP^+ cells are shown.

(H) Representative contour plots of MHC I and MHC II expression of hematopoietic ($CD45^+$) GFP^+ cells are shown.

$CD4^+$ T_{RM} cells in the SG required TGFβ signaling for CD69 expression. Type I interferons are also known to induce CD69 expression (Shiow et al., 2006), but type I interferon receptor signaling was not required for the sustained expression of CD69 on MCMV-specific T_{RM} cells in the SG.

In many cases, CD103 plays a central role in $CD8^+$ T_{RM} cell maintenance through two mechanisms: CD103 promotes T cell survival by upregulation of the anti-apoptotic molecule Bcl-2 (Mackay et al., 2013; Wakim et al., 2010) and exerts adhesive function by tethering T cells to E-cadherin (Cepek et al., 1994). Mechanistically, $CD103^+$ $CD8^+$ T_{RM} cells in the SG appear to profit from both mechanisms, indicated by elevated levels of Bcl-2 as well as preferential localization within epithelial ducts compared to $CD103^-$ $CD8^+$ T cells and $CD4^+$ T cells. However, in light of constant resupply by reactivated $CD8^+$ memory T cells from LNs (Torti et al., 2011) and vascular sources (Smith et al., 2014), CD103 is functionally dispensable in the SG. These data suggest that the importance of CD103 for $CD8^+$ T_{RM} maintenance might be dictated by the nature of infection. Most $CD4^+$ T_{RM} cells do not express CD103, excluding a function in $CD4^+$ T_{RM} maintenance. It remains to be determined whether $CD4^+$ T_{RM} retention is achieved by CD69 through its inhibitory effect on cell surface expression of the sphingosine-1-phosphate receptor 1 (S1P1), which is required for T cell egress from organs (Bankovich et al., 2010; Matloubian et al., 2004).

The fact that MCMV infection promotes the establishment of considerable populations of both MCMV-specific $CD4^+$ and $CD8^+$ T_{RM} cells specifically in the SG suggests that the strategic positioning of these cells at relevant re-entry sites of the virus affords immediate local protection and hence curtails horizontal transmission upon re-infection. During primary MCMV infection, $CD4^+$ T cells are the only cell type capable of controlling lytic infection in the SG (Jonjić et al., 1989). The inability of $CD8^+$ T cells to control lytic MCMV replication in the SG upon primary infection is due to the profound virally mediated downregulation of MHC I expression on the main long-term target cells of MCMV, the acinar glandular epithelial cells (Walton et al., 2011). We therefore hypothesized that MCMV-specific $CD8^+$ T_{RM} cells would also be incapable of controlling local re-infection events. Contrary to our expectations, memory $CD8^+$ T cells were superior in providing local protection compared to memory $CD4^+$ T cells. Detailed analysis of the cell types permissive for MCMV infection and replication at early time points in the SG revealed that MCMV targeted largely non-epithelial cells and, to a lesser extent, hematopoietic cells including tissue macrophages. MCMV failed to achieve complete MHC I downregulation in these early SG target cells, thereby providing a window of opportunity for $CD8^+$ T_{RM} cells to detect viral antigen and control virus replication. Thus, the potential of $CD8^+$ T_{RM} cells to confer immediate protection against localized SG infection illustrates that the effort of the SG in hosting a sizeable population of $CD8^+$ T_{RM} cells indeed has physiological advantages that are explained by the altered composition of MCMV-targeted cell types at early time points of localized infection.

So far, CMV superinfection studies were mainly conducted using the subcutaneous route of re-infection, which is likely leading to a systemic viral challenge (Böhm et al., 2008; Hansen et al., 2010; Holtappels et al., 2008; Reddehase et al., 1987). Systemic memory $CD8^+$ T cells were shown to protect against lethal challenge in immunocompromised murine hosts (Böhm et al., 2008; Holtappels et al., 2008) or to provide protection against systemic reactivation of CMV in immune-suppressed organ transplant recipients (Bunde et al., 2005; Nauerth et al., 2013; Scheinberg et al., 2009). Furthermore, expression of CMV-encoded immune evasion genes led to the inability of CMV-specific $CD8^+$ T cells to restrict subcutaneous CMV superinfection (Nauerth et al., 2013). Here, we show that CMV-encoded immune evasion genes do not compromise the ability of $CD8^+$ memory T cells in protecting against re-infection with CMV.

In summary, we delineate the extraordinary capacity and the mechanisms by which the SG accommodates T_{RM} memory cells in an epidemiologically relevant infection model and demonstrate the protective capacity of memory $CD8^+$ T cells upon localized MCMV infection of the SG, highlighting a yet-unappreciated role of $CD8^+$ T cells in this organ. The mechanisms by which SG $CD8^+$ T_{RM} cells can mediate this immunity may involve direct cytotoxicity and effector cytokine production or the establishment of a broad, organ-wide antiviral state, such as shown for $CD8^+$ T_{RM} cells in the female reproductive tract and the skin (Ariotti et al., 2014; Schenkel et al., 2014).

EXPERIMENTAL PROCEDURES

Ethics Statement

This study was conducted in accordance to the guidelines of the animal experimentation law (SR 455.163; TVV) of the Swiss Federal Government. The protocols were approved by the Cantonal Veterinary Office of the canton Zurich, Switzerland (permit number 109/2011, 110/2011, 127/2011).

Mice

All mice were housed and bred (C57BL/6 mice, $CCR7^{-/-}$ mice [Förster et al., 1999], and CD11c-YFP reporter mice [Lindquist et al., 2004]) in SPF facilities. $IL15^{-/-}$ mice (Kennedy et al., 2000) were provided by Prof. O. Boyman (University Hospital of Zurich). DnTGFβRII mice (Gorelik and Flavell, 2000) were provided by Dr. J. Herkel (Universitaetsklinikum Hamburg-Eppendorf). *Tgfbr2*$^{f/f}$ dLck-Cre mice (Zhang and Bevan, 2012) were a gift of Prof. M. Bevan (University of Washington). Maxi transgenic ($Ly5.1^+$) mice express a TCR specific for the MCMV peptide $M38_{316–323}$ (Torti et al., 2011) M25 transgenic ($Ly5.1^+$) mice express a TCR specific for the MCMV peptide $M25_{410–425}$ (Mandaric et al., 2012). P14 transgenic ($Ly5.1^+$) mice express a TCR specific for the LCMV peptide $gp_{33–41}$ (Pircher et al., 1990). Smarta transgenic ($Ly5.1^+$) mice express a TCR specific for the LCMV peptide $gp_{61–80}$ (Oxenius et al., 1998).

Viruses and Infections

Recombinant MCMV deficient in m157 (MCMV-Δm157) was previously described (Walton et al., 2008) and is referred to as MCMV in this study. The MCMV mutant expressing GFP under the m157 promoter was described in Hasan et al. (2005). MCMV strains were propagated on MEFs as described (Brune et al., 2001). Virus titers of organs were determined on M2-10B4 cells as described recently (Zurbach et al., 2014). For systemic infection, mice were infected intravenously with 5×10^6 pfu MCMV-Δm157. Focused i.g. infection was performed with modifications as previously described (Pilgrim et al., 2007; see Supplemental Experimental Procedures). The LCMV isolate WE and Docile were provided by Dr. R.M. Zinkernagel (University Hospital) and propagated at a low MOI on L929 fibroblast cells. Mice were intravenously infected with 200 ffu LCMV-WE for low-dose infection and 10^6 ffu LCMV Docile for chronic infection. Recombinant Vaccinia virus expressing the LCMV glycoprotein (VV-G2) was originally obtained from Dr. D.H.L. Bishop (Oxford University) and was grown on BSC40 cells at low MOI. Infection of VV-G2 was performed by intraperitoneal injection of 5×10^6 pfu VV-G2.

Adoptive Transfer

$CD8^+$ and $CD4^+$ T cells were isolated from naive Maxi, $CD103^{-/-}$ Maxi, P14, M25, Smarta (all $Ly5.1^+$), or $INFAR^{-/-}$ P14 ($Thy1.1^+$) mice using magnetic anti-CD8 or anti-CD4 beads (Miltenyi Biotech). 10^6 M25 cells or 10^4 of all other transgenic cells were adoptively transferred into naive recipient mice 1 day prior to infection. To study the migration and maintenance of in-vivo-activated T cells, splenocytes were isolated 1 wpi and 10^6 T cells purified by MACS were transferred into naive recipient mice. To study the maintenance of $CD8^+$ T cells in the SG, mice were treated with 5 μg/ml FTY720 (US Biological) in the drinking water, starting 1 wpi.

Lymphocyte Isolation, i.v., Intracellular, and Cell Surface Staining

Lymphocytes were isolated from spleen and SG as previously described (Joller et al., 2007), with the modification that cell suspensions were not subjected to a Percoll gradient. Staining for flow cytometry was performed on whole-blood or single-cell suspensions from the indicated organs as described in the Supplemental Experimental Procedures. Data were acquired on a LSRII flow cytometer (BD Bioscience) and analyzed using Flowjo software (Treestar).

Statistical Analysis

Statistical significance was determined as indicated either by two-tailed unpaired t test or Fisher's exact test using GraphPad Prism.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.09.082.

AUTHOR CONTRIBUTIONS

J.T.T. and T.C.W. conducted the experiments, J.T.T. and A.O. designed the experiments, J.T.T. and A.O. wrote the paper, and S.M.W. and N.T. contributed important reagents.

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REFERENCES

Anderson, K.G., Mayer-Barber, K., Sung, H., Beura, L., James, B.R., Taylor, J.J., Qunaj, L., Griffith, T.S., Vezys, V., Barber, D.L., and Masopust, D. (2014). Intravascular staining for discrimination of vascular and tissue leukocytes. Nat. Protoc. *9*, 209–222.

Ariotti, S., Hogenbirk, M.A., Dijkgraaf, F.E., Visser, L.L., Hoekstra, M.E., Song, J.Y., Jacobs, H., Haanen, J.B., and Schumacher, T.N. (2014). T cell memory. Skin-resident memory $CD8^+$ T cells trigger a state of tissue-wide pathogen alert. Science *346*, 101–105.

Bachmann, M.F., Wolint, P., Schwarz, K., and Oxenius, A. (2005). Recall proliferation potential of memory CD8+ T cells and antiviral protection. J. Immunol. *175*, 4677–4685.

Bankovich, A.J., Shiow, L.R., and Cyster, J.G. (2010). CD69 suppresses sphingosine 1-phosophate receptor-1 (S1P1) function through interaction with membrane helix 4. J. Biol. Chem. *285*, 22328–22337.

Böhm, V., Podlech, J., Thomas, D., Deegen, P., Pahl-Seibert, M.F., Lemmermann, N.A., Grzimek, N.K., Oehrlein-Karpi, S.A., Reddehase, M.J., and Holtappels, R. (2008). Epitope-specific in vivo protection against cytomegalovirus disease by CD8 T cells in the murine model of preemptive immunotherapy. Med. Microbiol. Immunol. (Berl.) *197*, 135–144.

Britt, W. (2007). Virus entry into host, establishment of infection, spread in host, mechanisms of tissue damage. In Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis, A. Arvin, G. Campadelli-Fiume, E. Mocarski, P.S. Moore, B. Roizman, R. Whitley, and K. Yamanishi, eds. (Cambridge University Press).

Bromley, S.K., Thomas, S.Y., and Luster, A.D. (2005). Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics. Nat. Immunol. *6*, 895–901.

Brune, W., Hengel, H., and Koszinowski, U.H. (2001). A mouse model for cytomegalovirus infection. Curr. Protoc. Immunol. *Chapter 19*, 7.

Bunde, T., Kirchner, A., Hoffmeister, B., Habedank, D., Hetzer, R., Cherepnev, G., Proesch, S., Reinke, P., Volk, H.D., Lehmkuhl, H., and Kern, F. (2005). Protection from cytomegalovirus after transplantation is correlated with immediate early 1-specific CD8 T cells. J. Exp. Med. *201*, 1031–1036.

Casey, K.A., Fraser, K.A., Schenkel, J.M., Moran, A., Abt, M.C., Beura, L.K., Lucas, P.J., Artis, D., Wherry, E.J., Hogquist, K., et al. (2012). Antigen-independent differentiation and maintenance of effector-like resident memory T cells in tissues. J. Immunol. *188*, 4866–4875.

Cepek, K.L., Shaw, S.K., Parker, C.M., Russell, G.J., Morrow, J.S., Rimm, D.L., and Brenner, M.B. (1994). Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the alpha E beta 7 integrin. Nature *372*, 190–193.

Debes, G.F., Bonhagen, K., Wolff, T., Kretschmer, U., Krautwald, S., Kamradt, T., and Hamann, A. (2004). CC chemokine receptor 7 expression by effector/memory CD4+ T cells depends on antigen specificity and tissue localization during influenza A virus infection. J. Virol. *78*, 7528–7535.

El-Asady, R., Yuan, R., Liu, K., Wang, D., Gress, R.E., Lucas, P.J., Drachenberg, C.B., and Hadley, G.A. (2005). TGF-beta-dependent CD103 expression by CD8(+) T cells promotes selective destruction of the host intestinal epithelium during graft-versus-host disease. J. Exp. Med. *201*, 1647–1657.

Förster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Müller, I., Wolf, E., and Lipp, M. (1999). CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell *99*, 23–33.

Gebhardt, T., and Mackay, L.K. (2012). Local immunity by tissue-resident CD8(+) memory T cells. Front. Immunol. *3*, 340.

Gebhardt, T., Wakim, L.M., Eidsmo, L., Reading, P.C., Heath, W.R., and Carbone, F.R. (2009). Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. Nat. Immunol. *10*, 524–530.

Gebhardt, T., Whitney, P.G., Zaid, A., Mackay, L.K., Brooks, A.G., Heath, W.R., Carbone, F.R., and Mueller, S.N. (2011). Different patterns of peripheral migration by memory CD4+ and CD8+ T cells. Nature *477*, 216–219.

Gorelik, L., and Flavell, R.A. (2000). Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. Immunity *12*, 171–181.

Hansen, S.G., Powers, C.J., Richards, R., Ventura, A.B., Ford, J.C., Siess, D., Axthelm, M.K., Nelson, J.A., Jarvis, M.A., Picker, L.J., and Früh, K. (2010). Evasion of CD8+ T cells is critical for superinfection by cytomegalovirus. Science *328*, 102–106.

Hasan, M., Krmpotic, A., Ruzsics, Z., Bubic, I., Lenac, T., Halenius, A., Loewendorf, A., Messerle, M., Hengel, H., Jonjic, S., and Koszinowski, U.H. (2005). Selective down-regulation of the NKG2D ligand H60 by mouse cytomegalovirus m155 glycoprotein. J. Virol. *79*, 2920–2930.

Hawke, S., Stevenson, P.G., Freeman, S., and Bangham, C.R. (1998). Long-term persistence of activated cytotoxic T lymphocytes after viral infection of the central nervous system. J. Exp. Med. *187*, 1575–1582.

Hofmann, M., and Pircher, H. (2011). E-cadherin promotes accumulation of a unique memory CD8 T-cell population in murine salivary glands. Proc. Natl. Acad. Sci. USA *108*, 16741–16746.

Holtappels, R., Simon, C.O., Munks, M.W., Thomas, D., Deegen, P., Kühnapfel, B., Däubner, T., Emde, S.F., Podlech, J., Grzimek, N.K., et al. (2008). Subdominant CD8 T-cell epitopes account for protection against cytomegalovirus independent of immunodomination. J. Virol. *82*, 5781–5796.

Jeitziner, S.M., Walton, S.M., Torti, N., and Oxenius, A. (2013). Adoptive transfer of cytomegalovirus-specific effector CD4+ T cells provides antiviral protection from murine CMV infection. Eur. J. Immunol. *43*, 2886–2895.

Jiang, X., Clark, R.A., Liu, L., Wagers, A.J., Fuhlbrigge, R.C., and Kupper, T.S. (2012). Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. Nature *483*, 227–231.

Joller, N., Spörri, R., Hilbi, H., and Oxenius, A. (2007). Induction and protective role of antibodies in Legionella pneumophila infection. Eur. J. Immunol. *37*, 3414–3423.

Jonjić, S., Mutter, W., Weiland, F., Reddehase, M.J., and Koszinowski, U.H. (1989). Site-restricted persistent cytomegalovirus infection after selective long-term depletion of CD4+ T lymphocytes. J. Exp. Med. *169*, 1199–1212.

Karrer, U., Sierro, S., Wagner, M., Oxenius, A., Hengel, H., Koszinowski, U.H., Phillips, R.E., and Klenerman, P. (2003). Memory inflation: continuous accumulation of antiviral CD8+ T cells over time. J. Immunol. *170*, 2022–2029.

Kennedy, M.K., Glaccum, M., Brown, S.N., Butz, E.A., Viney, J.L., Embers, M., Matsuki, N., Charrier, K., Sedger, L., Willis, C.R., et al. (2000). Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. J. Exp. Med. *191*, 771–780.

Kim, S.K., Reed, D.S., Heath, W.R., Carbone, F., and Lefrançois, L. (1997). Activation and migration of CD8 T cells in the intestinal mucosa. J. Immunol. *159*, 4295–4306.

Klonowski, K.D., Williams, K.J., Marzo, A.L., Blair, D.A., Lingenheld, E.G., and Lefrançois, L. (2004). Dynamics of blood-borne CD8 memory T cell migration in vivo. Immunity *20*, 551–562.

Lee, Y.T., Suarez-Ramirez, J.E., Wu, T., Redman, J.M., Bouchard, K., Hadley, G.A., and Cauley, L.S. (2011). Environmental and antigen receptor-derived signals support sustained surveillance of the lungs by pathogen-specific cytotoxic T lymphocytes. J. Virol. *85*, 4085–4094.

Lindquist, R.L., Shakhar, G., Dudziak, D., Wardemann, H., Eisenreich, T., Dustin, M.L., and Nussenzweig, M.C. (2004). Visualizing dendritic cell networks in vivo. Nat. Immunol. *5*, 1243–1250.

Lu, X., Pinto, A.K., Kelly, A.M., Cho, K.S., and Hill, A.B. (2006). Murine cytomegalovirus interference with antigen presentation contributes to the inability of CD8 T cells to control virus in the salivary gland. J. Virol. *80*, 4200–4202.

Lucin, P., Pavić, I., Polić, B., Jonjić, S., and Koszinowski, U.H. (1992). Gamma interferon-dependent clearance of cytomegalovirus infection in salivary glands. J. Virol. *66*, 1977–1984.

Mackay, L.K., Stock, A.T., Ma, J.Z., Jones, C.M., Kent, S.J., Mueller, S.N., Heath, W.R., Carbone, F.R., and Gebhardt, T. (2012). Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation. Proc. Natl. Acad. Sci. USA *109*, 7037–7042.

Mackay, L.K., Rahimpour, A., Ma, J.Z., Collins, N., Stock, A.T., Hafon, M.L., Vega-Ramos, J., Lauzurica, P., Mueller, S.N., Stefanovic, T., et al. (2013). The developmental pathway for CD103(+)CD8+ tissue-resident memory T cells of skin. Nat. Immunol. *14*, 1294–1301.

Mandaric, S., Walton, S.M., Rülicke, T., Richter, K., Girard-Madoux, M.J., Clausen, B.E., Zurunic, A., Kamanaka, M., Flavell, R.A., Jonjic, S., and Oxenius, A. (2012). IL-10 suppression of NK/DC crosstalk leads to poor priming of MCMV-specific CD4 T cells and prolonged MCMV persistence. PLoS Pathog. *8*, e1002846.

Matloubian, M., Lo, C.G., Cinamon, G., Lesneski, M.J., Xu, Y., Brinkmann, V., Allende, M.L., Proia, R.L., and Cyster, J.G. (2004). Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature *427*, 355–360.

Munks, M.W., Cho, K.S., Pinto, A.K., Sierro, S., Klenerman, P., and Hill, A.B. (2006). Four distinct patterns of memory CD8 T cell responses to chronic murine cytomegalovirus infection. J. Immunol. *177*, 450–458.

Nauerth, M., Weißbrich, B., Knall, R., Franz, T., Dössinger, G., Bet, J., Paszkiewicz, P.J., Pfeifer, L., Bunse, M., Uckert, W., et al. (2013). TCR-ligand koff rate correlates with the protective capacity of antigen-specific CD8+ T cells for adoptive transfer. Sci. Transl. Med. *5*, 192ra87.

Oxenius, A., Bachmann, M.F., Zinkernagel, R.M., and 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.

Pilgrim, M.J., Kasman, L., Grewal, J., Bruorton, M.E., Werner, P., London, L., and London, S.D. (2007). A focused salivary gland infection with attenuated MCMV: an animal model with prevention of pathology associated with systemic MCMV infection. Exp. Mol. Pathol. *82*, 269–279.

Pircher, H., Moskophidis, D., Rohrer, U., Bürki, K., Hengartner, H., and Zinkernagel, R.M. (1990). Viral escape by selection of cytotoxic T cell-resistant virus variants in vivo. Nature *346*, 629–633.

Reddehase, M.J., Mutter, W., Münch, K., Bühring, H.J., and Koszinowski, U.H. (1987). CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity. J. Virol. *61*, 3102–3108.

Sallusto, F., Lenig, D., Förster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature *401*, 708–712.

Scheinberg, P., Melenhorst, J.J., Brenchley, J.M., Hill, B.J., Hensel, N.F., Chattopadhyay, P.K., Roederer, M., Picker, L.J., Price, D.A., Barrett, A.J., and Douek, D.C. (2009). The transfer of adaptive immunity to CMV during hematopoietic stem cell transplantation is dependent on the specificity and phenotype of CMV-specific T cells in the donor. Blood *114*, 5071–5080.

Schenkel, J.M., Fraser, K.A., Beura, L.K., Pauken, K.E., Vezys, V., and Masopust, D. (2014). T cell memory. Resident memory CD8 T cells trigger protective innate and adaptive immune responses. Science *346*, 98–101.

Schön, M.P., Arya, A., Murphy, E.A., Adams, C.M., Strauch, U.G., Agace, W.W., Marsal, J., Donohue, J.P., Her, H., Beier, D.R., et al. (1999). Mucosal T lymphocyte numbers are selectively reduced in integrin alpha E (CD103)-deficient mice. J. Immunol. *162*, 6641–6649.

Shiow, L.R., Rosen, D.B., Brdicková, N., Xu, Y., An, J., Lanier, L.L., Cyster, J.G., and Matloubian, M. (2006). CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. Nature *440*, 540–544.

Smith, C.J., Turula, H., and Snyder, C.M. (2014). Systemic hematogenous maintenance of memory inflation by MCMV infection. PLoS Pathog. *10*, e1004233.

Smith, C.J., Caldeira-Dantas, S., Turula, H., and Snyder, C.M. (2015). Murine CMV infection induces the continuous production of mucosal resident T cells. Cell Rep. *13*, this issue, 1137–1148.

Steinert, E.M., Schenkel, J.M., Fraser, K.A., Beura, L.K., Manlove, L.S., Igyártó, B.Z., Southern, P.J., and Masopust, D. (2015). Quantifying memory CD8 T cells reveals regionalization of immunosurveillance. Cell *161*, 737–749.

Teijaro, J.R., Turner, D., Pham, Q., Wherry, E.J., Lefrançois, L., and Farber, D.L. (2011). Cutting edge: Tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection. J. Immunol. *187*, 5510–5514.

Thom, J.T., Walton, S.M., Torti, N., and Oxenius, A. (2014). Salivary gland resident APCs are Flt3L- and CCR2-independent macrophage-like cells incapable of cross-presentation. Eur. J. Immunol. *44*, 706–714.

Torti, N., Walton, S.M., Brocker, T., Rülicke, T., and Oxenius, A. (2011). Non-hematopoietic cells in lymph nodes drive memory CD8 T cell inflation during murine cytomegalovirus infection. PLoS Pathog. *7*, e1002313.

Wakim, L.M., Woodward-Davis, A., and Bevan, M.J. (2010). Memory T cells persisting within the brain after local infection show functional adaptations to their tissue of residence. Proc. Natl. Acad. Sci. USA *107*, 17872–17879.

Walton, S.M., Wyrsch, P., Munks, M.W., Zimmermann, A., Hengel, H., Hill, A.B., and Oxenius, A. (2008). The dynamics of mouse cytomegalovirus-specific CD4 T cell responses during acute and latent infection. J. Immunol. *181*, 1128–1134.

Walton, S.M., Mandaric, S., Torti, N., Zimmermann, A., Hengel, H., and Oxenius, A. (2011). Absence of cross-presenting cells in the salivary gland and viral immune evasion confine cytomegalovirus immune control to effector CD4 T cells. PLoS Pathog. *7*, e1002214.

Zhang, N., and Bevan, M.J. (2012). TGF-β signaling to T cells inhibits autoimmunity during lymphopenia-driven proliferation. Nat. Immunol. *13*, 667–673.

Zhang, N., and Bevan, M.J. (2013). Transforming growth factor-β signaling controls the formation and maintenance of gut-resident memory T cells by regulating migration and retention. Immunity *39*, 687–696.

Zurbach, K.A., Moghbeli, T., and Snyder, C.M. (2014). Resolving the titer of murine cytomegalovirus by plaque assay using the M2-10B4 cell line and a low viscosity overlay. Virol. J. *11*, 71.