Differential Microenvironment Localization of Effector and Memory CD8 T Cells

Joseph G. Dauner, Ifor R. Williams and Joshy Jacob

http://www.jimmunol.org/content/180/1/291

---

**References**  This article cites 44 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/180/1/291.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Differential Microenvironment Localization of Effector and Memory CD8 T Cells

Joseph G. Dauner,* Ifor R. Williams,† and Joshy Jacob2*

CD8 T cells are critical for the clearance of intracellular pathogens. Upon infection, naive CD8 T cells differentiate into effector cells that target and eliminate infected cells. Following clearance of the pathogen, most effector cells die, although a small fraction survives to establish a memory population. Subsequent exposure to the same pathogen induces a rapid response of memory T cells and efficient elimination of the pathogen. Although much is known about the CD8 T cell response, the precise microenvironment location of effector and memory CD8 T cells in secondary lymphoid organs is not well characterized. In this study, we present an in situ analysis of the localization of effector and memory CD8 T cells during the murine immune response to lymphocytic choriomeningitis virus. We identified the location of these cells using a transgenic mouse model system in which CD8 T cells are irreversibly tagged with yellow fluorescent protein (YFP) after activation. After infection, YFP+ CD8 T cells were initially observed within T cell zones. Later, these cells were found in the red pulp and a disruption of all CD8 T cell zones was observed. After resolution of the immune response, YFP+ memory CD8 T cells were observed primarily in T cell zones. Thus, in the spleens of mice, effector CD8 T cells localize to the red pulp and memory CD8 T cells localize to the T cell zones. Upon rechallenge, memory CD8 T cells rapidly proliferate and the secondary effector CD8 T cells are found in the red pulp. The Journal of Immunology, 2008, 180: 291–299.

The adaptive immune response is a dynamic process that involves the participation of various cell types, including phagocytes, B cells, and T cells. B cells are important in the production of pathogen-specific Abs, which can neutralize the pathogen and prevent infection. CD4 T cells, typically referred to as helper T cells, serve several important functions in the immune response; they provide help to B cells by secreting cytokines and providing costimulation within germinal centers (1). In addition, CD4 T cells play a critical role in generating high quality memory CD8 T cells (2). CD8 T cells are crucial for the clearance of intracellular pathogens such as viruses and some bacteria (3). APCs present pathogen-derived peptides in the context of MHC class I to naive CD8 T cells. Recognition of peptide-MHC complexes by the TCR coupled with costimulation provided by APCs leads to the initiation of a proliferation (4) and differentiation program that results in the generation of effector CD8 T cells (5). Effector CD8 T cells express the proteins perforin and granzyme B that allow them to lyse infected cells in a contact-dependent process (6). Once the pathogen is cleared, the majority of effector cells undergo an apoptotic contraction phase, and a small fraction of these cells persist as memory CD8 T cells (7). Memory CD8 T cells are capable of mounting a highly efficient and effective immune response against subsequent exposures to the pathogen because of their increased numbers and their ability to respond rapidly (8–10).

The trafficking of CD8 T cell populations between various tissues during Ag-induced differentiation is well understood (11). However, the in situ microenvironment localization of activated effector and memory T cells within secondary lymphoid organs has not been well characterized. The lack of specific markers to distinguish naive, effector, and memory T cells from each other in situ has complicated the design of these experiments. Therefore, studies attempting to track effector and memory T cell populations have relied on adoptive transfer of TCR transgenic CD8 T cells that can be identified using a congenic marker (12–14). These studies concluded that upon adoptive transfer into naive recipients, transgenic CD8 T cells activated either in vivo (13) or in vitro (15) were largely excluded from the white pulp regions and were found in the red pulp regions of the recipient spleens. In these experiments, effector CD8 T cells were tracked in the foreign environment of a naive mouse that may lead to unique results specific to the experimental design. In addition, viral infection with cytomegalovirus (16) and strains of lymphocytic choriomeningitis virus (LCMV)3 (17) lead to alterations in the splenic architecture and how effector CD8 T cells traffic in this altered landscape could provide us with valuable new insight. Another aspect that could affect effector CD8 T cell localization is the presence of infected cells. For instance, it is possible that effector T cell populations may be drawn near infected cells to efficiently mediate pathogen clearance, and this was not addressed in the adoptive transfer experiments. This is the first study to analyze the in situ localization of endogenous effector and memory CD8 T cells generated in response to a live viral infection.

In this study, we describe the localization of activated effector and memory CD8 T cells during acute LCMV infection. We used a transgenic mouse model in which Ag-specific CD8 T cells permanently express yellow fluorescent protein (YFP) after activation and can be identified for months after infection. During acute LCMV infection, we observed major alterations/destruction of the...
red pulp boundary, marginal zones, and T cell zones. In addition, we identified LCMV Ag-bearing cells on the border between the white and red pulp by staining frozen spleen sections with anti-serum raised against LCMV. Following infection, activated YFP\(^+\) CD8 T cells were found initially within T cell zones. Over time, T cells zones were dissolved, resulting in YFP\(^+\) and YFP\(^-\) CD8 T cells being relocated to the red pulp. Interestingly, YFP\(^+\) CD8 T cells were observed in close contact with the LCMV Ag\(^+\) cells. In the memory phase (>50 days), YFP\(^+\) memory CD8 T cells primarily located to the reestablished T cell zones. Upon subsequent exposure to LCMV, YFP\(^+\) memory CD8 T cells rapidly expanded and mobilized to the red pulp and, in contrast to the primary response, no disruption of the T cell zones occurred.

### Methods and Materials

#### Animals and viral infections

All animals were housed in an American Association of Laboratory Animal Care-accredited facility under specific pathogen-free conditions at the Emory University Vaccine Research Center. Granulysin B-cre and ROSA-EYFP mice were cross-bred and maintained at Emory University following the guidelines of the Institutional Animal Care and Use Committee. Mice were used at ages ranging from 4–8 wk. LCMV Armstrong and Clone 13 (gifts from Dr. Rafi Ahmed, Emory University, Atlanta, GA) was triple plaque purified on Vero cells and amplified on BHK-21 cells (American Type Culture Collection). For viral infections, mice were given one i.p. injection of 2 \times 10^5 PFU LCMV Armstrong diluted in PBS. For secondary challenge studies, mice were infected i.v. with 2 \times 10^6 PFU LCMV Clone 13.

#### Cell isolation, Abs, and flow cytometry

Mice were euthanized via cervical dislocation at desired time points postinfection and spleens were harvested. Single cell suspensions were prepared and RBCs were lysed using RBC lysis buffer (Sigma-Aldrich). To detect cell surface Ags, splenocytes were stained on ice with optimal concentrations of anti-CD8 (eBioscience) and GP33–41 or NP396–404-specific tetramers conjugated to allophycocyanin. For intracellular IFN-\(\gamma\) staining, a cell permeabilization reagent was used as per the manufacturer’s advice. Flow cytometry data were acquired using a FACSCalibur (BD Biosciences) flow cytometer running CellQuest software. For viral infections, mice were given one i.p. injection of 2 \times 10^5 PFU LCMV Armstrong diluted in PBS. For secondary challenge studies, mice were infected i.v. with 2 \times 10^6 PFU LCMV Clone 13.

#### Tissue preparation for immunofluorescence staining

At desired time points postinfection, mice were deeply anesthetized, followed by a two-stage perfusion. Mice were first perfused with 8 ml PBS followed by 8 ml paraformaldehyde (4% paraformaldehyde in PBS, pH 7.4). Tissues were isolated and embedded in OCT compound (Sakura). Tissue blocks were frozen in isopentane (Sigma-Aldrich) chilled by liquid nitrogen. Six micrometer sections were cut using a cryostat, air dried, and stored at -80°C until stained.

#### Immunofluorescence staining and microscopy

On the day of staining, slides were thawed and washed in PBS. Sections were blocked and Abs were diluted with PBS containing 2% normal goat serum. All staining was done at room temperature using Shandon Cover-plate disposable immunostaining chambers (Thermo Scientific). Sections were stained at one slice per slide using the following Abs: Alexa 350-, Alexa 488-, Alexa 546-, Alexa 647-conjugated streptavidin, Alexa 488-conjugated anti-rabbit IgG, Alexa 546-conjugated anti-rat IgG, and Alexa 546-conjugated anti-guinea pig IgG (Molecular Probes). Images were collected using a Nikon E600 fluorescence microscope and Spot Advanced software. Adobe Photoshop was used to identify and eliminate YFP\(^-\)CD8\(^+\) events from overlays of the CD8 and YFP single color images. Additional channels were overlaid and brightness and contrast were optimized using Adobe Photoshop.

### Results

#### Following LCMV infection, YFP expression in CD8 T cells correlates with Ag specificity

We have previously described the granulysin B-cre (GBC) \(\times\) Rosa26R mouse model system and its use in tracking Ag-specific CD8 T cells in the context of live viral infections (18). The GBC mouse (19) contains a transgene expressing cre recombinase under the control of a truncated human granulysin B promoter (20). In GBC \(\times\) ROSA26R double transgenic mice, viral infection leads to the activation of CD8 T cells and cre-lox recombination that induces permanent expression of \(\beta\)-galactosidase (\(\beta\)-gal). Although this model was powerful in studying effector and memory CD8 T cells by flow cytometry, analyzing these marked cells in tissues sections was difficult because costaining of \(\beta\)-gal and other cellular markers was technically challenging. We alleviated this issue by adopting a new cre-reporter mouse strain. The Rosa-EYFP mouse (21) expresses YFP instead of \(\beta\)-gal after cre-recombination, and thus can be easily visualized in tissue samples by either flow cytometric analysis or in situ staining techniques.

The restricted expression of YFP within the activated CD8 T cell compartment of GBC \(\times\) Rosa-EYFP (GBC \(\times\) RYPEF) mice should delineate naive from activated cells. To confirm this, we infected GBC \(\times\) RYPEF mice i.p. with 2 \times 10^5 PFU of the Armstrong strain of LCMV and analyzed the phenotype of YFP\(^+\) and YFP\(^-\) CD8 T cells on day 8 postinfection. At day 8, YFP\(^+\) and YFP\(^-\) CD8 T cells had distinctly different phenotypes. YFP\(^+\) CD8 T cells exhibited an activated phenotype of CD44\(^hi\)CD62L\(^lo\)CD11b\(^hi\)CD25\(^lo\)CD43\(^hi\)CD127\(^lo\) while YFP\(^-\) CD8 T cells displayed a CD44\(^lo\)CD62L\(^hi\)CD11b\(^lo\)CD25\(^hi\)CD43\(^lo\)CD127\(^lo\) naive phenotype (data not shown).

Next, we wanted to correlate YFP expression in CD8 T cells with their Ag specificity. We determined the specificity of CD8 T cells by virtue of MHC class I tetramer binding and the ability to produce IFN-\(\gamma\) in response to stimulation with immunodominant LCMV peptides. In brief, cohorts of GBC \(\times\) RYPEF mice were infected as previously described and sacrificed at various time points. We stained single cell splenocyte suspensions with LCMV peptide/MHC class I tetramers specific for two immunodominant LCMV epitopes (Db-GP33 and Db-NP396). Over the course of the infection, we observed a well-defined population of tetramer positive cells expressing YFP (Fig. 1A). By day 8 post infection, 79.9% \(\pm\) 12.3 of GP33 and 78.1% \(\pm\) 12.6 of NP396 tetramer\(^+\) CD8 T cells expressed YFP. This high level of marking was observed at all subsequent time points, with >75% of tetramer\(^+\) cells expressing YFP in the memory phase (Fig. 1B).

Analysis of the specificity of YFP\(^+\) CD8 T cells as determined by their ability to produce IFN-\(\gamma\) in response to LCMV peptides, showed that a majority of IFN-\(\gamma\)-producing CD8 T cells expressed YFP over the course of the infection (Fig. 1C). At day 8 of LCMV infection, nearly 85% of IFN-\(\gamma\)-producing CD8 T cells expressed YFP (83.9\% \(\pm\) 14.0 of GP33-specific and 84.4\% \(\pm\) 12.3 of NP396-specific, Fig. 1D). During the memory phase, this high level of correlation slightly increased, with 90% of GP33 and NP396-specific cells expressing YFP.

Taken together, these data demonstrate the usefulness of using the GBC \(\times\) RYPEF mouse to track polyclonal CD8 T cell responses after viral infection. The majority of Ag-specific CD8 T cells expressed YFP after LCMV infection, whereas naive CD8 T cells remained YFP\(^-\). The effector CD8 T cells tagged during the acute phase of the response survived to become YFP\(^+\) memory cells and can be identified within mice for several months after infection.
We conclude that the GBC/H11003 RYFP mouse is a good model to analyze the in situ microenvironmental localization of effector and memory CD8 T cells following infection. LCMV Ags localize to the boundary of the red and white pulp and are cleared by day 8 postinfection

Before studying the localization of effector and memory CD8 T cells, we first analyzed the spleen for the location of LCMV Ags, architectural changes, and the localization of APCs. In brief, we infected GBC × RYFP mice with LCMV and at various time points harvested their spleens and prepared frozen sections from them. These sections were then stained to analyze splenic structure and location of LCMV Ags. We used guinea pig anti-LCMV polyclonal sera to analyze the distribution of LCMV Ag-bearing cells over time and the Abs ER-TR7 and ER-TR9 to identify the red pulp and marginal zones, respectively. In the spleens of mice, the Ab ER-TR7 stains stromal cells located in the red pulp for clear delineation of red and white pulp regions (22). ER-TR9 binds the C-type lectin, SIGN-R1, expressed by a unique marginal zone resident macrophage subpopulation (23). Two days following infection, discrete foci of cells expressing LCMV Ags were detected at the border of the red and white pulp, and these cells did not express ER-TR9. By days 4 and 5 postinfection, the pattern of viral Ag staining changed to a ring pattern that lies on the border of the red and white pulp and a loss of ER-TR9 macrophages was observed (Fig. 2, left column). As the infection progressed, a deterioration of the boundary of red and white pulp steadily occurred such that we observed no separation, in terms of ER-TR7 staining, by day 8 (Fig. 2, left column). The loss of distinct ER-TR7 and ER-TR9 staining suggests that LCMV infection led to alterations in splenic stromal cells, potentially leading to disruptions in red and white pulp segregation and loss of distinct marginal zone macrophage populations.

Next, we examined the effect of acute LCMV infection on the localization of dendritic cells (DCs) within the spleen. DCs are important in the activation of T cells and help direct the type of immune response that ensues (25). Any potential changes in DC localization could provide insight into the kinetics of Ag presentation and T cell activation following infection. At day 2 postinfection, we observed an influx of CD11c+ DCs into the white pulp of the spleen (Fig. 2, right column) and these DCs did not contain LCMV Ags as detected by the LCMV-specific guinea pig serum. Interestingly, at day 5 postinfection, a significant number of CD11c+ DC were positive for LCMV Ags. These CD11c+ and LCMV Ag double positive cells were located at the outer edge of the white pulp (Fig. 2, right column). We conclude based on these data that LCMV Ags can be detected in the spleens of infected mice from day 2 to 6 and the viral Ag load peaks between days 4 and 5. Initially (days 2–4), the viral Ag-bearing cells are confined to the border region of the red and white pulp. In addition, at early
FIGURE 2. Following infection, LCMV Ags are restricted to the outer edge of the white pulp in the spleen of infected mice. GBC × RYFP mice were infected i.p. with $2 \times 10^5$ PFU LCMV and frozen spleen sections were analyzed by immunofluorescent microscopy from day 2 to 8. Representative two-color immunofluorescent images showing the location of LCMV Ags in relation to the red pulp (ER-TR7, left column), marginal zones (ER-TR9, middle column), and DCs (CD11c$^+$ cells, right column) over the course of LCMV infection on days 2, 5, and 8. Yellow color represents colocalization of the two markers. The dotted lines depict the boundary of the white pulp as determined by ER-TR7 staining. Images presented are at a magnification of ×10. Data presented are representative of two to eight mice, and multiple frozen sections per mouse were analyzed at each time point.
time points of infection, DCs, either those residing in the red pulp or migrating from the peripheral tissues, flood the white pulp.

The staining pattern for LCMV Ags over the time course was reminiscent of the structure of marginal zones. Several reports have linked LCMV infection to macrophages, and more specifically marginal zone macrophages (24, 26–28). We sought to identify what macrophage populations were infected and whether they could serve as an adequate landmark population to locate activated YFP⁺ CD8 T cells in future experiments. We chose to divide the analysis into an early infection time point, day 2, and a late infection time point, day 5, and used Abs against known macrophage and APC markers including CD11b, CD11c, CD169, ER-TR9, F4/80, MARCO, MOMA-1, and MOMA-2. At day 2 postinfection, LCMV Ags were largely excluded from cells expressing CD11b (Mac-1), MOMA-1 (marginal zone resident metallophilic macrophages), F4/80 (mature macrophages), and CD11c (Fig. 3). At day 2 postinfection, a majority (54.56% ± 2.71) of the LCMV Ag-bearing cells were CD169⁺ (Fig. 3). At day 5, we observed CD11c⁺ cells containing a significant amount of the LCMV Ags detected (60.85% ± 2.41). This is significant because previous reports concluded that the Armstrong strain of LCMV does not infect DCs while the Clone 13 strain does (24). When we expanded our analysis to include more phenotypic markers, we observed a broad spectrum of cells harboring viral Ags at late time points of infection. Cells expressing either CD11c, MOMA-1, or CD169 contained significant amounts of Ag, while CD11b⁺ and F4/80⁺ macrophages rarely contained LCMV Ags (Fig. 3).

**CD8 T cells are mobilized and interact with infected cells during acute LCMV infection**

CD8 T cells are critical for the clearance of LCMV (29, 30). Therefore, we wanted to visualize YFP⁺ effector CD8 T cells within the spleen in the presence of an ongoing, acute LCMV infection. Given that LCMV Ags were nearly eliminated by day 6 (data not shown), we decided to investigate the location of effector CD8 T cells between days 2 and 6 postinfection. At each time point, we analyzed the location of B cells, total CD8 T cell and YFP⁺ CD8 T cells in relation to LCMV Ags. During the first couple days of infection, the spleens of infected mice had discrete B cell follicles and T cell zones similar to naive mice. At day 2, the number of YFP⁺ CD8 T cells was more than that observed in naive mice, and these YFP⁺ CD8 T cells were restricted to T cell zones (Fig. 4). This increase in YFP⁺ CD8 T cells corresponds with the influx of CD11c⁺ DCs as shown in Fig. 2. This may reflect the initiation of Ag presentation to naive CD8 T cells. By day 5 postinfection, we observed a mobilization of YFP⁺ CD8 T cells from the white pulp to the red pulp of the spleen. Although B cell follicles remained intact, CD8 T cell zones were dissolving, and this resulted in the loss of CD8 T cell zones by day 5 (Fig. 4). It has been shown that activated CD8 T cells largely do not home to T cell zones in the spleens of naive mice (13, 15), but it is interesting to note that we do not see residual CD8 T cell zones composed of naive T cells in infected spleens (Fig. 4). Along with our earlier observations, these data demonstrate the dynamic nature of the spleen during LCMV infection, as many normal cellular compartments, including the red and white pulp, T cell zones, and marginal zones of the spleen are altered or eliminated all together.

We next examined the localization of YFP⁺ CD8 T cells in relation to LCMV Ags. Earlier, we had shown that the CD8 T cells marked in this model have cytolytic activity and, therefore, mediate viral clearance in vivo (18). At day 3, YFP⁺ CD8 T cells were located within T cell zones while LCMV Ags were localized in the outer edge of the white pulp (Fig. 4). With the dissolution of T cell zones starting at day 4, one might reason that activated CD8 T cells were then drawn to LCMV infected cells in the spleen. Consistent with this idea, we found that YFP⁺ effector CD8 T cells were interacting LCMV Ag-bearing cells (Fig. 4, Day 5). In an average field of view, ~25% YFP⁺ CD8 T cells were in contact with virally infected cells. This small but significant frequency may indicate that the interaction of effector CD8 T cells with infected cells is transient, but sufficient for lysis of target cells. Based on these data, we conclude during early LCMV infection, activated CD8 T cells are maintained in T cell zones. After YFP⁺ CD8 T cells were interacting LCMV Ag-bearing cells (Fig. 4, Day 5). In an average field of view, ~25% YFP⁺ CD8 T cells were in contact with virally infected cells. This small but significant frequency may indicate that the interaction of effector CD8 T cells with infected cells is transient, but sufficient for lysis of target cells. Based on these data, we conclude during early LCMV infection, activated CD8 T cells are maintained in T cell zones. After YFP⁺ CD8 T cells were interacting LCMV Ag-bearing cells (Fig. 4, Day 5). In an average field of view, ~25% YFP⁺ CD8 T cells were in contact with virally infected cells. This small but significant frequency may indicate that the interaction of effector CD8 T cells with infected cells is transient, but sufficient for lysis of target cells. Based on these data, we conclude during early LCMV infection, activated CD8 T cells are maintained in T cell zones. After YFP⁺ CD8 T...
cells leave T cell zones, they enter the red pulp and engage LCMV-infected cells.

**Memory CD8 T cells are evenly distributed throughout T cell zones**

In the LCMV infection model, Wherry et al. (31) identified Ag-specific CD8 T cells within lymphoid organs, including spleen and lymph nodes, and peripheral tissues such as the brain, lung, and liver. The in situ location of memory CD8 T cells in LCMV-immune mice has not been well characterized. We analyzed the spleens of immune (>day 55 postinfection) GBC × RYFP mice to locate memory CD8 T cells and to determine whether they reside in any specific niches. In LCMV-immune spleens, we found that normal B cells follicles and T cells zones are restored and that memory CD8 T cells are dispersed throughout T cell zones (Fig. 5A). Memory CD8 T cells were found among the naive T cell population within T cell zones and they did not preferentially localize near B cells (Fig. 5A), CD11c+ DCs, or marginal zone macrophage populations (ER-TR7+ or MOMA-1+ staining, data not shown). Effector memory T cells have been identified as being CCR7− (32) and therefore, it can be reasoned that this memory population could be found in the red pulp of the spleen. However, ER-TR7 staining revealed a majority of memory CD8 T cells were
observed massive CD8 T cell activation and proliferation. This proliferation occurred in the absence of detectable LCMV Ags in situ, in contrast to the primary response to Armstrong where LCMV Ag-bearing cells were abundant in the spleen (Fig. 3). This was not due to inefficient infection because, in control naive mice that were infected with Clone 13, we observed massive numbers of Ag-bearing cells.

On day 1 of postsecondary challenge, most YFP+ CD8 T cells were found in T cells zones, but small foci of YFP+ CD8 T cells were found on the edge of B cells follicles in 64% of the microscopic fields analyzed (Fig. 6). Through day 2 postsecondary infection, YFP+ CD8 T cells were in T cells zones, however on days 3 and 4 of the secondary response, the numbers of YFP+ CD8 T cells in the T cell zones decrease, and there was a concomitant accumulation of these cells in the red pulp (Fig. 6). By day 8, the YFP+ CD8 T cells are distributed in the red pulp as well as the T cell zones. During the primary immune response to LCMV, we observed a total loss of T cell zones (Fig. 4), but we did not observe this loss during the secondary response (Fig. 6). Starting at day 4 of the primary response, CD8 T cells zones began to dissolve and were gone by day 5. In contrast, at all time points analyzed after secondary infection with Clone 13, CD8 T cell zones were clearly present (Fig. 6).

**Discussion**

Our analysis of the in situ localization of activated LCMV-specific, YFP+ CD8 T cells revealed their presence in T cell zones and the eventual migration of both YFP+ and YFP+ CD8 T cells out of the T cell zones between days 4 and 5 after a primary infection. Approximately 25% of the YFP+ effector CD8 T cells found in the red pulp were in close proximity with LCMV Ag-bearing cells. After the immune response subsided and normal splenic architecture was restored, a majority of memory CD8 T cells homed back to the T cell zones of the spleen. The re-established T cell zones were composed of an even distribution of naive and memory cells. In this study, we have shown that the GBC × RYFP mouse is a good model to study polyclonal CD8 T cell responses both ex vivo and in situ during an immune response.

Our results agree with published data that have analyzed the location of effector and memory CD8 T cells (13, 15). These reports showed that after adoptive transfer to naive recipients, effector TCR-transgenic CD8 T cells specific for LCMV were excluded from the white pulp and found in the red pulp, while memory CD8 T cells homed to the T cell zones. We observed similar results; however, our system has some key differences. The T cells we tracked in our study are derived from the endogenous naive CD8 T cell pool and not TCR-transgenic T cells. The precursor frequency of adoptively transferred, naive T cells has been implicated in affecting the resulting phenotype of memory CD8 T cells (37), which may also lead to altered trafficking properties. Another difference is that we tracked Ag-specific YFP+ CD8 T cells in the context of acute infection, which allowed us to analyze the location of responding CD8 T cells in relation to cells bearing viral Ag. We showed that during acute LCMV infection, CD8 T cell zones and marginal zone macrophages are lost. This was accompanied by a disruption of the boundary between the red and white pulp. When analyzing trafficking of transferred effector and memory CD8 T cells in naive mice, these changes in splenic architecture were not considered. Despite the different experimental approaches, our studies and CD8 TCR-transgenic transfer studies show that effector CD8 T cells migrate to the red pulp while memory cells reside in T cell zones.

We showed that a majority of splenic memory CD8 T cells were found within the T cell zones. It is an interesting observation that
naive and memory CD8 T cells occupy the same niche within the spleen, as this is not true for other lymphoid cells. Memory B cells are currently thought to colonize marginal zones (38), while naive B cells are located in B cells follicles. The marginal zone is the entry site for blood into the spleen, and therefore is important in early detection of blood-borne pathogens. The differential location of naive and memory B cells allows memory B cells the first opportunity to interact with a pathogen which provides the host with an advantage. We predicted that a similar phenomenon would occur with naive and memory CD8 T cells; however, we did not observe this. Upon careful consideration, key differences between T cells and B cells and their ability to detect Ag could explain our observation of memory CD8 T cell location. Unlike B cells, which can bind unprocessed Ags, CD8 T cells can only detect antigenic peptides in the context of MHC class I molecules. The T cells zones of the spleen and lymph nodes are the primary locations where this interaction occurs. Activated DCs, bearing processed Ags, express CCR7 and are attracted to T cells zones by the CCL21 gradient present (39). Therefore, the ideal location for memory CD8 T cells would be the T cell zone and they would not have preferential exposure to newly arriving Ags over naive T cells.

Analysis of secondary LCMV infection showed an increase in the frequency of YFP+ CD8 T cells. Unlike the primary response, small clusters of YFP+ CD8 T cells were found initially (day 1) at the outer edge of B cell follicles. Interestingly, in naive mice infected with either LCMV Armstrong or Clone 13, this is where viral Ags are located (data not shown). These clusters of YFP+ CD8 T cells are most likely rapidly generated secondary effectors responsible for eliminating the low levels of virus not neutralized by circulating Abs. During the secondary response to LCMV Clone 13, the lymphoid structure of the spleen, specifically CD8 T cell zones, was maintained, in contrast to what occurs in a primary LCMV response. A plausible explanation could be that the expression of homeostatic chemokines, such as CCL21 or CCL19, in the spleen may be differentially controlled dependent upon the viral load. Recently, Mueller et al. (40) reported that after infection with a variety of pathogens, CCL21 expression within the spleen was decreased and this resulted in naive T cells being unable to enter T cells zones. We have also observed a loss of CCL21 staining in primary but not in secondary response (data not shown) and this may contribute to maintenance of CD8 T cell zones.

At an early time point (day 2), we were able to determine the identity of the cells harboring LCMV Ags as being CD169+ macrophages. A broader analysis also showed that these cells were MOMA-1+, ER-TR9−, CD11c+, ER-TR7−, and found in discrete foci in the border of the white and red pulp regions. But by day 5, we observed that a large fraction (60.85% ± 2.41) of the cells containing LCMV Ags were CD11c+ DC as well as CD169+ cells, and these were, as in the early time points, confined to the border of the red and white pulp. It has been reported that the Armstrong strain of LCMV does not infect DCs (24); however, these studies only compared the infection pattern of different strains of LCMV on days 1 through 3 postinfection. Their conclusion was that the red pulp and CD169− metallocophilic macrophages within the marginal zones were the sites of LCMV infection, and our data agree with this. The previous study used the SER-4 mAb that recognizes CD169 and we used the 3D6 clone (41). The presence of LCMV Ags within CD11c+ cells late during infection may reflect one of three scenarios. One possibility is that LCMV Armstrong infects two different cell populations during the course of infection. The first population is macrophages expressing CD169 that are infected from days 1 through 3 postinfection and at later time points CD11c+ DCs appeared infected. This switch in cell tropism to DCs may reflect an elimination of the initial infected population. The receptor of LCMV is α-dystroglycan (42) and is expressed by a wide variety of cells, including DCs, and tissues (43). It is possible once its original reservoir is destroyed, the Armstrong strain can infect DCs as evidenced by the staining we observed (Fig. 2). However, the large deposits of LCMV Ags found in CD169+ cells at day 5 could suggest that the original reservoir may not be completely eliminated. A second scenario is that DCs located near virally infected cells take up cellular debris containing LCMV Ags. In our analysis, we then may be detecting exogenous Ags before they are processed by DCs for potential Ag presentation. A third possibility is that the cells infected at the start of the infection include a CD11c− DC precursor population (44). As the infection proceeds, these cells differentiate into CD11c+ DCs, and at day 5 we are able to detect LCMV-harboring DCs. Our observations could be a due to one of these scenarios or any combination of the three.

Our current study was confined to the spleen, but it would be interesting to analyze architectural changes of lymph nodes as well as mucosal lymphoid tissues during acute viral infection. LCMV Ag presentation after i.p. inoculation may primarily occur in certain draining lymph node (mesenteric lymph nodes) rather than others (inguinal lymph nodes). It would be beneficial to know whether T cell activation and/or viral infection leads to local architectural alterations of the lymphoid compartment or whether more systemic mechanisms are in play. Activated CD8 T cells within the spleen displayed a tendency to interact with infected cells. During an LCMV infection, virus is not only found in the spleen but several other tissues also, and it would be interesting to investigate the interactions of CD8 T cells and infected cells in these organs. Activated CD8 T cells may enter infected tissues in a more restricted manner than the spleen, and therefore be more isolated around infected cells. Lastly, the GBC × RYFP mouse model could be used to study antitumor immune responses and a variety of pathogenic infections.

Acknowledgments
We thank Drs. Jin Hyang Kim and Bali Pulendran for helpful suggestions and Leela Thomas for maintaining the mouse colony.

Disclosures
The authors have no financial conflict of interest.

References


