Interleukin 15 Is Required for Proliferative Renewal of Virus-specific Memory CD8 T Cells

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Abstract

The generation and efficient maintenance of antigen-specific memory T cells is essential for long-lasting immunological protection. In this study, we examined the role of interleukin (IL)-15 in the generation and maintenance of virus-specific memory CD8 T cells using mice deficient in either IL-15 or the IL-15 receptor α chain. Both cytokine- and receptor-deficient mice made potent primary CD8 T cell responses to infection with lymphocytic choriomeningitis virus (LCMV), effectively cleared the virus and generated a pool of antigen-specific memory CD8 T cells that were phenotypically and functionally similar to memory CD8 T cells present in IL-15$^{+/+}$ mice. However, longitudinal analysis revealed a slow attrition of virus-specific memory CD8 T cells in the absence of IL-15 signals.This loss of CD8 T cells was due to a severe defect in the proliferative renewal of antigen-specific memory CD8 T cells in IL-15$^{-/-}$ mice. Taken together, these results show that IL-15 is not essential for the generation of memory CD8 T cells, but is required for homeostatic proliferation to maintain populations of memory cells over long periods of time.

Key words: CD8 T cell • immunological memory • IL-15 • homeostasis • viral immunity

Introduction

Long-term CD8 T cell memory, as determined by faster recall responses in vivo, is a characteristic feature of most acute viral infections (1). These rapid anamnestic responses to reinfection are the result of both qualitative and quantitative changes in virus-specific T cells (2–6). During primary infection there is extensive expansion of antigen-specific CD8 T cells and, even though the vast majority of these activated effector CD8 T cells undergo apoptosis, the end result is a net increase in the numbers of virus-specific T cells (7). For example, after infection of mice with lymphocytic choriomeningitis virus (LCMV)* there is up to a 1,000-fold increase in the precursor frequency of virus-specific CD8 T cells (7, 8). Substantial increases (200–500-fold) in the numbers of antigen-specific CD8 T cells are also seen after clearance of acute vaccinia virus or vesicular stomatitis virus infections (9, 10). This numerical advantage alone can account for the faster recall response, but several recent studies have shown that upon reexposure to antigen, memory T cells can also elaborate effector functions such as cytotoxicity and cytokine production much more quickly than naive T cells (2–4, 6). Thus, it is this combination of increased numbers and faster responsiveness that forms the cellular basis of long-term T cell immunity.

A central question of immunological memory is understanding how memory T cells are maintained. The availability of TCR transgenic T cells, the development of sensitive techniques for assessing T cell function at the single cell level, and perhaps most importantly, the ability to physically identify antigen-specific CD8 T cells using MHC class I tetramers, has greatly facilitated studies addressing the important question of memory T cell maintenance (7, 11). Several studies using a variety of antigenic systems have now clearly established that memory CD8 T cells can persist in the absence of specific antigen (5, 12–24). It has also been shown that memory CD8 T cells undergo homeostatic proliferation to replenish their numbers and that this proliferative renewal does not require stimulation

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**Abbreviation used in this paper:* LCMV, lymphocytic choriomeningitis virus.

with specific antigen (14,15), or even MHC class I (16, 24). It also appears that costimulatory signals including B7-CD28 are not essential for maintaining memory CD8 T cells (25, 26). Recently, attention has turned toward the possible role of cytokines in this process and in particular to the role of IL-15 (20, 27–29). Knockout mice lacking IL-15 or the high affinity IL-15Rα contain reduced numbers of memory phenotype CD8 $CD44^{hi}$ T cells (28, 29). Also, exogenous IL-15 or IL-15–inducing agents selectively stimulate the division of $CD44^{hi}$ CD8 T cells (27, 30) and IL-15 overexpressing transgenic animals contain increased numbers of memory phenotype CD8 T cells (31). Despite these elegant studies implicating IL-15 in the development of memory CD8 T cells, several important questions remain unanswered. First, it is not precisely known whether IL-15 is needed for the generation of memory CD8 T cells or for their maintenance. Second, and perhaps more importantly, all studies done so far using the cytokine ($IL\text{-}15^{-/-}$) or the receptor ($IL\text{-}15R\alpha^{-/-}$) knockout mice have been confined to the analysis of memory phenotype $CD44^{hi}$ CD8 T cells whose antigenic specificity is not known, and so far no studies have examined the generation and maintenance of antigen-specific CD8 T cells. While the study of memory phenotype $CD44^{hi}$ CD8 T cells is certainly of value, the antigenic stimuli and signals that result in their generation are not known. It is also not clear whether these $CD44^{hi}$ CD8 T cells present in "clean" (specific pathogen-free) mice truly represent the functional characteristics of antigen-specific memory CD8 T cells induced after infection or vaccination.

In this study, we have investigated the role of IL-15 in the generation and maintenance of virus-specific CD8 T cells. Our results show that after infection with LCMV mice deficient in either IL-15 or IL-15Rα mount robust primary CD8 T cell responses, clear the virus, and generate a pool of virus-specific memory CD8 T cells. These memory CD8 T cells appear phenotypically and functionally normal and can make potent recall responses in vivo upon viral rechallenge. However, homeostatic proliferation of LCMV-specific memory CD8 T cells is almost completely lost in $IL\text{-}15^{-/-}$ mice and memory CD8 T cell numbers undergo a slow attrition in the absence of IL-15. Thus, IL-15 is not required to generate a virus-specific CD8 T cell response or to develop a pool of antigen-specific memory CD8 T cells, but IL-15 signals appear crucial for homeostatic proliferation and long-term maintenance of memory CD8 T cells.

Materials and Methods

Mice. 4–6-wk-old female C57Bl/6 were purchased from The National Cancer Institute (Frederick, MD). The generation and description of $IL\text{-}15R\alpha^{-/-}$ and $IL\text{-}15^{-/-}$ have been described previously (28, 29). $IL\text{-}15^{-/-}$ mice were obtained from Michael Caligiuri (Ohio State University, Columbus, OH). Both $IL\text{-}15\alpha^{-/-}$ and $IL\text{-}15^{-/-}$ mice and littermate controls were backcrossed to B6 mice for >10 generations.

Viral Infections. Stocks of the Armstrong strain of LCMV were plaque purified on Vero cells and grown in BHK-21 cells as described previously (32). Mice were infected by intraperitoneal injection with 2×10^5 PFU of LCMV. For rechallenge experiments, mice were infected intravenously with 2×10^6 PFU of LCMV clone 13, a strain that causes chronic infection in naive adult mice (32).

Antibodies and MHC Class I Tetramers. All antibodies were purchased from BD PharMingen. MHC class I/peptide tetramers were prepared and used as described previously (7).

Cell Preparation and Staining. Mice were bled retroorbitally into 4% sodium citrate under isofluorane anesthesia. PBMCs were purified on a histopaque density gradient (Sigma-Aldrich). Single cell suspensions were prepared from spleens and red blood cells were lysed using 0.83% ammonium chloride. Cells were washed and stained for four-color flow cytometry on a FACSCalibur™ Flow Cytometer (Becton Dickinson).

Isolation of Hepatic Lymphocytes. Mice were killed and the liver was perfused with 5 ml ice cold PBS via the hepatic artery. Liver tissue was homogenized using a wire screen and incubated with 0.25 mg/ml collagenase B (Boehringer Mannheim) and 1 U/ml DNase (Sigma-Aldrich) at 37°C for 45 min. Digested liver was centrifuged and the pellet was resuspended in 5–10 ml 44% Percoll (Sigma-Aldrich). This solution was underlaid with 56% Percoll and spun for 20 min at 20°C. The interface was harvested, RBCs were lysed using 0.83% ammonium chloride, washed, and counted. This procedure was found to have little impact on the expression of most cell surface molecules, including TCR (data not shown).

Intracellular Cytokine Staining. Intracellular cytokine staining was performed as described previously (7). Briefly, 10^6 splenocytes were incubated in 96-well, flat-bottomed plates in the presence of 1 μl/ml Golgistop (BD PharMingen) with or without 0.1 μg/ml of the indicated peptides. After 5 h at 37°C, cells were washed 1× in FACS® buffer (0.5% FCS, 0.2% sodium azide in PBS) and stained for surface markers for 30 min on ice. After two washes in FACS® buffer, cells were fixed and permeabilized using the BD PharMingen Cytofix/Cytoperm kit, according to the manufacturer's instructions, and stained for intracellular cytokines. After two washes in perm/wash and two washes in FACS® buffer, cells were fixed in 2% PFA and samples acquired as described previously.

CFSE Labeling and Adoptive Transfer. B6 or $IL\text{-}15^{-/-}$ mice that had been infected with LCMV 140 d previously were killed. Spleens were removed, RBCs lysed, and single cell suspensions were generated. Splenocytes were labeled with CFSE (Molecular Probes) as described previously (16, 33) and 3×10^7 labeled splenocytes were transferred by intravenous injection into naive recipients. After 30 d, recipients were killed and splenocytes were prepared and analyzed as described above.

Results and Discussion

$IL\text{-}15^{-/-}$ and $IL\text{-}15R\alpha^{-/-}$ Mice Generate Robust Primary CD8 T Cell Responses to LCMV Infection, but Virus-specific Memory CD8 T Cell Numbers Slowly Decline Over Time. Previous reports have demonstrated that mice lacking either IL-15 or IL-15Rα have reduced CD8 T cell numbers, particularly those of a $CD44^{hi}$ memory phenotype (28, 29). To investigate the induction and maintenance of virus-specific memory CD8 T cells in the absence of IL-15 signals, groups of $IL\text{-}15R\alpha^{-/-}$ and $IL\text{-}15^{-/-}$ mice and their re-

spective controls were infected with LCMV, and virus-specific CD8 T cell responses were monitored longitudinally in the PBMCs of individual mice. Fig. 1 A shows staining with MHC class I/peptide tetramers for a dominant (NP396) and a subdominant (GP276) LCMV epitope from representative IL-15$^{-/-}$ and IL-15$^{+/-}$ mice. At the peak of the response, day 8, the frequency of tetramer-positive cells was similar between IL-15$^{-/-}$ and IL-15$^{+/-}$ mice, whether expressed as a percentage of total PBMCs (in bold) or as a percentage of the total CD8 T cells (in parentheses). This demonstrates that potent virus-specific CD8 T cell responses can be generated in the absence of IL-15. Indeed, functional virus-specific responses were not affected by the absence of IL-15 signals since effector CD8 T cells from IL-15$^{+/-}$ and IL-15$^{-/-}$ mice were equally proficient at eliminating the viral infection and exhibited similar effector responses (data not shown). Also, the persistence of readily detectable numbers of tetramer-positive cells at days 30, 50, and 140 demonstrates that memory CD8 T cells were generated in the absence of IL-15 (Fig. 1). However, it became evident during longitudinal tracking that LCMV-specific memory T cells were inefficiently maintained in IL-15$^{-/-}$ mice (Fig. 1 B). This is illustrated by examining the magnitude of the response in IL-15$^{-/-}$ mice as a percentage of the response in IL-15$^{+/-}$ mice. While only slightly reduced (in most cases) or not affected at day 8, the responses in IL-15$^{-/-}$ mice fell to <25–50% of that in controls by 140 d after infection for all epitopes that were examined (Fig. 1 B). This longitudinal analysis examining virus-specific CD8 T cells in the PBMCs of the same animals over time is an excellent way of monitoring memory T cell maintenance. A similar trend was seen in

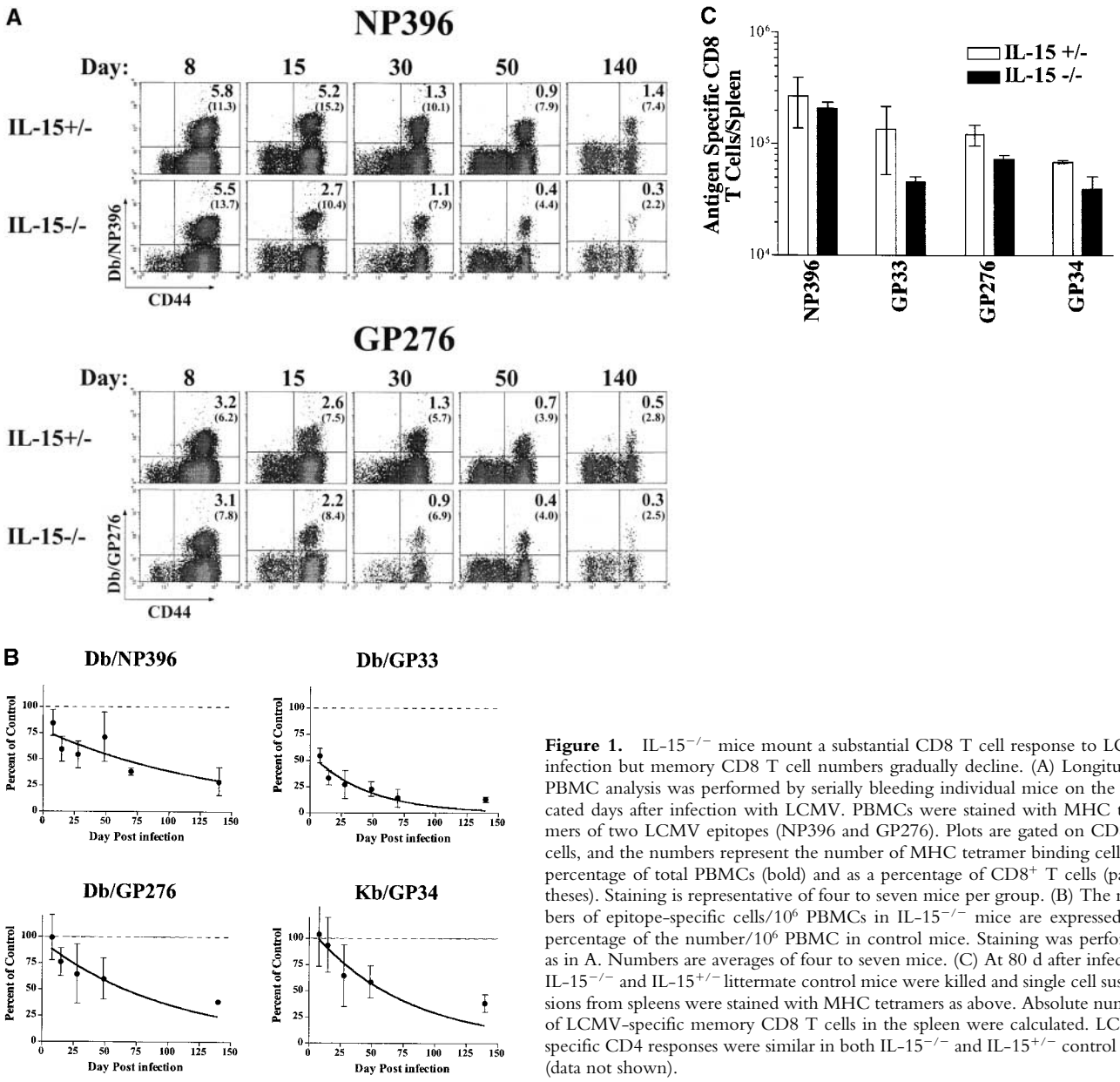

Figure 1. IL-15$^{-/-}$ mice mount a substantial CD8 T cell response to LCMV infection but memory CD8 T cell numbers gradually decline. (A) Longitudinal PBMC analysis was performed by serially bleeding individual mice on the indicated days after infection with LCMV. PBMCs were stained with MHC tetramers of two LCMV epitopes (NP396 and GP276). Plots are gated on CD8$^+$ T cells, and the numbers represent the number of MHC tetramer binding cells as a percentage of total PBMCs (bold) and as a percentage of CD8$^+$ T cells (parentheses). Staining is representative of four to seven mice per group. (B) The numbers of epitope-specific cells/10^6 PBMCs in IL-15$^{-/-}$ mice are expressed as a percentage of the number/10^6 PBMC in control mice. Staining was performed as in A. Numbers are averages of four to seven mice. (C) At 80 d after infection, IL-15$^{-/-}$ and IL-15$^{+/-}$ littermate control mice were killed and single cell suspensions from spleens were stained with MHC tetramers as above. Absolute numbers of LCMV-specific memory CD8 T cells in the spleen were calculated. LCMV-specific CD4 responses were similar in both IL-15$^{-/-}$ and IL-15$^{+/-}$ control mice (data not shown).

both lymphoid and nonlymphoid tissues as was observed in the longitudinal PBMC analysis; fewer virus-specific memory CD8 T cells were present in both lymphoid and nonlymphoid tissues of IL-15$^{-/-}$ mice compared with IL-15$^{+/-}$ littermates. Data from a representative experiment quantitating the number of LCMV-specific CD8 T cells for four different epitopes in the spleens at day 80 after infection are shown in Fig. 1 C.

In addition to analyzing CD8 T cell responses in the cytokine knockout mice, we also examined the ability of IL-15R$\alpha^{-/-}$ mice to generate virus-specific CD8 T cell responses. The receptor knockout mice exhibited a phenotype very similar to the cytokine-deficient mice; virus-specific CD8 T cell responses of IL-15$^{-/-}$ mice were comparable to IL-15$^{+/-}$ mice at day 8 but then slowly declined over time. A representative set of data quantitating antigen-specific CD8 T cells in the spleen and liver are shown in Fig. 2 A and B. Taken together the results of Fig. 1 and Fig. 2 show that both IL-15$^{-/-}$ and IL-15R$\alpha^{-/-}$ mice mount potent primary antiviral CD8 T cell responses, but the magnitude of the LCMV-specific memory population declines in lymphoid as well as nonlymphoid tissues over time in the absence of IL-15 signals.

Since virus-specific memory CD8 T cell numbers declined in IL-15$^{-/-}$ mice, we next examined whether these cells were qualitatively similar to those induced and maintained in normal mice. First, IFN-γ production by memory CD8 T cells from IL-15$^{+/+}$ and IL-15$^{-/-}$ mice was assessed after a 5-h stimulation with peptides corresponding to a dominant and a subdominant LCMV epitope. Fig. 3 A shows MHC tetramer staining along with intracellular cytokine staining. The frequencies obtained by IFN-γ production were similar to those obtained by MHC tetramer staining, indicating that antigen-specific CD8 T cells from IL-15$^{-/-}$ mice were capable of rapidly synthesizing the effector cytokine IFN-γ in this short term assay. In addition, LCMV-specific memory CD8 T cells from IL-15$^{+/+}$ and IL-15$^{-/-}$ mice were also similar with respect to TNF-α production (Fig. 3 A). Similar results were obtained for IL-15R$\alpha^{-/-}$ and IL-15R$\alpha^{+/+}$ mice 80 d after infection (data not shown). Fig. 3 B shows that expression of two surface markers characteristic of memory CD8 T cells is comparable. Despite the deficiency in CD44^{hi} CD8 T cells in naive IL-15$^{-/-}$ mice, "true" LCMV-specific tetramer positive memory CD8 T cells induced in IL-15$^{-/-}$ mice by viral infection expressed levels of CD44 similar to those observed in +/+ LCMV immune mice (Fig. 3 B). In addition, cells generated in IL-15$^{-/-}$ mice showed an equivalent increase in their expression of CD122, the IL-2/15Rβ chain (Fig. 3 B). The latter is significant, since CD122 is thought to facilitate the response of these cells to IL-15. Staining with an additional panel of antibodies to surface markers, including CD132, Ly6C, and CD62L, and adhesion molecules including CD49b, CD54, and CD103, showed no differences between IL-15$^{-/-}$ and wild-type antigen-specific cells (data not shown).

To determine whether memory CD8 T cells maintained in an IL-15–deficient environment were competent to respond to a viral challenge in vivo, mice that had been immunized 3 mo previously with the Armstrong strain of LCMV were infected intravenously with the more virulent LCMV clone 13 strain. The memory CD8 T cells in both IL-15$^{+/-}$ and IL-15$^{-/-}$ mice expanded rapidly after reinfection and made potent secondary responses (Fig. 4). By 8 d after infection, virus was undetectable in the sera, spleens, and livers of immunized IL-15$^{+/-}$ and IL-15$^{-/-}$ animals (<50 PFU/ml) while naive (unimmunized) animals had

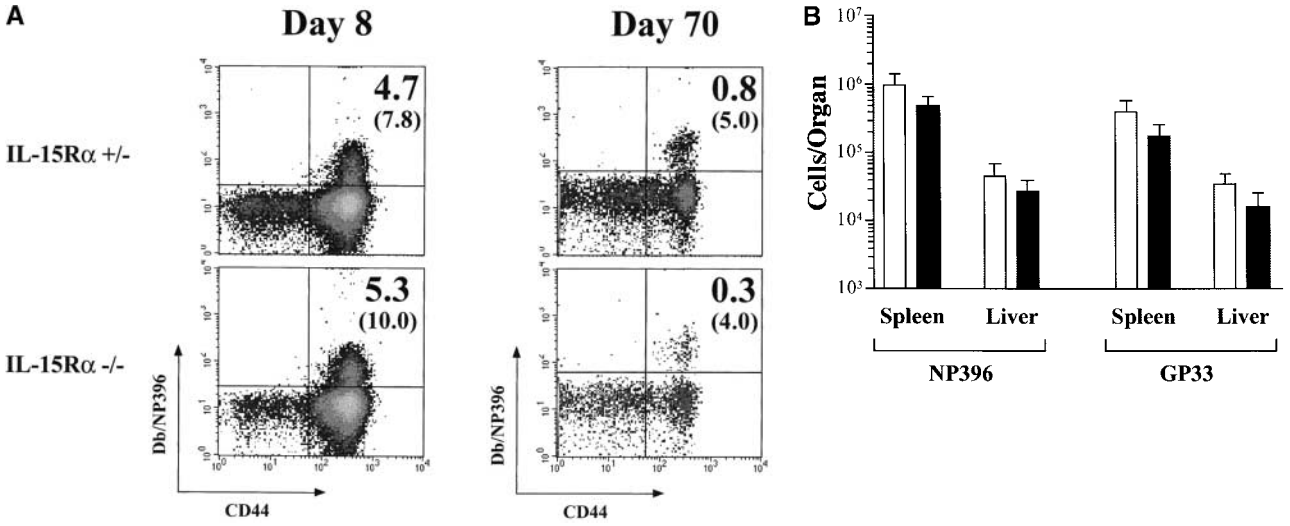

Figure 2. IL-15R$\alpha^{-/-}$ mice generate a potent LCMV-specific CD8 T cell response but memory CD8 T cell numbers are reduced relative to controls at day 70 after infection. (A) NP396-specific CD8 T cells from the PBMCs were stained as in Fig. 1. Staining is representative of five mice per group. (B) Total numbers of memory LCMV-specific CD8 T cells were determined in the spleen and liver by tetramer staining as described in Fig. 1 C. Numbers indicate the average of three to four mice per group. LCMV-specific CD4 responses were similar in both IL-15R$\alpha^{-/-}$ and IL-15R$\alpha^{+/-}$ control mice (data not shown).

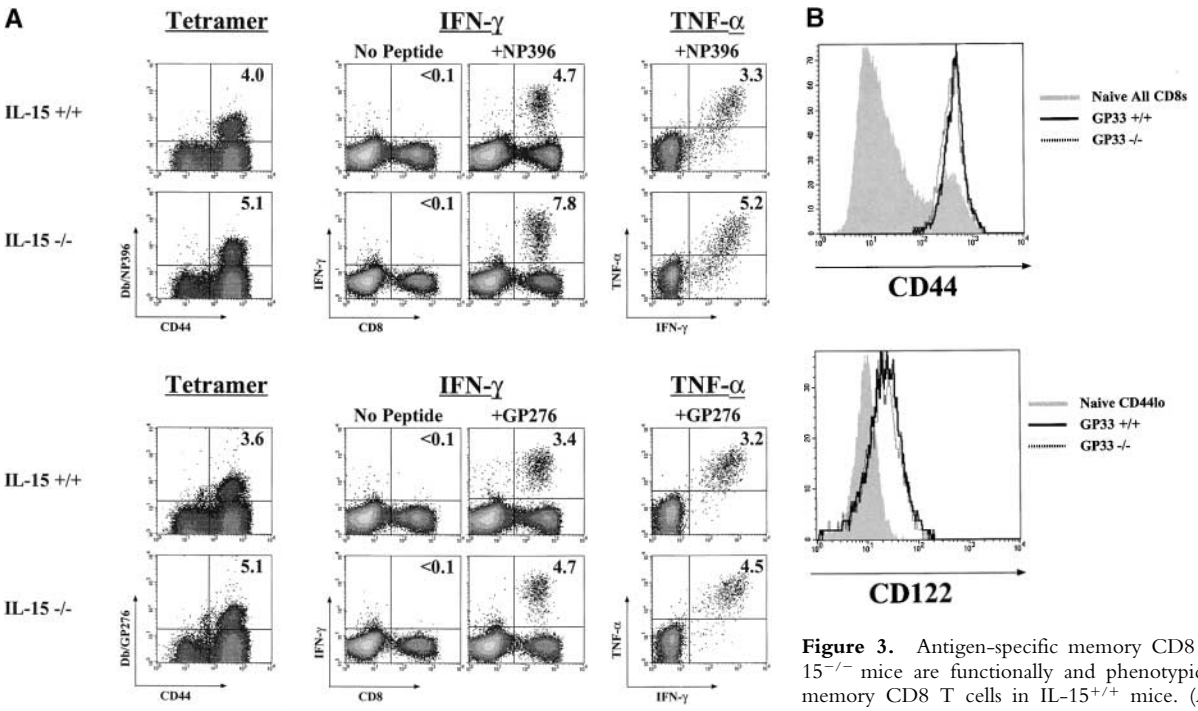

Figure 3. Antigen-specific memory CD8 T cells in IL-$15^{-/-}$ mice are functionally and phenotypically similar to memory CD8 T cells in IL-$15^{+/+}$ mice. (A) Splenocytes from IL-$15^{-/-}$ or IL-$15^{+/+}$ control mice were prepared 40 d after infection. Cells were stimulated in vitro for 5 h with NP396 or GP276 peptides, then intracellular cytokine staining was performed for IFN-γ and TNF-α. The percentage of CD8 T cells producing either cytokine is essentially equivalent to the percentage of tetramer-binding cells in all cases. Tetramer staining and IFN-γ/TNF-α dual staining is shown gated on CD8 T cells. Numbers in the top right represent the percentage of CD8 T cells (B) LCMV-specific GP33 tetramer$^+$ CD8 T cells from the spleens of IL-$15^{-/-}$ mice or IL-$15^{+/+}$ controls were stained for CD44 and CD122 at 40 d after infection. The dark line indicates GP33 tetramer$^+$ cells from a IL-$15^{+/+}$ mouse while the dashed line represents GP33 tetramer$^+$ CD8 T cells from an IL-$15^{-/-}$ mouse. Naive controls are shown in light gray; all CD8s from a naive mouse for the CD44 plot and CD44^{lo}CD8s for the CD122 plot.

high levels of virus in the sera (3.5×10^4 PFU/ml), liver (5.5×10^7 PFU/g), and spleen (10^8 PFU/g).

Together these results demonstrate that virus-specific memory CD8 T cells can be generated in the absence of IL-15 signals and these cells are phenotypically and functionally similar to memory T cells generated in IL-$15^{+/-}$ mice. However, LCMV-specific CD8 T cell numbers decline over time in receptor and cytokine knockout mice, suggesting a defect in memory maintenance.

Memory CD8 T Cells Are Unable to Undergo Homeostatic Proliferation in an IL-$15^{-/-}$ Environment. Based on the gradual decline in antigen-specific memory CD8 T cell numbers in the absence of IL-15 signals, we hypothesized that ineffective memory CD8 T cell maintenance might reflect a defect in proliferative renewal. To test this hypothesis memory CD8 T cells from B6 mice were labeled with CFSE and adoptively transferred into intact, nonirradiated, B6 or IL-$15^{-/-}$ hosts. Homeostatic proliferation of these memory CD8 T cell populations was assessed by the loss of CFSE fluorescence 30 d after transfer. When antigen-specific CD8 T cells generated in B6$^{+/+}$ animals were transferred into B6$^{+/+}$ recipient mice, their numbers were maintained and they underwent normal homeostatic prolif-

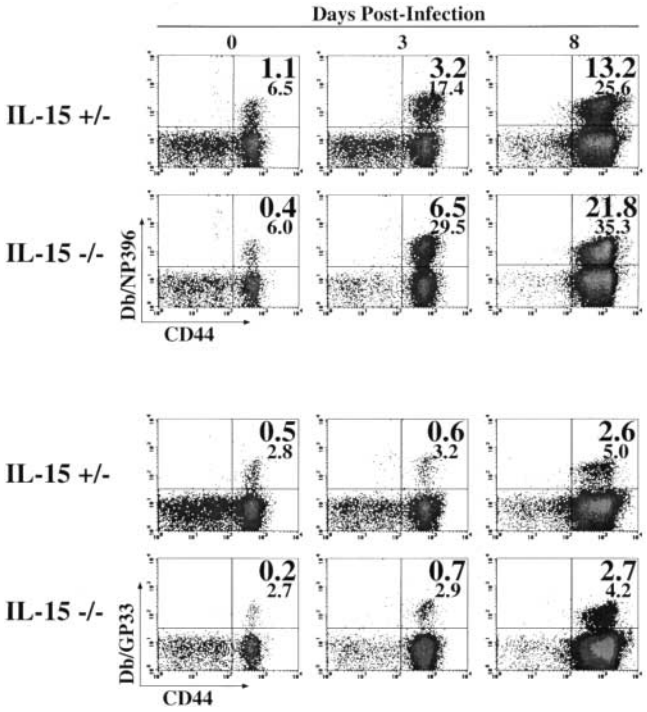

Figure 4. Memory CD8 T cells in IL-$15^{-/-}$ mice generate a potent recall response. IL-$15^{+/-}$ and IL-$15^{-/-}$ mice were immunized with the Armstrong strain of LCMV. 3 mo later, mice were challenged with the virulent LCMV clone 13 strain intravenously. PBMCs were stained using MHC class I tetramers at days 0, 3, and 8 after rechallenge. Plots are gated on CD8$^+$ T cells, and the numbers indicate the percentages of MHC tetramer positive cells as a percentage of total PBMCs (top number) and as a percentage of CD8$^+$ T cells (bottom number).

eration (Fig. 5 A). A large proportion of the transferred LCMV NP396 and GP33-specific CD8 T cells had divided between one and four times. In striking contrast, when memory CD8 T cells from B6 mice were transferred into IL-15$^{-/-}$ mice little division of antigen-specific memory CD8 T cells was detected (Fig. 5 A). Similar observations were made for total CD44^{hi} "memory phenotype" CD8 T cells; CD44^{hi} CD8 T cells divided in a +/+ environment but showed minimal to no division in IL-15$^{-/-}$ recipients. These transfer experiments clearly demonstrate that efficient homeostatic proliferation of antigen-specific memory CD8 T cells is compromised in an IL-15$^{-/-}$ environment.

The above experiments show that memory CD8 T cells generated in IL-15$^{+/+}$ mice required IL-15 for homeostatic proliferation. However, it is possible that memory CD8 T cells generated in IL-15$^{-/-}$ mice might adopt a partially or fully IL-15–independent phenotype that allows them to undergo homeostatic proliferation in an IL-15–deficient environment. To test this, memory CD8 T cells from LCMV immune IL-15$^{-/-}$ mice were CFSE-labeled and transferred into IL-15$^{-/-}$ or normal IL-15$^{+/+}$ hosts. Under these conditions, virtually no proliferation was detectable at 1 mo after transfer into IL-15$^{-/-}$ mice (Fig. 5 A). Thus, the LCMV-specific memory CD8 T cells generated in IL-15–deficient animals had not adapted to undergo IL-15–independent homeostatic proliferation. Moreover, antigen-specific memory CD8 T cells from IL-15$^{-/-}$ mice underwent substantial proliferation when transferred to normal B6$^{+/+}$ recipients, indicating that memory cells generated in IL-15$^{-/-}$ mice were still competent to respond to the IL-15 signals for homeostatic proliferation.

The total numbers of memory CD8 T cells recovered from the spleen at 30 d after adoptive transfer into +/+ or −/− mice are shown in Fig. 5 B. In all instances (+/+ memory CD8 T cells into +/+ or −/− mice or −/− memory cells into +/+ or −/− mice) there were fewer memory CD8 T cells present in IL-15$^{-/-}$ recipients. This is consistent with the data obtained from the longitudinal analysis of memory CD8 T cells shown in Fig. 1. However, a particularly interesting pattern emerged when we analyzed the number of memory CD8 T cells present in the "divided" versus "undivided" cell populations. There was no loss in the number of undivided memory CD8 T cells present in IL-15$^{-/-}$ recipients compared with IL-15$^{+/+}$. In fact, in most cases the number was slightly higher in IL-15–deficient mice. This suggests that memory CD8 T cells that are in G_0/G_1 can survive in the absence of IL-15. In striking contrast to the persistence of undivided memory CD8 T

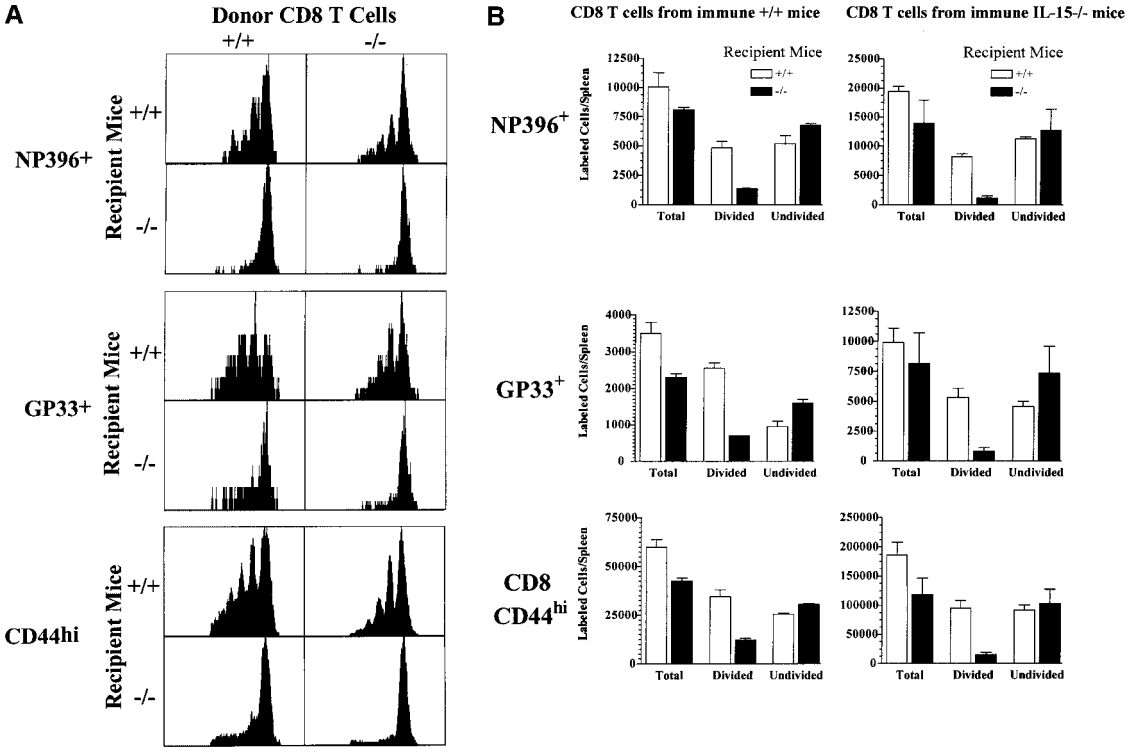

Figure 5. Memory CD8 T cells do not undergo homeostatic proliferation, but undivided cells are maintained in an IL-15$^{-/-}$ environment. (A) Splenocytes from LCMV immune mice were labeled with CFSE and transferred into naive recipients. 30 d after transfer, spleens were removed from recipient animals and cells were stained using MHC class I tetramers to identify antigen specific memory CD8 T cells. Transferred antigen-specific cells proliferated in IL-15$^{+/+}$ but not IL-15$^{-/-}$ mice over a 30-d period. In the right column, the same experiment was performed using splenocytes from immune IL-15$^{-/-}$ mice and similar results were observed. Homeostatic proliferation of CD4 T cells occurred and was normal in all environments (data not shown) (B) Labeled cells recovered from the recipient spleens at 30 d after transfer were quantified. In addition, the number of undivided cells (i.e., zero division CFSE peak) and the number of divided cells (sum of cells in divisions 1–4) are graphed. Results are similar for transfers of memory cells from +/+ mice (left panel) and IL-15$^{-/-}$ mice (right).

cells in IL-15$^{-/-}$ mice, very few memory cells had undergone division in the absence of IL-15. The decrease in total numbers of memory CD8 T cells after adoptive transfer into IL-15$^{-/-}$ mice was solely due to the absence of this divided population of memory cells. These results show that IL-15 plays an important role in cell cycle progression of memory CD8 T cells. Alternatively, it is equally plausible that IL-15 is essential for the survival of "dividing" memory CD8 T cells. These two are not mutually exclusive and it is possible that IL-15 is needed for initiating entry into the cell cycle and also for the survival of memory CD8 T cells in cycle.

T cells undergo two distinct types of proliferation; antigen-driven and homeostatic (7, 16, 17, 19, 34). After antigenic stimulation, both naive and memory CD8 T cells undergo rapid cell division (6–8 h doubling time) and also initiate a program that results in at least seven consecutive divisions (5, 7, 23). This proliferation results in substantial increases in the number of antigen-specific T cells. Thus, the primary function of antigen-driven proliferation is clonal expansion. The results of our study show that IL-15 does not play a major role in this process and that at least after a viral infection antigen-driven proliferation of CD8 T cells appeared to proceed normally in the absence of IL-15. In striking contrast, the second type of T cell proliferation termed "homeostatic" proliferation was dependent on IL-15. Only memory T cells undergo homeostatic proliferation under normal physiological conditions, naive cells do not. Also, in contrast to antigen-driven proliferation, homeostatic proliferation does not result in a net increase in the number of memory T cells and the primary function of this proliferation is maintaining the pool of memory T cells; hence the term "homeostatic" proliferation accurately describes this type of cell division. Our study not only identifies a key cytokine involved in memory CD8 T cell proliferation, but also shows that this proliferative renewal is critical for maintaining memory T cell numbers. In the absence of IL-15 signals, memory CD8 T cells in G_0/G_1 were able to survive as well as in IL-15$^{+/+}$ mice, but homeostatic proliferation was severely compromised, and this resulted in a net decrease in the total number of memory CD8 T cells. It will be interesting to determine whether the loss of T cell memory that is seen during aging and in certain other conditions is related to IL-15 deficiency and whether treatment with IL-15 can restore CD8 T cell memory. Also, it will be of interest to address in future studies which are the critical cell types that produce IL-15, how IL-15 regulates the proliferation and survival of memory CD8 T cells, whether IL-15 is acting directly on memory CD8 T cells or mediating its effects by acting on non-T cells, and, in particular, to determine how IL-15 effects the expression and function of genes involved in cell cycle regulation.

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