# The Journal of Immunology

## Cutting Edge: Requirement for IL-15 in the Generation of Primary and Memory Antigen-Specific CD8 T Cells

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# Cutting Edge

# Cutting Edge: Requirement for IL-15 in the Generation of Primary and Memory Antigen-Specific CD8 T Cells[1]

Kimberly S. Schluns,* Kristina Williams,* Averil Ma,† Xin X. Zheng,‡ and Leo Lefrançois[2]*

**IL-15 and IL-15Rα are required for generation of memory-phenotype CD8 T cells in unimmunized mice. However, the role of IL-15 in primary expansion and generation of Ag-specific memory CD8 T cells in vivo has not been investigated. We characterized the CD8 T cell response against vesicular stomatitis virus (VSV) in IL-15$^{-/-}$ and IL-15Rα$^{-/-}$ mice. Surprisingly, IL-15 was required for primary expansion of VSV-specific CD8 T cells. The generation of VSV-specific memory CD8 T cells was also impaired without IL-15 signaling, and this defect correlated with a decrease in memory CD8 T cell turnover. Despite minimal proliferation without IL-15, a subset of memory cells survived long-term. IL-15Rα expression was low on naive CD8 T cells, up-regulated on Ag-specific effector cells, and sustained on memory cells. Thus, IL-15 was important for the generation and the subsequent maintenance of antiviral memory CD8 T cells.   _The Journal of Immunology,_ 2002, 168: 4827–4831.**

I nterleukin-15 is a T cell-stimulating cytokine that while sharing some functions with IL-2 also mediates unique functions (1, 2). IL-2 and IL-15 receptor complexes are composed of the IL-2/IL-15Rβ subunit (CD122) and the common γ-chain (CD132), but each receptor complex combines with a private α-chain conferring high-affinity binding for either IL-2 or IL-15 (3–5). The expression patterns of IL-2Rα and IL-15Rα are quite distinct and this may contribute to differential functions mediated by these cytokines (1). Whereas IL-2Rα is expressed by immature T and B lineage cells and mature T cells, IL-15Rα mRNA is expressed in many cell types including lymphocytes (6). IL-15Rα

protein expression has yet to be characterized in detail. Despite the fact that IL-15Rα mRNA is widely expressed outside the immune system, the majority of data indicate that one of the major roles of IL-15 is in regulating development of NK cells and CD8 T cells (7–9).

The precise mechanisms by which memory CD8 T cells survive are unknown but survival is due in part to their ability to undergo continuous low level proliferation (10) in which IL-15 may participate (11). Memory-phenotype CD8 T cells express elevated levels of IL-2/15Rβ compared with naive CD8 T cells (7) and this correlates with their ability to proliferate in response to IL-15 (7). Studies by Ku et al. (11) also show that proliferation of memory-phenotype CD8 T cells is blocked by in vivo treatment with an anti-IL-2/IL-15Rβ receptor Ab. Furthermore, mice lacking IL-15 or IL-15Rα expression are deficient in memory phenotype CD8 T cells. Thus, there is evidence that IL-15 is involved in the generation and/or the maintenance of memory phenotype CD8 T cells. However, whether the requirements for the production of these cells are the same as those for production of Ag-specific antimicrobial CD8 memory T cells has not been tested. Moreover, whether the importance of IL-15 is manifest during a primary response is unknown. In this study, we characterize the primary and memory CD8 T cell response to vesicular stomatitis virus (VSV)[3] infection in IL-15$^{-/-}$ and IL-15Rα$^{-/-}$ mice and analyze IL-15Rα expression during the response.

## Materials and Methods

### Mice

C57BL/6J (Ly5.1) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/6-IL-15$^{-/-}$ mice were provided by Dr. J. Peschon (Immunex, Seattle, WA) (9). IL-15Rα$^{-/-}$ mice (8) were used after five backcross generations to C57BL/6J.

### Infections, isolation of lymphocytes, and immunofluorescence analysis

Mice were infected i.v. with $1 \times 10^6$ PFU of VSV-Indiana (12). At the indicated times, lymphocytes from spleen, peripheral lymph nodes (PLN), mesenteric lymph nodes (MLN), lung, and liver, and small intestinal lamina propria (LP) were isolated as previously described (13, 14). VSV-N-specific CD8 T cells were detected using H-2K$^b$ tetramers containing the N protein-derived peptide RGYVYQGL (14) and were generated as previously described (15, 16). To identify VSV-N-specific CD8 T cells, cells were incubated with 100 µl of a properly diluted mixture of N-tetramer-APC and anti-CD8 PerCP mAb (clone 53.6.72; BD Biosciences, San Jose, CA) at 25°C for 1 h as previously described (14). To detect IL-15 binding,

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[3] Abbreviations used in this paper: VSV, vesicular stomatitis virus; BrdU, 5-bromo-2′-deoxyuridine; PLN, peripheral lymph node; MLN, mesenteric lymph node; LP, lamina propria.

cells were incubated with IL-15 mutant Fcγ2a fusion protein (5 μg/ml) (17) for 20 min at 4°C and the bound IL-15/Fc fusion protein was detected using goat anti-mouse IgG2a-PE (2.5 μg/ml; Southern Biotechnology Associates, Birmingham, AL). Relative fluorescence intensities were measured with a FACSCalibur (BD Biosciences). Data were analyzed using WinMDI software (J. Trotter, The Scripps Clinic, La Jolla, CA). Statistics were performed using Student's *t* test.

*Analysis of proliferation in vivo*

VSV-infected mice were given water daily containing 5-bromo-2′-deoxyuridine (BrdU, 0.8 mg/ml; Sigma-Aldrich, St. Louis, MO) for 4 wk. Spleen cells were stained with N-tetramer and anti-CD8 mAb and then treated according to the BrdU flow kit protocol (BD Biosciences). For transfer studies, B cell-depleted spleen cells labeled with CFSE (0.01 mM; Molecular Probes, Eugene, OR) (18) were transferred into control and IL-15$^{-/-}$ hosts by i.v. injection. The percentage of cells of the original population that had divided (the "responding" population, R) was calculated as described elsewhere (19).

## Results and Discussion

*IL-15 drives optimal expansion of primary antiviral CD8 T cells.*

Whereas both IL-15 and IL-15Rα are required for the generation of CD44$^{high}$CD8 T cells (8, 9), we asked whether there was a similar requirement for generation of primary Ag-specific CD44$^{high}$CD8 T cells in response to an infection. To examine the dynamics of an antiviral CD8 T cell response in the absence of IL-15, IL-15$^{-/-}$ and control mice were infected i.v. with VSV, and VSV-specific CD8 T cells were tracked by reactivity with an H-2K$^b$ tetramer containing the immunodominant VSV-derived nucleoprotein peptide (14). Lymphocytes from spleen, PLN, MLN, lung, liver, and LP were isolated and tested for tetramer reactivity. We extended this observation to extralymphoid tissues as we have previously demonstrated that virus-specific primary and memory CD8 T cells populate many nonlymphoid tissues (14). At the peak of the response, 7 days after infection (14), the percentage of tetramer-positive cells among CD8 T cells was decreased 40–50% in secondary lymphoid and tertiary tissues of IL-15$^{-/-}$ mice compared with wild-type mice (Fig. 1, *A* and *B*). In IL-15$^{-/-}$ and IL-15Rα$^{-/-}$ mice, the overall CD8 T cell pool is decreased by ~50% (8, 9). Therefore, we expressed our results as a percentage of the total CD8 T cell population to provide a valid comparison to controls since the total number of tetramer-positive cells will be decreased in IL-15/IL-15Rα-deficient mice due to the reduced starting population.

CD44 expression was also examined after infection to determine whether IL-15 was involved in regulation of this molecule. Tetramer-positive cells from both IL-15$^{-/-}$ and control mice had similarly high CD44 levels (Fig. 1*A*), indicating that IL-15$^{-/-}$ CD8 T cells do not have a defect in CD44 up-regulation. Interestingly, a population of tetramer-negative CD44$^{high}$ CD8 T cells was also induced after infection of control or IL-15$^{-/-}$ mice (Fig. 1*A*, *lower right quadrant*). It is possible that some of these cells were specific for VSV epitopes other than the N-peptide used in our tetramer, although no others have been described. This population may also be the result of bystander activation induced by the inflammation associated with virus infections (20).

To determine whether the defect in IL-15$^{-/-}$ mice was due to an abnormal precursor frequency or due to decreased expansion of activated T cells, the percentage of tetramer-positive cells from the peripheral blood was measured before the peak of the response. At 4 and 5 days after infection, there was no deficiency in tetramer-positive cells in the IL-15$^{-/-}$ mice compared with controls (Fig. 1*C*). However, 6 days after infection, a slight decrease in tetramer-positive cells became apparent and this decrease was dramatic after 7 days (Fig. 1*C*). These data indicated that IL-15 was important
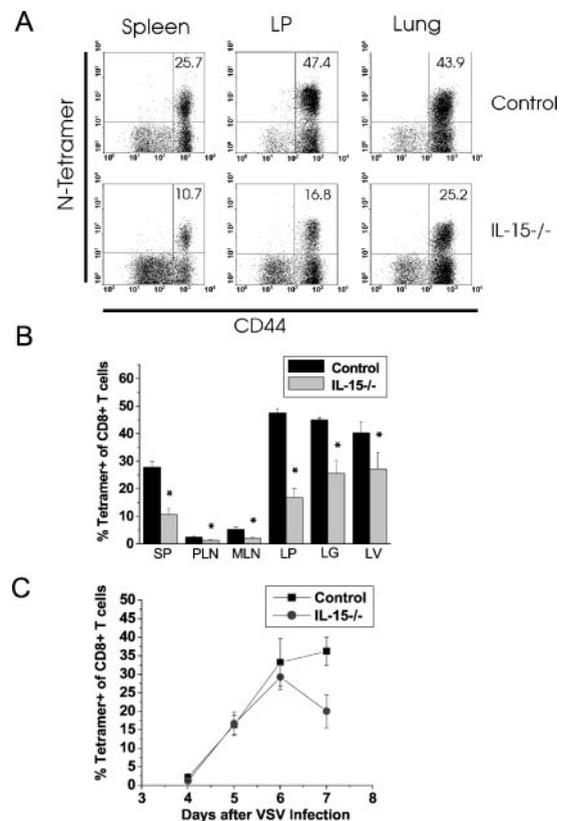


**FIGURE 1.** IL-15 is required during the primary CD8 T cell response to VSV infection. *A* and *B,* Seven days after VSV infection, lymphocytes were isolated from spleen (SP), PLN, MLN, intestinal LP, lung (LG), and liver (LV) and analyzed for expression of CD8, CD44, and for reactivity with N-tetramer. Values represent percent tetramer-positive cells among CD8 T cells. *B,* Mean percent ± SD of N-tetramer-positive among CD8 T cells (*n* = 3). *C,* Percent tetramer-positive among CD8 T cells in peripheral blood. ∗, Significance of $p < 0.05$. Data are representative of three experiments.

in determining the amplitude of the VSV-specific primary response and that the initial precursor frequency in IL-15$^{-/-}$ mice was likely normal. The burst size of the primary antiviral CD8 T cell response is thought to correlate with the size of the resulting memory population (21). Therefore, the defective primary CD8 T cell expansion noted in VSV-infected IL15$^{-/-}$ mice might be expected to yield a reduced memory population (see below).

Given our results using IL-15$^{-/-}$ mice, we wished to determine the requirement for IL-15Rα in the same response. To this end, IL-15Rα$^{-/-}$ and control mice were VSV-infected and 7 days later the percentage of tetramer-positive cells was determined. IL-15Rα$^{-/-}$ mice had an ~10–20% decrease in the percentage of tetramer-positive cells among CD8 T cells in the spleen but this difference was not statistically significant (Fig. 2) in this or several other experiments. There was no difference in the percentage of tetramer-positive cells in the PLN or MLN but, interestingly, the response in the intestine was decreased ~50% in IL-15Rα$^{-/-}$ mice (Fig. 2). In contrast, the lung and liver contained normal percentages of VSV-specific CD8 T cells (Fig. 2). These results demonstrated a minimal requirement for IL-15Rα in the primary expansion of antiviral CD8 T cells except in the intestinal mucosa.

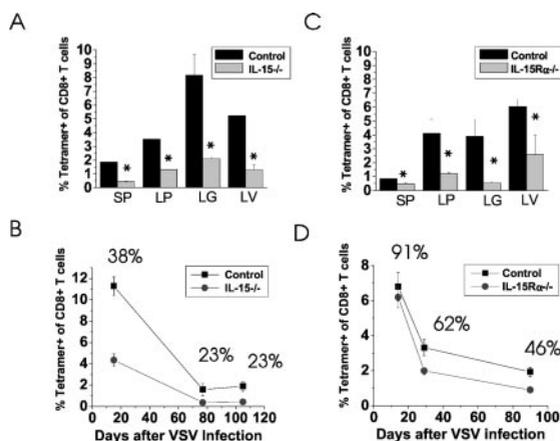*Role of IL-15 and IL-15Rα in generation of CD44$^{high}$ virus-specific memory CD8 T cells*

Considering the deficiency in memory-phenotype CD44$^{high}$CD8 T cells in IL-15$^{-/-}$ and IL-15Rα$^{-/-}$ mice, we investigated whether
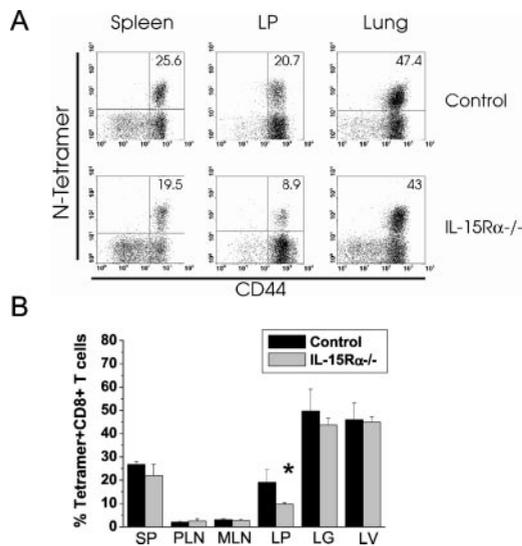
**FIGURE 2.** Primary expansion of virus-specific CD8 T cells does not require IL-15Rα. *A* and *B*, CD8 T cells 7 days postinfection. Values are percentage of tetramer-positive cells among CD8⁺ T cells. *B*, Mean percent ± SD of N-tetramer-positive CD8 T cells ($n = 3–4$). Data are representative of three experiments.

the absence of IL-15Rα or IL-15 affected the generation of Ag-specific memory CD8 T cells. The percentage of tetramer-positive memory cells was quantitated 38 days after VSV infection of IL-$15^{-/-}$ and control mice. Compared with memory levels in lymphoid and nonlymphoid tissues of control mice, memory populations were decreased 60–80% in the tissues of IL-$15^{-/-}$ mice and all differences were statistically significant (Fig. 3*A*). The response was also measured over time in the peripheral blood of a cohort of mice (Fig. 3*B*). At 15 days after infection, the percentage of Ag-specific cells in IL-$15^{-/-}$ mice was only 38% of control levels (Fig. 3*B*) and at 77 days was 23% of control levels, and this difference was maintained until at least 105 days after infection (Fig. 3*B*). These results suggested that although the generation of a substantial portion of antiviral memory CD8 T cells required IL-15, a subset of long-lived IL-15-independent CD8 memory T cells existed.
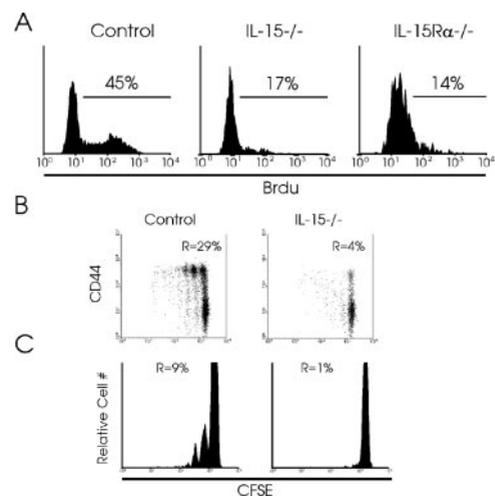
Ag-specific CD8 T cells were also quantitated in IL-15Rα$^{-/-}$ and control mice 75 days after infection (Fig. 3*C*). The percentage of tetramer-positive memory cells in the spleen, LP, lung, and liver of IL-15Rα$^{-/-}$ mice was decreased 50–75% as compared with controls (Fig. 3*C*). To determine the point in the immune response when IL-15Rα became important for CD8 memory generation, the level of tetramer-positive cells was tracked over time. Whereas there was no difference in the percentage of tetramer-positive cells between the lL-15Rα$^{-/-}$ mice and control mice 7 days after infection, differences emerged 14 days after infection and became progressively greater over time (Fig. 3*D*). These data indicated that IL-15Rα participates in the generation of memory CD8 T cells during the contraction phase and effects memory maintenance.

### Turnover of memory CD8 T cells requires IL-15 and IL-15Rα

Previous studies have demonstrated that IL-15 has a role in the low level proliferation of memory-phenotype CD8 T cells (11). Therefore, we determined whether IL-15 regulated proliferation of Ag-specific memory CD8 T cells. IL-$15^{-/-}$, IL-15Rα$^{-/-}$, and control mice that were infected with VSV at least 30 days earlier were administered BrdU for 4 wk. At that time, incorporation of BrdU into tetramer-positive CD8 T cells from the spleen was measured as an indicator of cell division. Among CD8⁺tetramer⁺ gated cells, 45% of the cells from control mice had incorporated BrdU (Fig. 4*A*). In contrast, only 17 and 14% of the tetramer-positive CD8 cells were BrdU positive from IL-$15^{-/-}$ and IL-15Rα$^{-/-}$ mice, respectively (Fig. 4*A*). In addition, the BrdU⁺ T cells in the IL-15 and IL-15Rα-deficient mice exhibited only low levels of incorporation as compared with controls.

To analyze the role of IL-15 in the turnover of memory cells generated in normal mice, we used CFSE labeling and adoptive transfer. To obtain increased numbers of memory cells, mice were infected with VSV-Indiana and then challenged with the cross-reactive VSV-New Jersey serotype 70 days later. Thirty days after the second infection, spleen cells labeled with CFSE were injected into IL-$15^{-/-}$ or control mice. Fifty days later, CFSE intensity was examined. Twenty-nine percent of CD44$^{high}$CD8 T cells had divided in control hosts with the majority having divided one or two



**FIGURE 3.** IL-15 and IL-15Rα are required for generation of normal numbers of antiviral memory CD8 T cells. *A* and *C*, Mean percent ± SD of tetramer-positive cells among CD8 T cells 38 days (*A*) and 75 days (*C*) after infection ($n = 3$). *B* and *D*, Different experiments showing percentage of tetramer-positive CD8 T cells in the peripheral blood. Numbers represent the percentage of control.



**FIGURE 4.** Proliferation of memory CD8 T cells is defective in IL-$15^{-/-}$ and IL-15R$^{-/-}$ mice. VSV-infected mice were given BrdU for 4 wk. *A*, BrdU intensity on N-tetramer-positive CD8⁺ gated cells. Splenocytes from VSV-infected mice were stained with CFSE and transferred into either control or IL-$15^{-/-}$ mice. *B* and *C*, CFSE intensity of total CD8 T cells (*B*) and N-tetramer-positive CD8⁺ gated T cells (*C*) 50 days after transfer. R is the calculated value of the percentage of the population responding.

times. In contrast, few dividing CD44$^{high}$CD8 T cells were detected in IL-15$^{-/-}$ hosts (Fig. 4*B*). Similarly, N-tetramer-positive CD8 T cells divided in control mice but not in IL-15$^{-/-}$ mice (Fig. 4*C*). In contrast to CD8 T cells, CD4 T cell division was similar in IL-15$^{-/-}$ and control mice (data not shown). Thus, IL-15/IL-15R$\alpha$ is critical for maintaining proliferation of virus-specific CD8 memory cells.

*Expression of IL-15R by naive and memory CD8 T cells*

Whether the differential effects of IL-15 on naive, primary effectors, or memory CD8 T cells was due to differences in IL-15R$\alpha$ expression are unknown and thus far the expression of the IL-15R$\alpha$ subunit on CD8 T cell subsets has not been examined. Therefore, we measured IL-15R$\alpha$ expression by using an IL-15 mutant/Fc$\gamma$2a fusion protein (17) (Fig. 5). In unimmunized mice, the highest level of IL-15/Fc binding was detected on CD44$^{high}$CD8 T cells which also correlates with high expression of CD122 (IL-2/15R$\beta$) on memory-phenotype CD8 T cells (7, 11). Interestingly, naive CD44$^{low}$CD8 T cells also expressed IL-15R$\alpha$, albeit at lower levels than memory-phenotype CD8 T cells. The binding of IL-15/Fc was attributed to reactivity with the IL-15R$\alpha$ chain, since naive IL-15R$\alpha^{-/-}$CD8 T cells did not bind the IL-15/Fc protein (Fig. 5, *left panel*). IL-15/Fc binding was also analyzed on Ag-specific CD8 T cells from normal and IL-15R$\alpha^{-/-}$ mice. Six days after VSV infection, tetramer-positive CD8 T cells from normal mice displayed high IL-15/Fc binding (Fig. 5). Although some low level binding of IL-15/Fc to tetramer-positive IL-15R$\alpha^{-/-}$ cells was detected, this binding was not blocked by preincubation with IL-15, while IL-15/Fc binding to normal naive CD8 T cells and to activated normal tetramer-positive cells was inhibited by IL-15 (data not shown). Similar to memory-phenotype CD8 T cells from unimmunized mice, tetramer-positive memory cells from normal mice 75 days after infection displayed high levels of IL-15/Fc binding. Tetramer-positive memory cells from IL-15R$\alpha^{-/-}$ mice exhibited no detectable binding of IL-15/Fc (Fig. 5). Therefore, IL-15/Fc binding to CD8 T cells is dependent on the expression of the IL-15R$\alpha$ molecule and thus our data show for the first time the expression of IL-15R$\alpha$ throughout an antiviral immune response.

Our data demonstrated that naive CD8 T cells expressed low levels of IL-15R$\alpha$ and thus may be targets of IL-15 action. Therefore, the deficiency of naive CD8 T cells in IL-15$^{-/-}$ and IL-15R$\alpha^{-/-}$ mice may be due to effects on naive CD8 T cells, although thymic defects could also contribute. Nevertheless, our examination of the early CD8 T cell response to VSV infection indicated that a proportionally normal frequency of Ag-specific cells was present in IL-15$^{-/-}$ mice since the percentage of tetramer-positive cells was similar in IL-15$^{-/-}$ and normal mice 4 days after infection (Fig. 1).

The increased levels of IL-15R$\alpha$ expressed by activated and memory CD8 T cells were likely responsible for the observed IL-15 augmentation of the primary response and the induction of memory cell proliferation by IL-15. Although we observed that the defect in the primary response in IL-15$^{-/-}$ mice was more severe than that in IL-15R$\alpha^{-/-}$ mice, it is possible that IL-15 delivers signals via the intermediate affinity IL-15R$\beta\gamma$ (5) and that the up-regulation of IL-15 during infection could augment this signaling. In addition, a second receptor for IL-15 has been described but is apparently expressed only in mast cells and has not been further characterized (22). In any case, the overall requirement for IL-15 during the antiviral response is exemplified by the defects observed in IL-15$^{-/-}$ mice in both the primary and memory phases of the response. It should also be noted that IL-15 was important for the generation of both central and effector memory cells, in that establishment of memory cells in lymphoid and nonlymphoid tissues was IL-15 dependent (Fig. 3).

Despite the fact that IL-15 was critical for the generation and maintenance of memory CD8 T cells, a subset of memory cells survived for several months in the absence of IL-15 signaling and with minimal proliferation. This is in contrast to CD44$^{high}$CD8 T cells which are deficient in 5-wk-old IL-15$^{-/-}$ mice. This finding suggests that the generation and/or survival of memory-phenotype CD8 T cells is more critically dependent on IL-15 than are virus-specific memory CD8 T cells. Furthermore, the presence of an IL-15-independent memory CD8 T cell population suggests that other factors may be involved in memory CD8 T cell maintenance. Since IL-7R$\alpha$ is highly expressed by memory CD8 T cells and is involved in T cell survival (23), it is possible that the IL-15-independent memory population is being maintained by IL-7 survival signals. Thus, growth-promoting effects of IL-15 in combination with the survival signals induced by IL-7 are likely to be in large part responsible for induction and maintenance of memory CD8 T cells.

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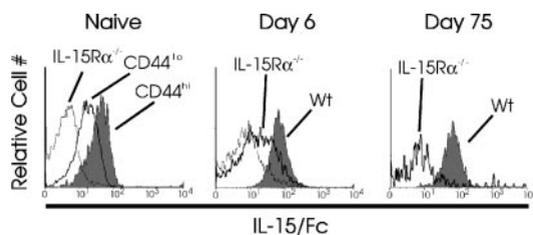
**FIGURE 5.** IL-15R$\alpha$ is expressed on naive CD8 T cells and is up-regulated on effector and memory CD8 T cells. Spleen cells from unimmunized normal mice and VSV-infected normal and IL-15R$\alpha^{-/-}$ mice were incubated with IL-15/Fc followed by anti-IgG2a-PE Ab to detect binding. Panel 1, IL-15/Fc binding of CD8 T cells from unimmunized mice: control CD44$^{high}$ (shaded histogram), control CD44$^{low}$ cells (bold black line), and IL-15R$\alpha^{-/-}$ CD8$^+$ cells (thin black line). Panels 2 and 3, IL-15/Fc binding on N-tetramer-positive CD8 T cells 6 and 75 days postinfection: control mice (shaded histograms) and IL-15R$\alpha^{-/-}$ mice (bold thick histograms). The negative control represents staining in the absence of the IL-15/Fc but in the presence of the anti-IgG2a secondary Ab (panel 2, thin black line).

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