# Interleukin 15 Controls both Proliferation and Survival of a Subset of Memory–Phenotype CD8$^+$ T Cells

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## Abstract

Previous work has shown that memory-phenotype CD44$^{hi}$ CD8$^+$ cells are controlled by a cytokine, interleukin (IL)-15. However, the dependency of CD44$^{hi}$ CD8$^+$ cells on IL-15 is partial rather than complete. Here, evidence is presented that CD44$^{hi}$ CD8$^+$ cells comprise a mixed population of IL-15–dependent and IL-15–independent cells. The major subset of CD122$^{hi}$ CD44$^{hi}$ CD8$^+$ cells is heavily dependent on IL-15 by three different parameters, namely (1) "bystander" proliferation induced via IFN-induced stimulation of the innate immune system, (2) normal "background" proliferation, and (3) T cell survival; IL-15 dependency is most extreme for the Ly49$^+$ subset of CD122$^{hi}$ CD44$^{hi}$ CD8$^+$ cells. In contrast to CD122$^{hi}$ cells, the CD122$^{lo}$ subset of CD44$^{hi}$ CD8$^+$ cells is IL-15 independent; likewise, being CD122$^{lo}$, CD44$^{hi}$ CD4$^+$ cells are IL-15 independent. Thus, subsets of memory-phenotype T cells differ radically in their sensitivity to IL-15.

Key words:   IL-15 • T cell subsets • CD122 • CD44 • memory

## Introduction

For typical naive T cells, the long lifespan of these cells requires contact with self-peptides bound to MHC molecules (1) plus exposure to a cytokine, IL-7 (2, 3). These findings have focused attention on the factors controlling the survival of T cells with a memory (CD44$^{hi}$) phenotype. The latter cells increase progressively with age and are presumed to represent memory cells primed to various environmental antigens (4).

Whereas naive T cells remain in interphase for prolonged periods, the turnover (background rate of proliferation) of memory-phenotype cells is appreciable, division of these cells being in the order of once every 1–3 wk (5–7). This relatively high rate of turnover might reflect that memory-phenotype T cells are engaged in chronic responses to environmental antigens. However, adoptive transfer experiments have shown that both the survival and turnover of memory-phenotype T cells are MHC independent (6, 8). The alternative possibility is that memory-phenotype T cells are maintained through contact with cytokines. Here, for memory-phenotype (CD44$^{hi}$) CD8$^+$ cells, indirect evidence suggests that these cells may be controlled by IL-15, an IL-2–like cytokine synthesized by a variety of cell types, though not by T cells (9). The evidence is as follows.

First, the background turnover of CD44$^{hi}$ CD8$^+$ cells is intensified after in vivo exposure to agents that stimulate the innate immune system, for example LPS, poly I:C, and CpG DNA (10–13). Such stimulation is much more pronounced for CD44$^{hi}$ CD8$^+$ cells than for CD44$^{hi}$ CD4$^+$ cells and is TCR independent, requires the release of IFNs, either type I IFN (IFN-I) or IFN-$\gamma$, and can be mimicked by injection of IFNs (10, 14). Since IFNs failed to stimulate purified CD44$^{hi}$ CD8$^+$ cells in vitro, we suggested previously that stimulation of these cells in vivo might be mediated through IFN-induced production of an effector cytokine, possibly IL-15 (15). Although circumstantial evidence favors this possibility (see below), proof that IFN-dependent "bystander" (TCR independent) proliferation of CD44$^{hi}$ CD8$^+$ cells is mediated by IL-15 rather than by other cytokines has yet to be obtained.

Other evidence that IL-15 influences CD44$^{hi}$ CD8$^+$ cells comes from the finding that numbers of CD44$^{hi}$ CD8$^+$ cells (but not CD44$^{hi}$ CD4$^+$ cells) are selectively reduced in IL-15$^{-/-}$ (16) and IL-15R$\alpha^{-/-}$ (17) mice. These findings are not definitive, however, because the reduction of CD44$^{hi}$ CD8$^+$ cells in IL-15$^{-/-}$ and IL-15R$\alpha^{-/-}$ mice is only partial (50–70%); moreover, the data do not indicate whether IL-15 is required for the initial generation of CD44$^{hi}$ CD8$^+$ cells or for their survival. Direct evidence that IL-15 affects CD44$^{hi}$ CD8$^+$ cells has come from the finding that the background turnover of these cells can be

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inhibited by injection of a mAb specific for CD122 (IL-2Rβ), an important component of the receptor for IL-15 and IL-2 (7, 18); by contrast, turnover of CD44hi CD8+ cells is enhanced by IL-2 mAb, implying a positive role for IL-15 and an inhibitory role for IL-2. However, it is notable that inhibition of CD8+ cell turnover by CD122 mAb is incomplete, i.e., ∼50% (tested in 1-y-old mice) (18), which suggests that stimulation may not be restricted to IL-15.

In this paper, we sought definitive information on the role of IL-15 on T cells with the aid of IL-15−/− mice. Evidence is presented that, for CD44hi CD8+ cells, IFN-induced bystander proliferation, normal turnover, and survival on adoptive transfer are all highly dependent on IL-15; by contrast, IL-15 has no obvious effect on CD44hi CD4+ cells. For CD44hi CD8+ cells, the influence of IL-15 is largely restricted to CD122hi cells and is most prominent for the Ly49+ subset of CD122hi cells, i.e., for T cells expressing a prominent natural killer (NK)* cell marker (19). In contrast to CD122hi cells, the subset of CD122lo CD44hi CD8+ cells is largely IL-15 independent. Significantly, these cells, which are Ly49−, account for nearly all of the residual CD44hi CD8+ cells found in IL-15−/− mice.

## Materials and Methods

*Mice.* C57BL/6J (B6), Thy1.1 (B6.PL), Ly5.1 (B6.SJL), CD122-deficient (CD122−/−), and IL-2–deficient (IL-2−/−) mice on a B6 background were purchased from The Jackson Laboratory. IL-15–deficient (IL-15−/−) mice generated on a B6 background were provided by Mary Kennedy, Immunex Corp., Seattle, WA (16) and maintained by homozygous × heterozygous breeding. Heterozygous (IL-15+) littermates or B6 mice were used as controls. No apparent differences have been reported between IL-15 heterozygotes and wild-type B6 mice (16). All mice were housed under specific pathogen-free conditions at The Scripps Research Institute and were used between 6–20 wk of age.

*Isolation of T Cells.* CD8+ T cells used for adoptive transfer or in vitro experiments were purified from LN cells by MACS®. In brief, LN cells were resuspended in PBS supplemented with 0.5% BSA and 2 mM EDTA and incubated with CD8α mAb-coated magnetic beads according to the manufacturer's protocol. Cells were then washed and separated by passing the cells through a charged MACS® column. CD8+ cells eluted from the column were typically 94–96% pure. Alternatively, CD8+ or CD4+ cells were purified by negative depletion of LN cells using MHC class II mAb plus either CD4 or CD8α mAb-coated magnetic beads, respectively. Similar purities were obtained by this method. For in vitro experiments, CD8+ T cells were fractionated further into CD44hi CD122hi, CD44hi CD122lo, or CD44lo CD122lo subsets by FACS® sorting. Typical purities of sorted populations were >98%.

*CFSE Labeling.* Purified CD8+ T cells were suspended in PBS containing 0.1% BSA at a concentration of 2 × 10⁷ cells per milliliter. CFSE (Molecular Probes) was added at a final concentration of 5 μM and the cells incubated for 10 min at 37°C. Labeled cells were washed twice then resuspended in

PBS at 4 × 10⁷ cells per milliliter before intravenous transfer into recipient mice.

*Mixed Bone Marrow Radiation Chimeras.* Bone marrow (BM) cells were obtained from 4–6-wk-old CD122−/− mice and T cells were depleted by treatment with anti-Thy 1.2 mAb followed by complement mediated lysis. 6–10-wk-old B6 Thy 1.1 mice were irradiated at 600 cGy before being injected intravenously with 5 × 10⁶ T cell–depleted BM cells. Mice were then left for 5 mo before being used in experiments.

*Bystander Proliferation Measured by BrdU Incorporation.* Mice were injected intraperitoneally with graded doses of LPS (from *E. coli* serotype 055:B5) or 50 μg Poly I:C (Sigma-Aldrich) in PBS. To assess BrdU incorporation, mice were coinjected with 2 mg BrdU in PBS at the time of immunization and placed on BrdU drinking water (0.8 mg/ml) for 3 d as described previously (5). Spleen and LN cells were harvested 3 d after injection of LPS or poly I:C, then stained for surface markers and for BrdU incorporation (see below). Mice that were treated with anti-CD122 mAb (TM-β1; provided by M. Miyasaka and M. Tanaka, Osaka University, Osaka, Japan) received 0.5 mg anti-CD122 mAb intravenously at the time of poly I:C injection and then 0.5 mg daily for 2 d.

*Adoptive Transfer of T Cells.* 10⁷ purified Ly5.1 or Thy1.1 T cells were transferred into nonirradiated IL-15+ or IL-15−/− hosts by intravenous injection. For purified CD44hi T cells, FACS®-sorted CD4+ and CD8+ cells were mixed at a 1:1 ratio before transferring 6 × 10⁶ cells intravenously. To examine bystander proliferation on adoptive transfer, mice were injected with normal or CFSE-labeled T cells, rested for 24 h after cell transfer, then challenged with either LPS or poly I:C; proliferation was measured at 3 d after injection (using BrdU incorporation for recipients of normal T cells). For experiments on T cell survival, spleen and LN were taken from cohorts of mice at days 3, 8, and 14 after transfer and the phenotype of donor T cells analyzed by flow cytometry. Analysis of purified CD44hi donor T cells was performed 20 d after transfer.

*In Vitro Response of CD8+ T Cells to IL-15.* FACS®-sorted subsets of CD8+ T cells were cultured with 0–100 ng/ml murine rIL-15 (eBioscience) in RPMI 1640 supplemented with 10% FCS, glutamine, 2-ME, and antibiotics. 200 μl of cells were plated at a concentration of 1.5 × 10⁵ cells per well in 96-well, flat-bottomed plates. Proliferation was assessed after 3 d by measuring the uptake of ³[H]thymidine (1 μCi/ml) during a 6-h pulse. All cultures were performed in triplicate. To assess the effect of IL-15 on CD8+ T cell survival, purified CD8+ T cells were cultured alone or with 4 ng/ml rIL-15 at 4 × 10⁵ cells per well in 500 μl in 48-well plates. Cell viability was assessed at the indicated times by staining with Annexin V-FITC and propidium iodide together with CD8α-APC and CD44-PE (BD PharMingen) mAbs. Viable CD8+ CD44hi cells were also stained for CD122 expression.

*Flow Cytometry.* Using standard protocols, cells were stained for FACS® analysis in PBS containing 1% FCS, 5% rat serum, and 0.09% sodium azide with the following mAbs: FITC-conjugated anti-CD44 (IM7); phycoerythrin-conjugated anti-CD122 (TM-β1); anti-Thy1.1 (OX-7); anti-Ly5.1 (A20); cychrome™-conjugated anti-CD44; and allophycocyanin-conjugated anti-CD8α (53–6.7) (all from BD PharMingen). Purified CD8α mAb was also conjugated to Cy™5 (Amersham Pharmacia Biotech) according to the manufacturer's instructions for use in conjunction with the BrdU staining protocol. After staining for surface markers, staining of permeabilized cells for BrdU with FITC-conjugated BrdU mAb (Becton Dickinson) was performed as described

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*Abbreviations used in this paper:* BM, bone marrow; NK, natural killer.

previously (5). Cells were analyzed by a FACSCaliber™ flow cytometer (Becton Dickinson) and CELLQuest™ software.

Analysis of Ly49 expression on CD8$^+$ cells was performed using a panel of mAb (BD PharMingen) to detect the family members Ly49A (A1), C/I (5E6), F (HBF-719), and G2 (4D11). These represent 5 of the 7 inhibitory Ly49 molecules characterized thus far in B6 mice and have previously been shown to be expressed by CD8$^+$ T cells (20). Each mAb was tested individually and in combination with each other; patterns and levels of expression were similar to those reported previously (20). To determine total expression levels of Ly49, spleen, and LN cells were stained with a cocktail of the four biotinylated mAbs, in conjunction with other surface markers, followed by streptavidin-APC (BD PharMingen).

*In Vivo Responses to Peptides.* Purified LN CD8$^+$ cells from 2C TCR transgenic mice (>95% CD122$^{lo}$, CD44$^{lo}$) were transferred intravenously in a dose of $5 \times 10^6$ cells to normal B6 mice (21). After 1 d, the host mice received an intraperitoneal injection of 50 μg SIYR peptide plus 50 μg poly I:C in PBS. Cells were removed from host LN on day 2 after peptide injection and typed for CD44 and CD122 expression; donor CD8$^+$ cells were detected with 1B2 anti-clonotypic antibody. Proliferation of the donor cells was measured by labeling the cells with CFSE before injection.

## Results

*Bystander Proliferation of CD44$^{hi}$ CD8$^+$ Cells.* Our prior suggestion that IFN-induced bystander proliferation in vivo might be mediated by IL-15 stemmed from three observations. First, IFNs and IFN-inducing agents elicit strong production of IL-15 mRNA by macrophages (15, 22) and dendritic cells (23) in vitro. Second, CD122 is expressed at a much higher level on CD44$^{hi}$ CD8$^+$ cells (the main targets for bystander proliferation) than on other T cells, including CD44$^{hi}$ CD4$^+$ cells (15, 18). Third, unlike IFNs, IL-15 is directly stimulatory for CD44$^{hi}$ CD8$^+$ cells, both in vivo and in vitro (15, 24). It is also notable that numbers of CD44$^{hi}$ CD8$^+$ cells are selectively increased in IL-15 transgenic mice (25, 26). Despite these findings, it has yet to be shown that bystander proliferation is mediated by IL-15 per se rather than by other cytokines.

To seek direct evidence on the role of IL-15, bystander proliferation was examined by adoptively transferring wild-type C57BL/6 (B6) T cells to IL-15$^{-/-}$ (16) versus IL-15$^+$ hosts. For these experiments, purified allotype-marked (Ly5.1$^+$ B6) T cells were transferred to IL-15$^{-/-}$ versus IL-15$^+$ hosts (both on a B6 Ly5.2$^+$ background). After 1 d, the host mice were injected with poly I:C and given water containing BrdU, a DNA precursor, for 3 d. The key finding was that, as measured by BrdU incorporation, bystander proliferation by the adoptively transferred T cells was high in normal IL-15$^+$ hosts but almost undetectable in IL-15$^{-/-}$ hosts (Fig. 1 a). Thus, in IL-15$^{-/-}$ hosts, BrdU incorporation after poly I:C injection was only marginally higher than in PBS-injected control mice. Similarly, poly I:C–induced proliferation of CFSE-labeled wild-type T cells (manifested as a step-wise loss of CFSE) was prominent in IL-15$^+$ hosts but minimal in IL-15$^{-/-}$ hosts (Fig. 1 b).

For BrdU incorporation, comparable results after adoptive transfer applied to bystander proliferation induced by LPS (Fig. 1 c). These data refer to CD44$^{hi}$ CD8$^+$ cells transferred from normal B6 mice. For the residual host CD44$^{hi}$ CD8$^+$ cells in IL-15$^{-/-}$ mice (see below), these cells showed a higher than normal turnover relative to IL-15$^+$ B6 mice (Fig. 1 d); this high turnover was only marginally enhanced after LPS injection (Fig. 1 d).

In addition to showing a paucity of CD44$^{hi}$ CD8$^+$ cells, IL-15$^{-/-}$ mice lack NK cells (16). Hence the poor bystander proliferation of T cells transferred to IL-15$^{-/-}$ hosts might reflect a defect in the innate immune system rather than simply an absence of IL-15. The following two observations are against this possibility. First, bystander proliferation induced by poly I:C in normal B6 mice was substantially inhibited by coinjection of anti-CD122 mAb (Fig. 1 e). Second, CD8$^+$ cells from CD122$^{-/-}$ mice were resistant to poly I:C–induced bystander proliferation (Fig. 1 f). Because CD122$^{-/-}$ mice display lymphadenopathy (27), bystander proliferation was tested in sublethally irradiated (600 cGy) B6.Thy 1.1 mice reconstituted with T cell–depleted BM cells taken from CD122$^{-/-}$ mice on a B6 (Thy 1.2) background; these chimeric mice did not show lymphadenopathy and their CD8$^+$ T cells comprised a 1:1 ratio of donor CD122$^{-/-}$ (Thy 1.2) and normal host (Thy 1.1) cells. Significantly, bystander proliferation of CD44$^{hi}$ CD8$^+$ cells elicited by injecting the chimeras with poly I:C applied only to host T cells and not to the donor CD122$^{-/-}$ T cells.

Collectively, the above data indicate that bystander proliferation of CD44$^{hi}$ CD8$^+$ cells is crucially dependent on IL-15 and requires CD122 expression on the responding cells.

*Bystander Proliferation of Subsets of CD44$^{hi}$ CD8$^+$ Cells.* As mentioned above, CD44$^{hi}$ CD8$^+$ cells express much higher levels of CD122 (an important component of the IL-15 receptor) than CD44$^{hi}$ CD4$^+$ cells (15, 18). However, CD122 expression on CD44$^{hi}$ CD8$^+$ cells is heterogeneous, ~60% of these cells being CD122$^{hi}$ in young mice and the remainder being CD122$^{lo}$ (Fig. 2, a and b). Confirming the results of others (20), a subset (20–30%) of CD122$^{hi}$ CD8$^+$ cells resembles NK cells in expressing high levels of the Ly49 marker (Fig. 2 c, see Figure legend for detecting Ly49 expression). Virtually all Ly49$^+$ CD8$^+$ cells are CD122$^{hi}$ CD44$^{hi}$ (Fig. 2 d).

As mentioned earlier, IL-15$^{-/-}$ and IL-15R$\alpha^{-/-}$ mice contain appreciable (30% of normal) numbers of CD44$^{hi}$ CD8$^+$ cells. This finding might indicate that CD44$^{hi}$ CD8$^+$ cells are only partially dependent on IL-15. Alternatively, the CD44$^{hi}$ CD8$^+$ cells found in these knockout mice might represent a separate subset of IL-15–independent cells. To assess this possibility, we typed IL-15$^{-/-}$ CD8$^+$ cells for CD122 and Ly49 expression. Significantly, for spleen, LN, and blood, the vast majority of the residual CD44$^{hi}$ CD8$^+$ cells found in IL-15$^{-/-}$ mice proved to be CD122$^{lo}$ (Fig. 2, a and e) and virtually all of these cells were Ly49$^-$ (Fig. 2 f). Hence, taking into consideration
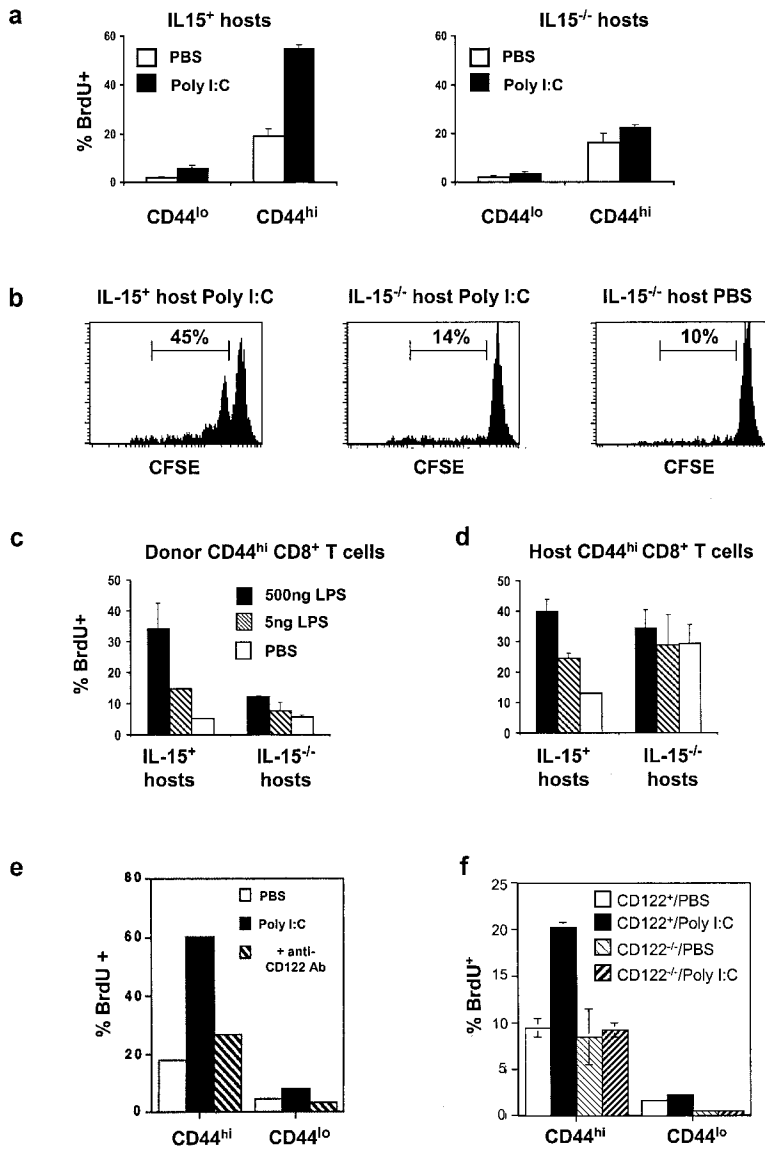
**Figure 1.** Bystander proliferation is IL-15 dependent. (a and b) Purified LN T cells from Ly5.1 B6 mice were transferred to normal B6 (IL-15$^+$) mice and IL-15$^{-/-}$ littermates (both Ly5.2). Hosts were then given 50 μg poly I:C intraperitoneally and proliferation was measured on day 3 by (a) BrdU incorporation after administration of BrdU given in the drinking water from day 0 or (b) dilution of CFSE by CFSE-labeled T cells; control mice received injections of saline (PBS). The data show proliferation measured on CD44$^{hi}$ (a and b) and CD44$^{lo}$ (a) subsets of gated donor (Ly5.1) CD8$^+$ cells recovered from host LN. For CFSE labeling (b), values refer to the percentage of cells that had divided one or more times. Mean of data ($\pm$ SD, four mice per group) (a) or representative data (b) are shown. Similar findings applied to cells recovered from host spleen (not shown). (c and d), as in (a) except that mice were injected with LPS, given in two different doses. The data show mean ($\pm$ SD) BrdU incorporation for four mice per group for (c) donor (Ly5.1$^+$) CD44$^{hi}$ CD8$^+$ cells and (d) host (Ly5.1$^-$) CD44$^{hi}$ CD8$^+$ cells. (e) Capacity of anti-CD122 mAb to inhibit proliferation induced by poly I:C. Normal B6 mice were injected with PBS, poly I:C, or a mixture of poly I:C and anti-CD122 mAb and placed on BrdU water for 3 d. The data show BrdU incorporation by gated CD44$^{hi}$ and CD44$^{lo}$ CD8$^+$ subsets from LN pooled from three mice per group; similar results were found in another experiment. (f) Poly I:C–induced proliferation of T cells in CD122$^{-/-}$ → CD122$^+$ chimeras. Irradiated (600 cGy) Thy 1.1 B6 mice were injected with T cell–depleted BM cells from CD122$^{-/-}$ mice on a B6 (Thy 1.2) background. After 5 mo, the chimeras were injected with poly I:C, placed on BrdU water for 3 d and stained. The data show BrdU incorporation ($\pm$ SD) by gated donor CD122$^{-/-}$ (Thy 1.2) versus host CD122$^+$ (Thy 1.1) CD44$^{hi}$ and CD44$^{lo}$ CD8$^+$ cells from LN from three mice per group. Similar results were found in another experiment.

total cell numbers (unpublished data), the reduction of CD8$^+$ cells in IL-15$^{-/-}$ mice is only moderate for total CD44$^{hi}$ cells (50–70%) but marked for CD122$^{hi}$ cells (90%) and near complete for Ly49$^+$ cells (99%), implying a step-wise increase in the dependency of these subsets on IL-15. For Ly49$^+$ cells, it is notable that, as in IL-15$^{-/-}$ mice, these cells were totally absent in CD122$^{-/-}$ mice (Fig. 2 g) but present in IL-2$^{-/-}$ mice (Fig. 2 h), thus confirming the extreme dependency of Ly49$^+$ cells on IL-15 (but not IL-2). In contrast to CD122$^{hi}$ Ly49$^+$ CD8$^+$ cells, the presence of near-normal numbers of CD44$^{hi}$ CD122$^{lo}$ Ly49$^-$ cells in IL-15$^{-/-}$ mice (and the above-normal numbers of these cells in CD122$^{-/-}$ mice, which are prone to lymphadenopathy; see above) indicates that this subset of cells is IL-15 independent.

In view of the above findings, we investigated the relative sensitivity of CD44$^{hi}$ CD8$^+$ cell subsets to IFN-induced bystander proliferation. In initial experiments we compared the sensitivity of CD44$^{hi}$ CD122$^{hi}$ and CD44$^{hi}$ CD122$^{lo}$ cells by injecting normal B6 mice with graded doses of LPS. Typing BrdU-labeled CD44$^{hi}$ CD8$^+$ cells for CD122 expression showed that bystander proliferation affected both CD122$^{lo}$ and CD122$^{hi}$ cells (Fig. 3 a). However, taking into consideration the much higher background turnover of CD122$^{lo}$ than CD122$^{hi}$ cells in uninjected control mice (Fig. 3 a), CD122$^{lo}$ cells were clearly much less sensitive to bystander proliferation than CD122$^{hi}$ cells. These data refer to BrdU incorporation. Similar findings applied when allotype-marked T cells were labeled with CFSE and subjected to bystander stimulation on adoptive transfer to normal B6 hosts. Thus, with poly I:C as the stimulus, proliferation of donor CD44$^{hi}$ CD8$^+$ cells was appreciably more marked for CD122$^{hi}$ than CD122$^{lo}$ cells (Fig. 3 b).

To examine the response of Ly49$^+$ versus Ly49$^-$ subsets of CD44$^{hi}$ CD8$^+$ cells, purified CFSE-labeled allotype-
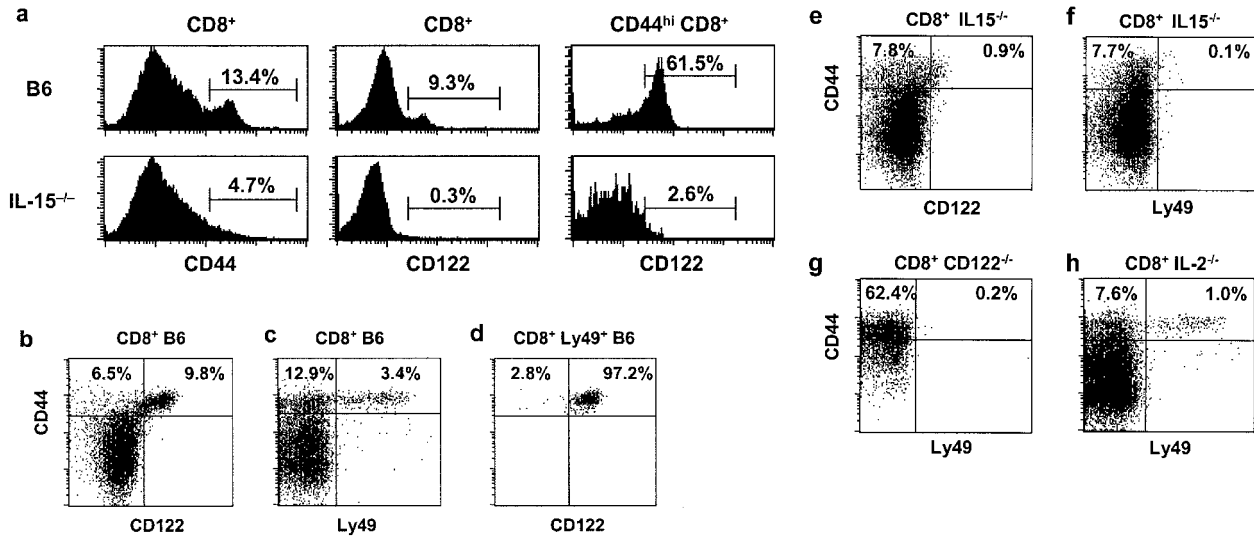
**Figure 2.** A subset of CD44^{hi} CD8^{+} T cells is IL-15 dependent. (a) Histograms show expression of CD44 on gated CD8^{+} cells and CD122 on gated CD8^{+} and CD44^{hi} CD8^{+} cells from LN of wild-type B6 versus IL-15^{−/−} mice. (b–h) The data show CD122 or Ly49 versus CD44 expression on gated CD8^{+} LN T cells from wild-type B6 mice (b–d), IL-15^{−/−} mice (e and f), CD122^{−/−} mice (g), and IL-2^{−/−} mice (h). The data are representative of >4 mice (aged 2–3 mo)/group and also applied to CD8^{+} spleen and peripheral blood cells (not shown). In d, data refer to gated Ly49^{+} CD8^{+} T cells. In all groups, Ly49 was detected using a panel of anti-Ly49A, C/I, F, and G2 mAbs that have previously been shown to be the most abundantly expressed Ly49 family members on CD8^{+} T cells in B6 mice (reference 20).

marked B6 T cells were transferred to IL-15^{+} versus IL-15^{−/−} hosts; the host mice were injected with poly I:C after 1 d and cell division was measured 3 d later. Confirming the above results (Fig. 3 b), poly I:C–induced bystander proliferation of donor CD44^{hi} CD8^{+} cells in IL-15^{+} hosts was minimal for CD122^{lo} cells but prominent for CD122^{hi} cells

(Fig. 3 c). The surprising finding, however, was that, for CD122^{hi} cells, bystander proliferation was substantially higher for Ly49^{+} cells than for Ly49^{−} cells (Fig. 3 c). For both of these subsets, bystander proliferation was very low in IL-15^{−/−} hosts (relative to background proliferation, measured in IL-15^{+} hosts), thus confirming the data in Fig. 1.
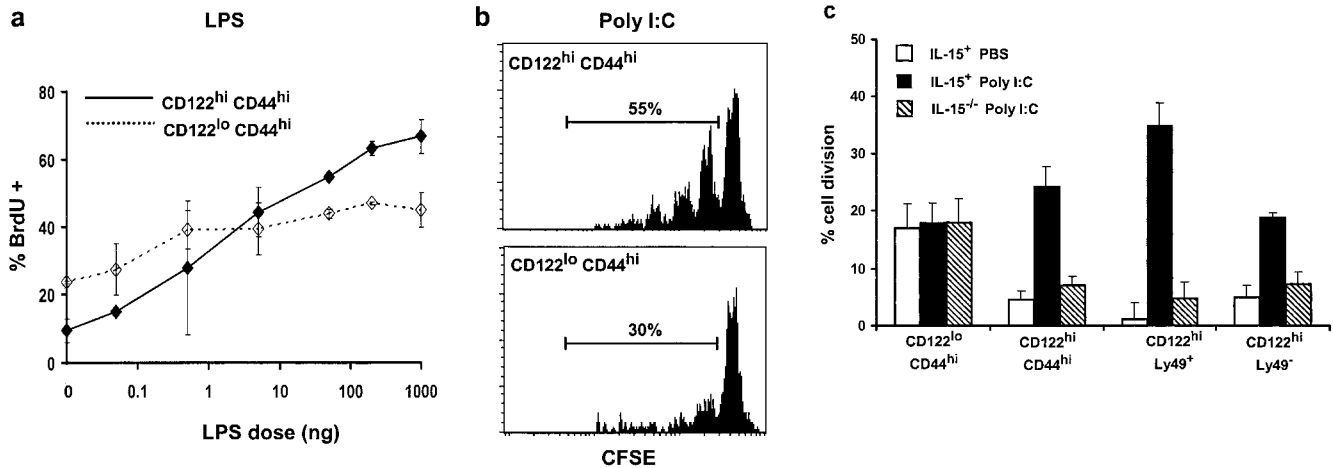


**Figure 3.** Bystander proliferation of CD44^{hi} CD8^{+} cells is largely restricted to CD122^{hi} subsets and most prominent for Ly49^{+} CD122^{hi} cells. (a) Bystander proliferation was measured in vivo by injecting mice with graded doses of LPS, placing mice on BrdU water for 3 d followed by FACS® analysis of LN and spleen cells. Injections of PBS were used as a control. The data show BrdU incorporation by CD122^{hi} CD44^{hi} CD8^{+} cells versus CD122^{lo} CD44^{hi} CD8^{+} cells from LN; mean values (± SD) of three mice per group. In further experiments, proliferation was measured by transferring CFSE-labeled Ly5.1 B6 T cells into (b) syngenic B6 and (c) B6 versus IL-15^{−/−} mice (both Ly5.2), injecting the hosts with 50 μg poly I:C, then using FACS® analysis to study division (loss of CFSE) by the donor cells 3 d later. In b, the data show CFSE labeling of donor-derived (Ly5.1^{+}) CD122^{hi} CD44^{hi} CD8^{+} versus CD122^{lo} CD44^{hi} CD8^{+} subsets recovered from spleen. Cells from LN showed a similar response. The data are representative of four mice tested individually. In c, the data show the percentage of donor T cell subsets that underwent one or more rounds of division (the percentage of cell division) after poly I:C injection. Data are shown for CD44^{hi} CD8^{+} spleen cells gated on CD122^{lo}, total CD122^{hi}, CD122^{hi} Ly49^{+}, and CD122^{hi} Ly49^{−} subsets after transfer to IL-15^{+} (black bars) or IL-15^{−/−} mice (striped bars); cells transferred to PBS-injected IL-15^{+} mice (white bars) were used as controls. The data show mean values (± SD) for four mice per group.

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Based on these findings, the graded dependency of the three CD44$^{hi}$ CD8$^+$ subsets on IL-15 (CD122$^{hi}$ Ly49$^+$ > CD122$^{hi}$ Ly49$^-$ > CD122$^{lo}$ Ly49$^-$) discussed earlier for their development and/or survival (Fig. 2) directly correlated with the sensitivity of these cells to IL-15–induced bystander proliferation.

*Responsiveness to IL-15 In Vitro.* Correlating with their sensitivity to bystander proliferation in vivo, CD122$^{hi}$ CD44$^{hi}$ cells were much more sensitive than CD122$^{lo}$ CD44$^{hi}$ cells to proliferation elicited by IL-15 in vitro (Fig. 4 a). Thus, when FACS®-sorted subsets of CD8$^+$ cells were cultured with graded concentrations of IL-15, proliferation ($^3$[H]thymidine incorporation) was high for CD122$^{hi}$ CD44$^{hi}$ cells, low though significant for CD122$^{lo}$ CD44$^{hi}$ CD8$^+$ cells and undetectable for naive CD122$^{lo}$ CD44$^{lo}$ cells. Likewise, when cultured with limiting concentrations of IL-15 (30 ng/ml), division of CFSE-labeled CD44$^{hi}$ CD8$^+$ cells was more prominent for CD122$^{hi}$ Ly49$^+$ cells than for CD122$^{hi}$ Ly49$^-$ cells (Fig. 4 b); with high concentrations of IL-15 (>100 ng/ml), >90% of the cells in both populations entered cell division (unpublished data).

These data on responsiveness to IL-15 in vitro thus closely mirror the above findings on bystander proliferation.

*IL-15 and T Cell Survival.* The virtual absence of the CD122$^{hi}$ subset of CD44$^{hi}$ CD8$^+$ cells in IL-15$^{-/-}$ mice may reflect that CD122$^{hi}$ cells require contact with IL-15 for their survival. Alternatively, IL-15 could be required only for the initial generation of CD122$^{hi}$ cells. To assess this second possibility, purified naive (CD122$^{lo}$) 2C TCR transgenic CD8$^+$ cells on a B6 (K$^b$) background were transferred to normal B6 versus IL-15$^{-/-}$ mice plus K$^b$-restricted SIYR peptide and poly I:C as a source of adjuvant (21). Examining cells recovered from host LN 2 d later showed that nearly all of the donor 2C CD8$^+$ cells had differentiated into CD122$^{hi}$ CD44$^{hi}$ cells, both in B6 and IL-15$^{-/-}$ hosts (Fig. 4 c and unpublished data); the density of CD122 expression on the donor cells on day 2 was as high as on the subset of host CD122$^{hi}$ CD8$^+$ cells found in the normal B6 (but not the IL-15$^{-/-}$) hosts (Fig. 4 c). Parallel experiments in which the donor cells were labeled with CFSE showed that division of the donor 2C CD8$^+$ cells was as high in IL-15$^{-/-}$ hosts as in normal B6 hosts (Fig. 4 d). Based on these and other findings, there was thus no evidence that IL-15 was required for the initial generation of CD122$^{hi}$ CD8$^+$ cells.

To assess whether IL-15 is required after the transition of CD122$^{lo}$ to CD122$^{hi}$ cells, we first tested whether IL-15 could promote survival of CD122$^{hi}$ cells in vitro. Purified
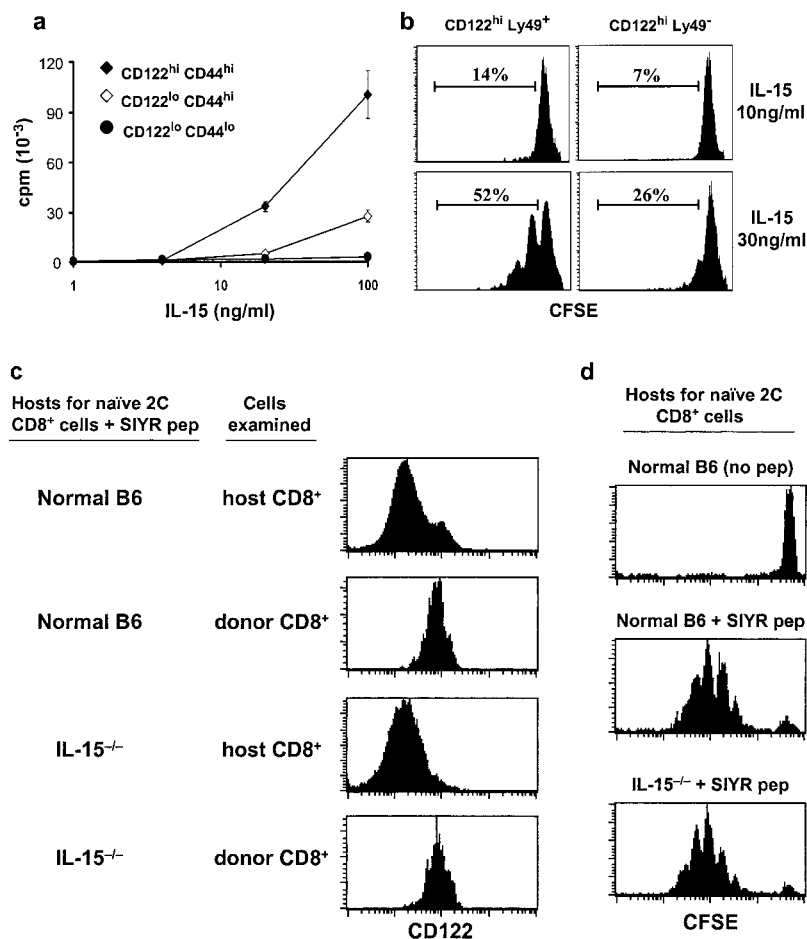


**Figure 4.** Sensitivity of CD44$^{hi}$ CD8$^+$ subsets to IL-15 in vitro and role of IL-15 on CD122 upregulation in vivo. (a) CD122$^{hi}$ CD44$^{hi}$, CD122$^{lo}$ CD44$^{hi}$ and naive CD122$^{lo}$ CD44$^{lo}$ CD8$^+$ cells from B6 mice were purified by FACS® sorting and cultured in vitro with increasing doses of IL-15. Proliferation was assessed after 3 d by the incorporation of $^3$[H]thymidine (mean cpm ± SD of triplicate cultures). (b) CFSE-labeled, purified CD8$^+$ cells were cultured with either 10 ng/ml (top panels) or 30 ng/ml (bottom panels) of IL-15 for 3 d. The data show division measured on gated Ly49$^+$ CD122$^{hi}$ (left panels) and Ly49$^-$ CD122$^{hi}$ cells (right panels). Values indicate the percentage of gated cells undergoing one or more divisions. Data are representative of duplicate cultures in three separate experiments. (c) CD122 upregulation by naive CD8$^+$ cells exposed to antigen in IL-15$^{-/-}$ mice. Purified naive LN CD8$^+$ cells from 2C TCR transgenic mice were transferred intravenously (5 × 10$^6$ cells per mouse) to normal B6 versus IL-15$^{-/-}$ mice. 1 d later the host mice received SIYR peptide (50 μg) and poly I:C (50 μg) as a source of adjuvant. The data show CD122 expression on gated donor (1B2$^+$) versus host (1B2$^-$) CD8$^+$ cells measured on day 2 after peptide injection; the data are representative of three mice per group, (d) as for c, except that the donor 2C cells were CFSE labeled before injection.

CD8^{+} cells from normal B6 mice were cultured in vitro with IL-15 at 4 ng/ml, i.e., at a concentration below the limit required to induce proliferation (Fig. 4 a). For control cells cultured in the absence of IL-15, CD44^{hi} CD8^{+} cells rapidly downregulated CD122 expression and died via apoptosis (Fig. 5 a). Significantly, addition of a submitogenic concentration of IL-15 caused the cells to survive well for 3 d and retain their CD122^{hi} phenotype (Fig. 5 a).

The above findings predicted that transferring normal T cells to IL-15^{−/−} hosts would cause selective disappearance of CD44^{hi} CD8^{+} cells and would be most pronounced for CD122^{hi} cells. This was indeed the case. Thus, after transfer of purified T cells (depleted of APC and B cells) to IL-15^{+} versus IL-15^{−/−} hosts, the CD44^{hi} CD8^{+} component of the donor T cells survived well in IL-15^{+} hosts but disappeared in IL-15^{−/−} hosts (Fig. 5 b). By 2 wk after transfer, donor CD44^{hi} CD8^{+} cells were rare in IL-15^{−/−} hosts and most of the surviving CD44^{hi} CD8^{+} cells were CD122^{lo} and

thus closely resembled the residual host CD44^{hi} CD8^{+} cells (Fig. 5 c, compare with Fig. 2 a). By contrast, donor CD122^{hi} CD8^{+} cells survived well in IL-15^{+} hosts. There was no reduction of donor CD44^{hi} CD4^{+} cells in IL-15^{−/−} hosts (Fig. 5, b and d), indicating that the disappearance of CD44^{hi} cells was restricted to CD8^{+} cells. This finding was expected because, in contrast to CD44^{hi} CD8^{+} cells, CD44^{hi} CD4^{+} cells are all CD122^{lo} (15) and IL-15^{−/−} mice show no reduction in numbers of CD44^{hi} CD4^{+} cells (16).

One concern in the above experiment was that the disappearance of CD44^{hi} CD8^{+} cells in IL-15^{−/−} hosts did not reflect death of these cells but reversion to naive-phenotype CD44^{lo} cells. To exclude this possibility, FACS®-purified CD44^{hi} cells, comprising a ∼1:1 mixture of CD8^{+} and CD4^{+} cells, were transferred to IL-15^{+} versus IL-15^{−/−} hosts and examined on day 20 after transfer. When transferred to IL-15^{+} hosts the donor cells survived and showed little or no change in the ratio of CD4^{+}/CD8^{+} cells (Fig.
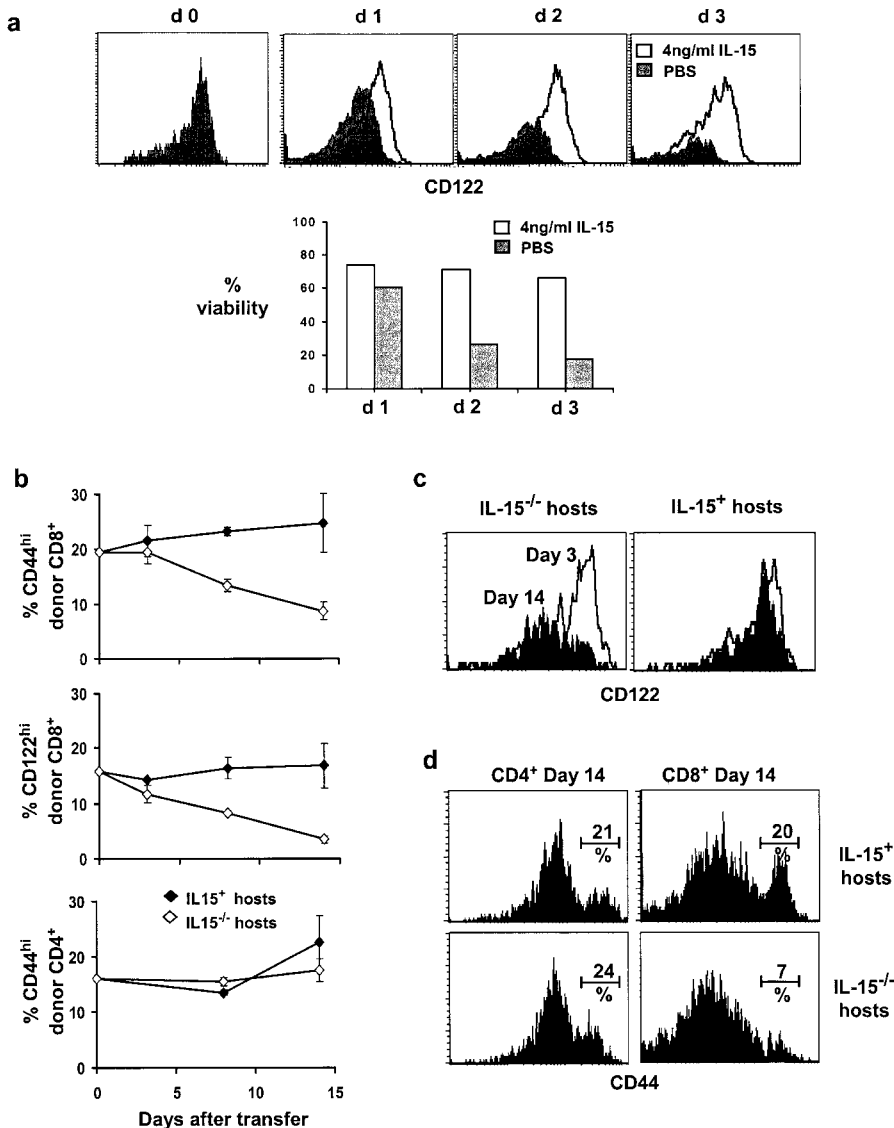


**Figure 5.** Antiapoptotic function of IL-15 in vitro and in vivo. (a) The capacity of submitogenic concentrations of IL-15 to maintain survival of CD122^{hi} CD8^{+} cells in vitro was assessed by culturing purified CD8^{+} LN cells from B6 mice for up to 3 d in the presence (open histograms) or absence (closed histograms) of a low concentration of IL-15 (4 ng/ml); at this concentration (see Fig. 4 a), IL-15 did not cause cell enlargement or proliferation. Cells were harvested at daily intervals and stained for CD122 expression and for Annexin V-binding and propidium iodide (PI) to measure viability. CD122 expression on viable CD44^{hi} cells (top) and the percentage of viable CD44^{hi} cells from pooled triplicate cultures (bottom) are shown. (b–d) Selective disappearance of CD122^{hi} CD44^{hi} CD8^{+} cells after transfer to IL-15^{−/−} hosts. Purified T cells from Thy 1.1 B6 mice were transferred to normal IL-15^{+} littermates or IL-15^{−/−} mice (both Thy 1.2). At the time periods shown, spleen and LN were removed from the recipients and stained for surface markers. The data show the surface markers on donor (Thy 1.1) cells recovered at various times after transfer. (b) The percentage of donor CD8^{+} and CD4^{+} cells that were CD44^{hi} or CD122^{hi} (mean data of three mice per group ± SD). (c) Expression of CD122 on donor CD44^{hi} CD8^{+} cells recovered 3 d (open histograms) and 14 d (closed histograms) after transfer. (d) CD44 expression on CD8^{+} and CD4^{+} donor cells 14 d after transfer (percentage of CD44^{hi} cells indicated). The data are representative of three mice per time point and two separate experiments.

6, a and c). In IL-15$^{-/-}$ hosts, by contrast, the donor cells showed a marked depletion of CD8$^+$ cells, thus leading to a high CD4$^+$/CD8$^+$ ratio; counting total cells (unpublished data) indicated that the increase in the CD4$^+$/CD8$^+$ ratio in IL-15$^{-/-}$ hosts did not reflect selective expansion of CD4$^+$ cells. Significantly, as in IL-15$^+$ hosts, the donor CD8$^+$ cells recovered from IL-15$^{-/-}$ hosts retained their CD44$^{hi}$ phenotype, i.e., the phenotype of the injected cells (Fig. 6 b). In addition, in contrast to IL-15$^+$ hosts, nearly all of the few donor CD44$^{hi}$ CD8$^+$ cells recovered from IL-15$^{-/-}$ hosts were CD122$^{lo}$ (Fig. 6 b).

These findings thus confirm the data in Fig. 5 and show that the disappearance of CD44$^{hi}$ CD8$^+$ cells in IL-15$^{-/-}$ hosts is selective for CD122$^{hi}$ CD8$^+$ cells, does not apply to CD44$^{hi}$ CD4$^+$ cells and cannot be attributed to reversion to CD44$^{lo}$ cells.

*IL-15 and Normal T Cell Turnover.* The above data indicate that, as for responsiveness to IFN-dependent bystander proliferation, the survival of CD122$^{hi}$ CD44$^{hi}$ CD8$^+$ cells is heavily dependent on IL-15; by contrast, CD122$^{lo}$ CD44$^{hi}$ CD8$^+$ cells are largely IL-15 independent. These findings raise the question whether IL-15 also plays a key role in maintaining the normal background turnover of CD44$^{hi}$ CD8$^+$ cells. As mentioned earlier, this possibility is supported by the finding that turnover of CD8$^+$ cells in normal mice can be blocked by injection of CD122 mAb (7, 18). However, the inhibition is only partial, i.e., ∼50%. Based on the data reported here, this partial effect of CD122 mAb could reflect that some proliferating CD44$^{hi}$ CD8$^+$ cells, namely CD122$^{lo}$ cells, are IL-15 independent. As pointed out above, the normal turnover of these cells is actually higher than for typical CD122$^{hi}$ cells (Fig. 3 a). Likewise, this high turnover of CD122$^{lo}$ cells applies to the residual CD44$^{hi}$ CD8$^+$ cells found in IL-15$^{-/-}$ mice (Fig. 1 d) and also to the large numbers of these cells present in lymphadenopathy-prone CD122$^{-/-}$ mice (unpublished data). Here, the stimulus for proliferation is clearly not IL-15.

To assess the role of IL-15 in controlling the normal turnover of CD44$^{hi}$ CD8$^+$ cell subsets, we measured division of CFSE-labeled CD8$^+$ cells transferred to IL-15$^+$ versus IL-15$^{-/-}$ hosts; here, in contrast to the experiments on IFN-dependent bystander proliferation discussed above, the hosts were not injected with IFN-inducing agents. When examined at day 14 after transfer, division of donor CD122$^{lo}$ CD44$^{hi}$ CD8$^+$ cells was high in both IL-15$^+$ and IL-15$^{-/-}$ hosts (Fig. 6 d, top panel), thus confirming that proliferation of this subset is IL-15 independent. Likewise, proliferation of CD44$^{hi}$ CD4$^+$ cells was high in both hosts (unpublished data). For CD122$^{hi}$ CD8$^+$ cells, by contrast, division of these cells was substantially lower in IL-15$^{-/-}$ than IL-15$^+$ hosts. This reduction in turnover was even
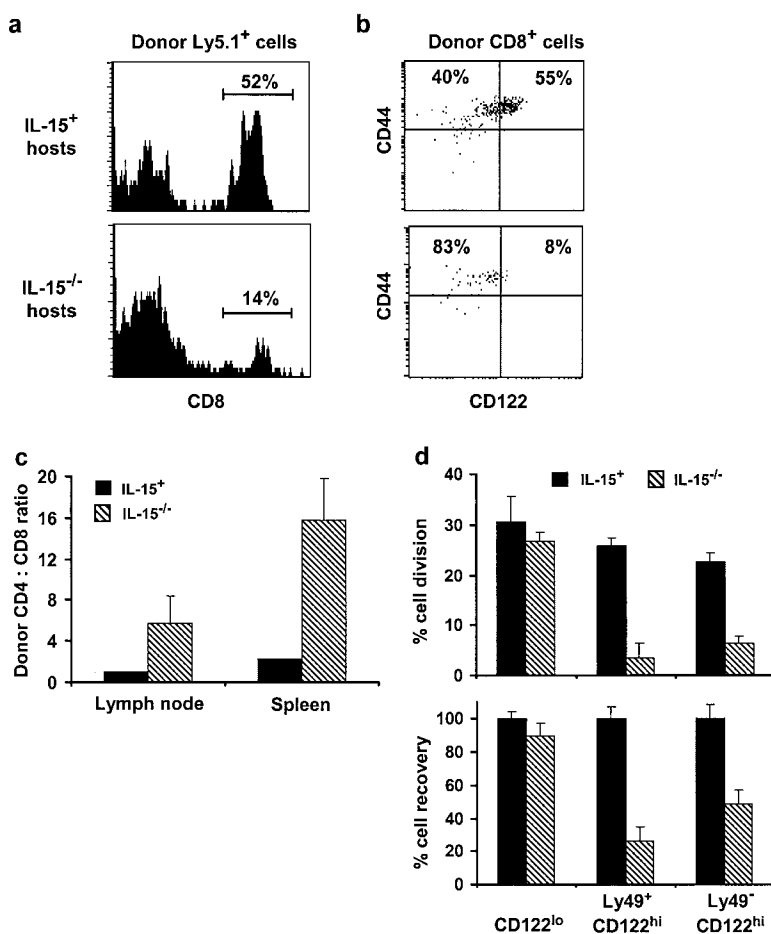


**Figure 6.** Disappearance of purified CD44$^{hi}$ T cells in IL-15$^{-/-}$ hosts is selective for CD122$^{hi}$ CD8$^+$ cells, most prominent for the Ly49$^+$ subset of these cells and parallels a decrease in the rate of cell division. (a–c) FACS® sorted CD44$^{hi}$ T cells comprising a 1:1 ratio of CD4$^+$ and CD8$^+$ T cells from Ly5.1 B6 mice were adoptively transferred into either IL-15$^+$ or IL-15$^{-/-}$ hosts. 20 d later the phenotype of donor cells recovered from spleen and LN was assessed. In a and b, the data show the percentage of donor (Ly5.1$^+$) cells recovered from host LN that were CD8$^+$ (a) and the proportion of these cells that were CD122$^{hi}$ and CD44$^{hi}$ (b). Phenotypes of cells recovered from the spleen were similar. In c, the ratio of donor CD4$^+$ to CD8$^+$ cells recovered from the host spleen and LN of two mice per group are shown. Data in a–c refer to one of two separate experiments. In d, purified CFSE-labeled T cells from Thy1.1 B6 mice were transferred to IL-15$^+$ versus IL-15$^{-/-}$ hosts and examined on day 14. The data (top panel) show mean percentage of cell division (± SD) (see Fig. 3 legend) of donor-derived CD44$^{hi}$ CD8$^+$ cells gated on CD122$^{lo}$, CD122$^{hi}$ Ly49$^+$, and CD122$^{hi}$ Ly49$^-$ subsets. The percentage of recovery of these subsets in IL-15$^{-/-}$ hosts relative to IL-15$^+$ controls is shown in the bottom panel. Data are from LN for three mice per group; similar results were found in the spleen.

more pronounced for the Ly49^{+} subset of CD122^{hi} cells (Fig. 6 d, top panel).

Thus, the data indicate that, as for bystander proliferation and survival, the differential sensitivity of individual subsets of CD44^{hi} CD8^{+} cells to IL-15 also applies to normal T cell turnover. For Ly49^{+} cells, it should be noted that the more-marked decrease in proliferation of Ly49^{+} CD122^{hi} cells (relative to Ly49^{−} CD122^{hi} cells) in IL-15^{−/−} hosts correlated with more extensive elimination of these cells (Fig. 6 d, bottom panel). Hence, onset of death coincides with a reduction in turnover.

## Discussion

To date, the notion that the turnover/survival of CD44^{hi} CD8^{+} cells in normal mice is controlled by IL-15 is based on three lines of evidence: (1) the capacity of recombinant human IL-15 injection to mimic the pattern of bystander activation of CD44^{hi} CD8^{+} cells induced by IFNs (15); (2) the ability of CD122 (but not IL-2) mAb to impair the background turnover of CD44^{hi} CD8^{+} cells (7, 18); and (3) the selective reduction of CD44^{hi} CD8^{+} cells in IL-15^{−/−} and IL-15Rα^{−/−} mice (16, 17). In considering these studies, it is notable that the inhibition of CD44^{hi} CD8^{+} cell turnover by CD122 mAb is moderate rather than marked. Likewise, the reduction of CD44^{hi} CD8^{+} cells in IL-15^{−/−} and IL-15Rα^{−/−} mice is only partial. At face value these findings suggest that the influence of IL-15 on CD44^{hi} CD8^{+} cells is significant but not essential. Alternatively, IL-15 could be crucially important for some CD44^{hi} CD8^{+} cells but largely irrelevant for others. In support of this second possibility, we show here that a subset of CD44^{hi} CD8^{+} cells is heavily dependent on IL-15, both for their survival and turnover. Significantly, however, some CD44^{hi} CD8^{+} cells are IL-15 independent.

In discussing the role of IL-15 on CD44^{hi} CD8^{+} cells, it is important to consider the expression of IL-15 receptors. According to the literature, the IL-15 receptor on T cells has three components, IL-15Rα, IL-2Rβ (CD122), and the γc chain (9). IL-15Rα and CD122 control binding, whereas CD122 and γc elicit signal transduction. Although IL-15Rα confers high binding affinity for IL-15, whether this chain is essential for IL-15 binding by CD44^{hi} CD8^{+} cells in mice, however, is unclear. IL-15Rα is reported to be expressed on a subset of dividing T cells (28, 29), but to our knowledge there is no definitive evidence that IL-15Rα is expressed by normal resting CD44^{hi} CD8^{+} cells. Hence, for resting CD44^{hi} CD8^{+} cells, IL-15 binding may be controlled largely or solely by CD122. As shown previously, γc expression on CD44^{hi} CD8^{+} and CD44^{hi} CD4^{+} cells is quite similar (15).

For CD44^{hi} CD8^{+} cells in normal mice, CD122 expression on these cells is heterogeneous, ∼60% of CD44^{hi} CD8^{+} cells being CD122^{hi} and the remainder being CD122^{lo} (15, 18). In support of CD122 being crucial for IL-15 binding (see above), we show here that proliferative responses by purified subsets of CD44^{hi} CD8^{+} cells to IL-15 in vitro are much higher for CD122^{hi} cells than for CD122^{lo} cells. Thus, in assessing the role of IL-15 on CD44^{hi} CD8^{+} cells in vivo, it became essential to compare CD122^{hi} versus CD122^{lo} cells separately.

By all parameters tested, the CD122^{hi} subset of CD44^{hi} CD8^{+} cells proved to be heavily dependent on IL-15. Thus, for proliferation, IFN-induced bystander proliferation of CD122^{hi} CD44^{hi} CD8^{+} cells after stimulation of the innate immune system with poly I:C or LPS was strong in normal hosts but very weak or undetectable in IL-15^{−/−} hosts. This finding provides direct proof for the previous suggestion (15) that bystander proliferation is mediated by IFN-dependent synthesis of IL-15. The data also support the suggestion that the lack of poly I:C–induced bystander proliferation in IL-15Rα^{−/−} hosts reflects a failure of these mice to synthesize IL-15 (24). Complementing the data on bystander proliferation, the normal turnover of CD122^{hi} cells in unstimulated mice was heavily dependent on IL-15. Thus, the background turnover of CD122^{hi} cells was maintained in normal hosts but declined to low levels in IL-15^{−/−} hosts. Similar dependence on IL-15 applied to cell survival. Thus, exposure to IL-15 maintained the survival of CD122^{hi} cells in vitro, and depriving these cells from contact with IL-15, i.e., after transfer to IL-15^{−/−} hosts, caused CD122^{hi} cells to disappear within 2–3 wk.

We caution that the data in this paper refer solely to normal memory-phenotype cells and not to memory cells specific for defined antigens. Since nearly all typical antigen-specific memory CD8^{+} cells are CD122^{hi} (30, 31), one would expect these cells, like memory-phenotype cells, to be IL-15 dependent. For proliferation, this is indeed the case. Thus, very recent studies on IL-15^{−/−} mice and TCR transgenic T cells showed that antigen-specific memory CD8^{+} cells were heavily dependent on IL-15 for background turnover (32–34). For survival, however, the data are less clear. Thus, contrary to our findings on memory-phenotype CD8^{+} cells (this paper), antigen-specific memory CD8^{+} cells were reported to survive for 1–2 mo in IL-15^{−/−} hosts, albeit in reduced numbers relative to IL-15^{+} hosts (32–34). By contrast, memory CD8^{+} cells derived from antigen-stimulated T cells in vitro failed to survive for more than a few weeks in vivo unless the cells upregulated CD122, suggesting an important role for IL-15 in survival (35). In our own hands, preliminary experiments showed that some antigen-specific memory CD8^{+} cells (2C) seem to survive well in IL-15^{−/−} hosts, whereas others (OT-1) do not (unpublished data). Clearly, further work will be needed to resolve this issue.

For both proliferation and survival, IL-15 had minimal effects on the CD122^{lo} component of CD44^{hi} CD8^{+} cells. Thus, in contrast to CD122^{hi} cells, CD122^{lo} CD44^{hi} CD8^{+} cells responded poorly in terms of bystander stimulation in vivo, which paralleled the poor response of these cells to IL-15 in vitro. Likewise, CD122^{lo} CD44^{hi} CD8^{+} cells survived well and maintained a high rate of turnover in IL-15^{−/−} hosts. It is also notable that almost all of the residual host CD44^{hi} CD8^{+} cells present in IL-15^{−/−} mice proved to be CD122^{lo} cells. Whether CD122^{lo} cells also account for the residual CD44^{hi} CD8^{+} cells in IL-15Rα^{−/−}

mice has yet to be tested. If this were the case, it could explain the poor response of IL-15R$^{-/-}$ CD44$^{hi}$ CD8$^{+}$ cells to IL-15 (24).

The observation that CD44$^{hi}$ CD8$^{+}$ cells comprise two discrete subsets of IL-15–dependent (CD122$^{hi}$) and IL-15–independent (CD122$^{lo}$) cells could explain why treating mice with blocking CD122 mAb caused only partial (50%) inhibition of background CD8$^{+}$ cell turnover (18). Based on the present findings, CD122 mAb-resistant proliferation of CD44$^{hi}$ CD8$^{+}$ cells is probably mediated by the CD122$^{lo}$ subset of CD44$^{hi}$ CD8$^{+}$ cells, i.e., by IL-15–independent cells. As shown here, these cells have a high rate of turnover in vivo.

For CD122$^{hi}$ cells, the sensitivity of these cells to IL-15 was especially high for the Ly49$^{+}$ subset of these cells. Thus, both for IFN–induced bystander proliferation in vivo and proliferation in response to IL-15 in vitro, the Ly49$^{+}$ subset of CD122$^{hi}$ CD44$^{hi}$ CD8$^{+}$ cells gave higher responses than the Ly49$^{-}$ subset. Likewise, the decline in background turnover and survival of CD122$^{hi}$ CD44$^{hi}$ CD8$^{+}$ cells after transfer to IL-15$^{-/-}$ hosts was most pronounced for Ly49$^{+}$ cells. Whether the heightened IL-15 sensitivity of Ly49$^{+}$ cells is controlled by Ly49 molecules per se or reflects slightly higher levels of CD122 and/or $\gamma_c$ on these cells is unclear. It is of interest that the Ly49 marker is also expressed on NK cells (19) and that, like Ly49$^{+}$ CD8$^{+}$ cells, NK cells are almost undetectable in IL-15$^{-/-}$ mice (16). Currently, the significance of this correlation is obscure. It is also intriguing that the survival/turnover of CD122$^{hi}$ CD8$^{+}$ cells is enhanced in transgenic mice expressing a human NK receptor plus the MHC ligand for the receptor (36). Based on the present data, this receptor/ligand interaction may potentiate responsiveness to IL-15, but the mechanisms involved have yet to be elucidated.

Since responsiveness to IL-15 requires high expression of CD122, it is perhaps not surprising that the residual CD44$^{hi}$ CD8$^{+}$ cells in IL-15$^{-/-}$ mice were found to be almost devoid of CD122$^{hi}$ cells and also Ly49$^{+}$ cells; likewise, Ly49$^{+}$ cells were undetectable in CD122$^{-/-}$ mice, though present in IL-2$^{-/-}$ mice. These data are clearly consistent with the view that CD122$^{hi}$ (and Ly49$^{+}$) cells require contact with IL-15 for their survival. However, IL-15 might be equally important for the initial formation of these cells. Against this possibility, we have found that upregulation of CD122 expression on naive CD8$^{+}$ cells responding to specific antigen does not require the presence of IL-15, either in vivo (this paper) or in vitro (unpublished data); likewise, others have reported that primary proliferative responses of CD8$^{+}$ cells are largely IL-15 independent (32–34). Hence, although IL-15 can have a qualitative influence on the primary response (37, 38), the main role of IL-15 is probably to act on preformed memory cells. The role of IL-15 on Ly49$^{+}$ cells is less clear. Thus, to date, we have been unable to induce Ly49 expression on CD8$^{+}$ cells responding to antigen plus cytokines, either in vitro or in vivo (unpublished data). Likewise, we have found that injecting IL-15 into IL-15$^{-/-}$ mice leads to rapid appearance of Ly49$^{+}$ NK cells but not Ly49$^{+}$ CD8$^{+}$ cells. Hence, whether IL-15 is

required for the initial formation of Ly49$^{+}$ CD8$^{+}$ cells is unresolved. Nevertheless, it is clear from the present data that, once formed, Ly49$^{+}$ CD8$^{+}$ cells are heavily dependent on IL-15.

Under steady-state conditions in unstimulated animals, the role of IL-15 in controlling the survival and intermittent division of CD122$^{hi}$ CD44$^{hi}$ CD8$^{+}$ cells presumably reflects low-level constitutive release of IL-15 by various stromal cells. As mentioned previously, levels of IL-15 (IL-15 mRNA by macrophages and dendritic cells) increase considerably when mice are injected with IFNs or other stimulators of the innate immune system (15, 22, 23). We envisage that this transient elevation in IL-15 accentuates division of IL-15–responsive CD122$^{hi}$ cells, thus causing bystander proliferation. The assumption here is that the mechanisms controlling bystander proliferation and normal turnover are essentially the same, the only difference being that bystander proliferation is more intense because of higher exposure to IL-15.

The observation that CD122$^{hi}$ CD8$^{+}$ cells are under the control of a single cytokine, IL-15, raises the question why other cytokines, e.g., IL-7, cannot substitute for the absence of IL-15. On this point, two observations indicate that raising the concentration of IL-7 in vivo to above-normal levels causes CD122$^{hi}$ CD8$^{+}$ cells to lose their sole dependency on IL-15; here, the cells can utilize either IL-7 or IL-15. First, homeostatic proliferation of CD122$^{hi}$ CD8$^{+}$ cells in irradiated hosts can occur in either IL-7$^{-/-}$ or IL-15$^{-/-}$ hosts but not in combined IL-7$^{-/-}$/IL-15$^{-/-}$ hosts (39). Our interpretation of this finding is that levels of IL-7 rise appreciably in irradiated hosts (reflecting diminished absorption of IL-7 because of T cell depletion), thus allowing the donor T cells to utilize either IL-15 or IL-7 for proliferation. Second, in marked contrast to IL-15$^{-/-}$ mice, IL-7 transgenic mice on an IL-15$^{-/-}$ background contain large numbers of CD122$^{hi}$ CD8$^{+}$ cells (40); here, high levels of IL-7 compensate for the absence of IL-15. In light of these findings, the sole dependency of CD122$^{hi}$ CD8$^{+}$ cells on IL-15 in normal mice may simply reflect that the concentration of other cytokines, notably IL-7, is too low to be significant. By contrast, the observation that some antigen-specific CD122$^{hi}$ CD8$^{+}$ memory cells can survive in the absence of IL-15 (see above) might reflect heightened sensitivity of these cells to IL-7.

The finding that one subset of memory-phenotype cells, namely CD122$^{hi}$ CD44$^{hi}$ CD8$^{+}$ cells, is heavily dependent on a single cytokine, IL-15, poses the question whether cytokines control proliferation/survival of other memory-phenotype cells. For the CD122$^{lo}$ component of CD44$^{hi}$ CD8$^{+}$ cells, it was mentioned earlier that these cells comprise ~40% of CD44$^{hi}$ CD8$^{+}$ cells in normal young mice and account for nearly all of the residual CD44$^{hi}$ CD8$^{+}$ cells found in IL-15$^{-/-}$ mice. Yet, despite being IL-15 independent, the baseline turnover of CD122$^{lo}$ CD44$^{hi}$ CD8$^{+}$ cells in both normal and IL-15$^{-/-}$ mice is appreciably higher than for typical CD122$^{hi}$ CD44$^{hi}$ CD8$^{+}$ cells. What then is the stimulus for these CD122$^{lo}$ cells? One possibility is that CD122$^{lo}$ CD44$^{hi}$ CD8$^{+}$ cells are stimu-

lated via chronic low-level, TCR-dependent responses to environmental antigens, thereby explaining their high rate of turnover. If so, CD122$^{lo}$ CD44$^{hi}$ CD8$^+$ cells might represent a subset of short-lived effector cells rather than true memory cells. Adoptive transfer studies with MHC class I$^{-/-}$ hosts will be needed to assess this possibility.

For CD44$^{hi}$ CD4$^+$ cells, we found no evidence that proliferation/survival of these cells was influenced by IL-15. This finding is to be expected because CD44$^{hi}$ CD4$^+$ cells are present in normal numbers in IL-15$^{-/-}$ mice and, being CD122$^{lo}$, are poorly equipped to respond to IL-15. Whether these cells are under the control of other cytokines is unclear, although it is striking that long-lived CD44$^{hi}$ CD4$^+$ memory cells can be generated from $\gamma_c^{-/-}$ mice (41).

In conclusion, the data in this paper demonstrate that the major subset of CD122$^{hi}$ CD44$^{hi}$ CD8$^+$ cells found in normal mice is crucially dependent on IL-15, both for survival and turnover. In addition, the data show that other CD44$^{hi}$ CD8$^+$ cells, namely CD122$^{lo}$ cells, are IL-15 independent and comprise nearly all of the residual CD44$^{hi}$ CD8$^+$ cells found in IL-15$^{-/-}$ mice; these cells may represent the immediate precursors of typical memory cells. The division of CD44$^{hi}$ CD8$^+$ cells into discrete subsets of IL-15–dependent and IL-15–independent cells may resolve the previous finding that background proliferation of CD44$^{hi}$ CD8$^+$ cells in vivo is only partially inhibited by CD122 mAb (18) and that IL-15$^{-/-}$ mice are only partially depleted of CD44$^{hi}$ CD8$^+$ cells (16).

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