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# IL-15 Promotes the Survival of Naive and Memory Phenotype CD8<sup>+</sup> T Cells<sup>1</sup>

Marion Berard,\* Katja Brandt,<sup>†</sup> Silvia Bulfone Paus,<sup>†</sup> and David F. Tough<sup>2\*</sup>

**IL-15 stimulates the proliferation of memory phenotype CD44<sup>high</sup>CD8<sup>+</sup> T cells and is thought to play a key role in regulating the turnover of these cells in vivo. We have investigated whether IL-15 also has the capacity to affect the life span of naive phenotype (CD44<sup>low</sup>) CD8<sup>+</sup> T cells. We report that IL-15 promotes the survival of both CD44<sup>low</sup> and CD44<sup>high</sup> CD8<sup>+</sup> T cells, doing so at much lower concentrations than required to induce proliferation of CD44<sup>high</sup> cells. Rescue from apoptosis was associated with the up-regulation of Bcl-2 in both cell types, whereas elevated expression of Bcl-x<sub>L</sub> was observed among CD44<sup>high</sup> but not CD44<sup>low</sup> CD8<sup>+</sup> cells. An investigation into the role of IL-15R subunits in mediating the effects of IL-15 revealed distinct contributions of the  $\alpha$ - and  $\beta$ - and  $\gamma$ -chains. Most strikingly, IL-15R $\alpha$  was not essential for either induction of proliferation or promotion of survival by IL-15, but did greatly enhance the sensitivity of cells to low concentrations of IL-15. By contrast, the  $\beta$ - and  $\gamma$ -chains of the IL-15R were absolutely required for the proliferative and pro-survival effects of IL-15, although it was not necessary for CD44<sup>high</sup>CD8<sup>+</sup> cells to express higher levels of IL-15R $\beta$  than CD44<sup>low</sup> cells to proliferate in response to IL-15. These results show that IL-15 has multiple effects on CD8 T cells and possesses the potential to regulate the life span of naive as well as memory CD8<sup>+</sup> T cells. *The Journal of Immunology*, 2003, 170: 5018–5026.**

**L**ong-term persistence of naive vs memory T cells is accompanied by fundamental differences in their kinetic behavior. Although naive cells divide only rarely in normal hosts, memory T cells undergo substantially higher rates of turnover (proliferation and death); these general characteristics were shown initially for naive vs memory phenotype T cell populations (1–3) and later confirmed for naive and memory T cells of defined specificity (4–6).

One of the factors that may contribute to the distinct kinetic behavior of naive and memory T cells is differential stimulation by cytokines. For CD8<sup>+</sup> T cells, one cytokine that is of particular interest is IL-15, which has been shown to stimulate proliferation of memory phenotype (CD44<sup>high</sup>) but not naive phenotype (CD44<sup>low</sup>) CD8<sup>+</sup> T cells when injected into mice or when added to purified CD8<sup>+</sup> T cells in vitro (7). IL-15 was originally identified as a cytokine with IL-2-like activity and belongs to the four  $\alpha$  helix bundle family of cytokines (8–11). Similarities in the functional properties of IL-15 and IL-2 stem from the fact that the receptors for these two cytokines employ the same  $\beta$ - and  $\gamma$ -chains (i.e., IL-2R $\beta$  and IL-2R $\gamma$ ), while the specificity of the receptors is provided by unique  $\alpha$ -chains (12). However, despite their similarities, there is evidence suggesting that IL-2 and IL-15 play different roles in regulating T cell turnover. Thus, although injection of an anti-IL-2R $\beta$  Ab (which blocks the activity of both IL-2 and IL-15) into normal mice reduced the background proliferation rate of CD44<sup>high</sup>CD8<sup>+</sup> T cells, administration of anti-IL-2R $\alpha$  plus anti-

IL-2 (which blocks IL-2 selectively) enhanced the turnover of these cells (13). These results implied that IL-15 and IL-2 promote and inhibit memory CD8 T cell turnover, respectively.

Direct evidence that IL-15 makes an important contribution to the maintenance of CD8 memory T cells has come from the description of IL-15- and IL-15R $\alpha$ -deficient mouse strains (14, 15). Strikingly, in both types of mice there is a dramatic reduction in the number of CD44<sup>high</sup>CD8<sup>+</sup> T cells. Furthermore, following virus infection, both the number of Ag-specific CD8 memory T cells and their rate of proliferation are reduced in the knockout mice compared with controls (16, 17). This contrasts with mice constitutively expressing an IL-15 transgene, which have a substantial increase in memory phenotype CD8<sup>+</sup> T cells and maintain higher numbers of Ag-specific CD8 memory cells after immunization (18–20). Overall, therefore, the data support the hypothesis that proliferation among CD8 memory cells plays an important role in maintaining this population and that IL-15 makes a key contribution to this process. However, it should be noted that IL-15 dependence appears to apply only to a subset of memory phenotype CD8<sup>+</sup> cells, since residual populations of CD44<sup>high</sup> cells are present in IL-15<sup>-/-</sup> and IL-15R $\alpha$ <sup>-/-</sup> mice (14, 15, 21), and these cells exhibit a high rate of background turnover (21).

It is less clear whether IL-15 has any role in supporting the long-term maintenance of naive CD8 T cells. As mentioned above, IL-15 does not induce proliferation of CD44<sup>low</sup>CD8<sup>+</sup> T cells when injected into mice or when added to purified CD8<sup>+</sup> T cells in vitro (7). Nevertheless, it is notable that IL-15<sup>-/-</sup> and IL-15R $\alpha$ <sup>-/-</sup> mice have substantially reduced numbers of naive phenotype as well as memory phenotype CD8 cells (14, 15, 22). Although decreased thymic output could play a part in this deficit in IL-15R $\alpha$ <sup>-/-</sup> mice, which have reduced numbers of CD8 single-positive thymocytes (14, 22), this does not appear to be the case in IL-15<sup>-/-</sup> mice (15). Given the seeming unresponsiveness of naive CD8 cells to IL-15, however, it is unclear how IL-15 could contribute to the persistence of these cells in the secondary lymphoid organs.

In this article, we sought to determine whether IL-15 has the potential to regulate the homeostasis of naive as well as memory

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CD8 T cells. Consistent with this idea, we found that IL-15 acted as a survival factor for both naive and memory phenotype CD8 cells, inhibiting apoptosis at much lower concentrations than that required to stimulate proliferation of CD44<sup>high</sup> cells. Evidence is presented showing that the response of CD8 cells to IL-15 is determined by multiple factors, including the expression of IL-15R subunits, the concentration of IL-15 and the previous activation history of the cell.

## Materials and Methods

### Mice

C57BL/6 mice were purchased from Charles River Breeding Laboratories-U.K. (Margate, Kent, U.K.). In most experiments, these mice were used at >6 mo of age because of the higher proportion of CD44<sup>high</sup>CD8<sup>+</sup> T cells present in aged vs young mice. Similar results, however, were obtained when cells were isolated from young (6- to 10-wk-old) mice. F5RAG mice (23) (originally obtained from D. Kioussis, National Institute for Medical Research, London, U.K.) were bred in the specific pathogen-free unit at the Institute for Animal Health (Compton, U.K.), while IL-15R $\alpha$ <sup>-/-</sup> mice (14) (originally obtained from The Jackson Laboratory, Bar Harbor, Maine USA) and their wild-type (WT)<sup>3</sup> controls were bred at the Research Center Borstel (Borstel, Germany).

### Reagents and mAbs

Recombinant human IL-15 was purchased from R&D Systems (Minneapolis, MN) and used at the indicated concentrations. CFSE was purchased from Molecular Probes (Leiden, The Netherlands). The following mAbs were purchased from BD Biosciences (Cowley, U.K.): FITC-conjugated mAbs against 5-bromo-2'-deoxyuridine (BrdU), CD25, CD69, Ly6C, Ly6A/E; PE-conjugated anti-CD44; CyChrome-conjugated anti-CD44; purified anti-Bcl-2 and anti-Bcl-x<sub>L</sub>, anti-CD122, anti-CD132, and rat IgG2b isotype controls. Anti-CD8 (YTS.169) was conjugated to Cy5 using a Cy5-labeling kit (Amersham Pharmacia Biotech, Little Chalfont, U.K.).

### Flow cytometric analysis

Aliquots of 2–5 × 10<sup>5</sup> cells were stained with Abs diluted in PBS containing 2% FCS and 0.1% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and analyzed on a FACSCalibur flow cytometer with the use of CellQuest software (BD Biosciences).

### Preparation of CD8<sup>+</sup> T cells

Lymph nodes (LN) and spleens were cut into small pieces and digested, with agitation, in RPMI 1640 medium containing 5% FCS, 1 mg/ml type III collagenase (Lorne Laboratories, Reading, U.K.), and 0.6 mg/ml DNase I (Sigma-Aldrich, St. Louis, MO) for 5 min at 37°C followed by 15 min at room temperature. Unless stated otherwise, cells from LN and spleen were pooled. For all the experiments except those using IL-15R $\alpha$ <sup>-/-</sup> or IL-15<sup>-/-</sup> mice (see below), CD44<sup>high</sup> and CD44<sup>low</sup> CD8<sup>+</sup> T cells were purified in a two-step procedure. First, CD8<sup>+</sup> T cell populations were obtained by negative selection after labeling with anti-CD4 (clone GK1.5), anti-MHC class II (clone TIB120), anti-B220 (clone RA3-6B2), and anti-CD11b (clone M1/70) mAbs followed by incubation with magnetic beads coated with anti-rat and anti-mouse IgG Abs (Dynabeads; Dynal, Oslo, Norway). Subsequently, the resultant cell population was labeled with anti-CD8-Cy5 and either anti-CD44-PE or anti-CD44-CyChrome and the CD44<sup>high</sup> and CD44<sup>low</sup>CD8<sup>+</sup> T cells sorted on a MoFlow flow cytometer (Cytomation, Fort Collins, CO). The resulting populations were >98% pure in all cases.

In experiments using IL-15R $\alpha$ <sup>-/-</sup> mice, highly purified populations of CD8<sup>+</sup> T cells (>96% CD8<sup>+</sup>) were obtained from pooled spleen and LN cells by two successive rounds of selection. In the first, CD4<sup>+</sup>, MHC class II<sup>+</sup>, B220<sup>+</sup>, and CD11b<sup>+</sup> cells were removed by negative selection as described above. In the second, CD8<sup>+</sup> cells were positively selected using anti-CD8-conjugated magnetic microbeads (Miltenyi Biotec, Bisley, U.K.).

### CFSE labeling

Cells (1 × 10<sup>7</sup>/ml) were incubated for 5 min in PBS containing 0.1% BSA and 1  $\mu$ M CFSE at 37°C and then washed three times.

### Culture of CD8<sup>+</sup> T cells

All cultures were made in complete medium (RPMI 1640 supplemented with 2-ME, penicillin, streptomycin, L-glutamine, and 10% heat-inactivated FCS; all from Life Technologies, Grand Island, NY). Cells were seeded at 5 × 10<sup>4</sup> cells/well in round-bottom 96-well microtiter plates in a final volume of 0.1 ml. Except where indicated, IL-15 was added at the onset of the culture. Where indicated, cells were preincubated with anti-CD122, anti-CD132, or isotype control Abs at the specified concentrations for 30 min before the addition of IL-15 to the culture. The determination of DNA and RNA synthesis was performed in triplicate for each culture point by pulsing the cells with 1  $\mu$ Ci/well [<sup>3</sup>H]thymidine or [<sup>3</sup>H]uridine, respectively (Amersham Pharmacia Biotech) for the last 16 h of the culture period. The phenotypic and cell cycle analysis of the cultured cells was performed on cells pooled from several wells of 96-well plates to ensure that the same concentration of IL-15 per density of cells was used in the different types of assays.

### Assessment of proliferation by BrdU labeling

Purified CD8<sup>+</sup> T cells were cultured in the presence or absence of IL-15 as indicated in medium containing 2.5  $\mu$ g/ml BrdU (Sigma-Aldrich) for 36 h. Subsequently, cells were harvested from wells and stained for the incorporation of BrdU into DNA as previously described (3).

### Measurement of apoptosis

Quantitation of apoptotic cells was performed using three different assays: 1) Staining with 3,3'-dihexyloxycarbocyanine iodide (DiOC6; Molecular Probes), which reveals the disruption of the mitochondrial transmembrane potential, was performed as described (24). In this assay, apoptotic cells are identified by their decreased staining with DiOC6 (DiOC6<sup>low</sup>). 2) Staining with annexin V conjugated to FITC (Roche Diagnostics, Lewes, U.K.) or Cy-3 (Biovision Research Products, Palo Alto, CA) according to the manufacturers' protocols, which detects translocation of phosphatidylserine from the inner side to the outer leaflet of the plasma membrane on apoptotic cells (25). 3) Propidium iodide staining, which identifies cells with sub-G1 DNA content (26). In this study, cells were washed in PBS, resuspended in 1 vol of saline buffer (6 mM glucose, 140 mM NaCl, 5 mM KCl, 1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1 mM KH<sub>2</sub>PO<sub>4</sub>, and 0.2 mM EDTA), fixed overnight in 3 vol of 95% ethanol, washed in PBS plus 2% FCS, and incubated in PBS plus 2% FCS with 0.5 mg/ml RNase A and 5  $\mu$ g/ml propidium iodide (Sigma-Aldrich). Flow cytometric analysis was performed on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences). The percentage of inhibition of apoptosis induced by IL-15 stimulation was calculated as 100 × ((percent spontaneous apoptosis – percent apoptosis in the presence of IL-15)/percent spontaneous apoptosis).

### Western blot analysis

Briefly, 5 × 10<sup>6</sup> cells were pelleted, washed twice with PBS, resuspended in 100  $\mu$ l of lysis buffer (50 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.25% Nonidet P-40, and 0.1% SDS) supplemented with a protease inhibitor mixture (Roche Diagnostics) and incubated for 15 min on ice. The protein concentration in cleared lysates was determined with the DC protein assay kit (Bio-Rad, Richmond, CA) and 20  $\mu$ g of protein from each sample was electrophoresed on 15% SDS polyacrylamide gels. Following transfer to nitrocellulose membrane (Amersham Pharmacia Biotech), the immunoblots were blocked by incubating with 5% skimmed-dry milk, TBST, and probed overnight with the anti-Bcl-2 or the anti-Bcl-x<sub>L</sub> mAbs at a dilution of 1/500 prepared in 15% FCS TBST. The immunoblots were then probed with HRP-conjugated goat anti-mouse IgG Abs and developed using the ECL system (Amersham Pharmacia Biotech). Band intensity was determined by densitometry using Quantity One software (Bio-Rad).

## Results

### IL-15 stimulates phenotypic activation and proliferation of CD44<sup>high</sup> but not CD44<sup>low</sup> CD8<sup>+</sup> T cells

Selective stimulation of memory but not naive phenotype CD8<sup>+</sup> T cell proliferation by IL-15 has been shown previously by examining the phenotype of cells that divided after either injection of IL-15 into mice or addition of IL-15 to purified CD8<sup>+</sup> T cells *in vitro* (7). However, because IL-15 was acting on mixed cell populations in these studies, it could not be excluded that some CD44<sup>low</sup>CD8<sup>+</sup> T cells were activated by IL-15 and acquired a CD44<sup>high</sup> phenotype concomitant with cell division. To assess this possibility, we generated highly purified populations of CD44<sup>high</sup>

<sup>3</sup> Abbreviations used in this paper: WT, wild type; BrdU, 5-bromo-2'-deoxyuridine; LN, lymph node; DiOC6, 3,3'-dihexyloxycarbocyanine iodide; FSC, forward scatter; SSC, side scatter; RAG, recombinant-activating gene; TRAF, TNFR-associated factor.

and CD44<sup>low</sup>CD8<sup>+</sup> T cells by cell sorting and examined the response of these cells to IL-15. Our initial experiments showed that treatment of purified CD44<sup>low</sup> cells with high concentrations of IL-15 failed to induce any up-regulation of CD44, indicating that IL-15 did not induce phenotypic activation of the naive phenotype CD8<sup>+</sup> T cells (data not shown). Furthermore, while culture of purified CD44<sup>high</sup>CD8<sup>+</sup> cells in the presence of IL-15 for 2 days resulted in up-regulation of a number of cell surface activation markers, including CD25, CD69, Ly-6C, and Ly-6A/E, no such changes were observed on IL-15-treated CD44<sup>low</sup>CD8<sup>+</sup> T cells (Fig. 1A).

To assess directly the ability of IL-15 to stimulate proliferation of these phenotypically defined subpopulations, graded doses of IL-15 were added to cultures of purified CD44<sup>high</sup> or CD44<sup>low</sup>CD8<sup>+</sup> T cells and cell division was measured by [<sup>3</sup>H]thymidine incorporation. Proliferation of CD44<sup>high</sup>CD8<sup>+</sup> T cells was evident at 12.5 ng/ml IL-15 and increased in a dose-dependent manner at higher concentrations (Fig. 1B); a similar dose-response curve was observed for phenotypic activation (Fig. 1C). In contrast, purified CD44<sup>low</sup>CD8<sup>+</sup> T cells exhibited virtually no proliferation, even at doses of IL-15 up to 200 ng/ml (Fig. 1, A and B, and data not shown). Taken together, these results confirm that IL-15 does indeed selectively induce activation and proliferation of pre-existing CD44<sup>high</sup> cells with little or no effect on the naive phenotype CD8<sup>+</sup> T cells.

#### IL-15 rescues naive phenotype CD8<sup>+</sup> T cells from undergoing spontaneous apoptosis in vitro

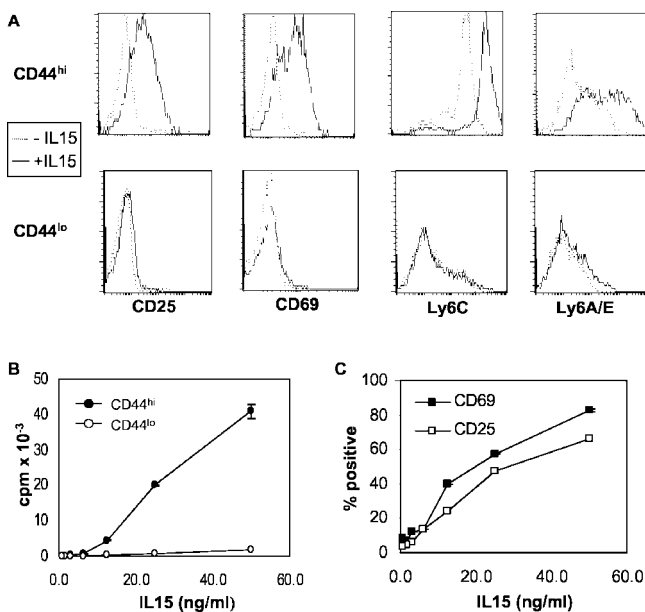
Although unable to stimulate overt activation of CD44<sup>low</sup>CD8<sup>+</sup> T cells, it was evident that IL-15 may be having more subtle effects

on these cells. This was suggested by differences in the morphological characteristics of cells cultured with and without IL-15. Thus, when the forward and side scatter (FSC/SSC) properties of the cells were assessed during flow cytometric analysis, two major differences were noted (Fig. 2A). First, a much higher proportion of the cells from cultures including IL-15 had the FSC/SSC properties expected of viable cells. Second, the cells falling into the “viable cell” gate were on average larger when obtained from cultures including IL-15. Although these differences were much more marked for CD44<sup>high</sup> cells, the same qualitative changes occurred among CD44<sup>low</sup> cells. These observations implied that although IL-15 was unable to trigger either activation or proliferation of the CD44<sup>low</sup>CD8<sup>+</sup> T cells, it could still signal through the IL-15R in these cells.

The modification in the FSC/SSC profile of CD44<sup>low</sup>CD8<sup>+</sup> T cells by IL-15 treatment suggested that IL-15 was affecting the viability of the naive phenotype CD8<sup>+</sup> cells at two levels. First, the larger size of the “viable” cells indicated that IL-15 inhibited the cellular atrophy that occurs before apoptosis (27). Second, the higher percentage of cells in the viable gate suggested that IL-15 also reduced their rate of apoptosis. To investigate this second possibility more directly, we determined the percentage of apoptotic cells among CD44<sup>low</sup>CD8<sup>+</sup> T cells that had been cultured in the absence or presence of IL-15 (50 ng/ml) for 48 h using three assays that detect different aspects of the apoptosis process (see *Materials and Methods*). Irrespective of the assay used, it was clear that the percentage of CD44<sup>low</sup>CD8<sup>+</sup> T cells undergoing spontaneous apoptosis in vitro decreased significantly upon addition of IL-15 to the culture (Fig. 2B). Importantly, this also applied to CD44<sup>low</sup>CD8<sup>+</sup> T cells from F5 TCR-transgenic mice (on a recombinant-activating gene (RAG)-1-deficient background; Fig. 2C). Since these cells express a transgenic TCR specific for a peptide in the nucleoprotein of influenza virus, they should be bona fide naive cells in mice that have not been infected with flu (23). Therefore, IL-15 was indeed capable of acting on naive CD8<sup>+</sup> T cells and in doing so delivered pro-survival signals.

#### IL-15 rescues CD44<sup>high</sup>CD8<sup>+</sup> T cells from undergoing spontaneous apoptosis in vitro at doses that do not induce proliferation

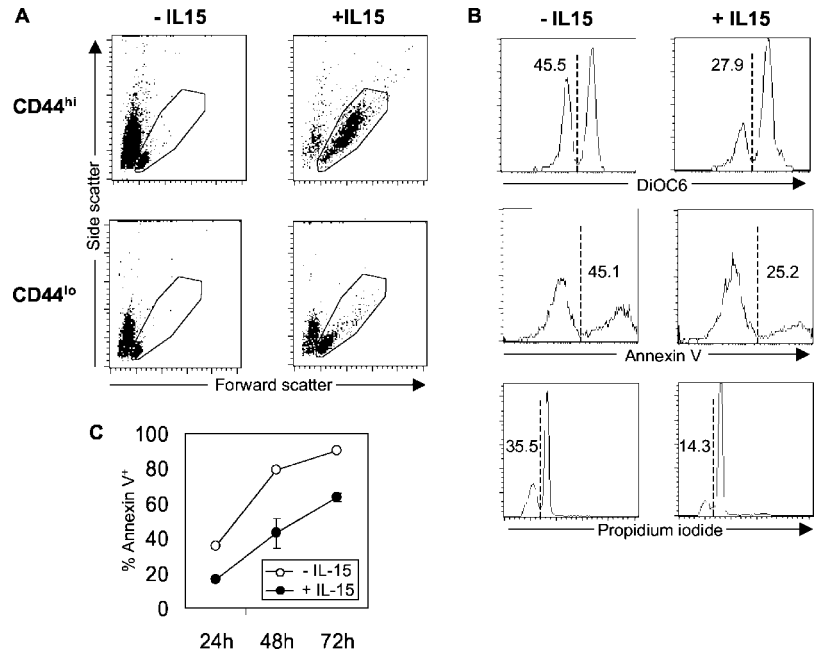
Based on FSC/SSC properties, IL-15 also appeared to enhance the viability of CD44<sup>high</sup>CD8<sup>+</sup> T cells (Fig. 2A). However, the greater proportion of viable cells in cultures containing IL-15 may have been due to the extensive proliferation that had occurred in the culture, diluting out apoptotic, nonresponsive cells. To avoid this complication, we examined whether a dose of IL-15 that did not induce significant proliferation of CD44<sup>high</sup> cells (6 ng/ml, see Fig. 1B) affected CD8<sup>+</sup> T cell apoptosis. Although this concentration of IL-15 was insufficient to induce [<sup>3</sup>H]thymidine incorporation by either CD44<sup>high</sup> or CD44<sup>low</sup>CD8<sup>+</sup> T cells, it was notable that [<sup>3</sup>H]uridine incorporation (i.e., RNA synthesis) was increased in both subsets (Fig. 3A), indicating that IL-15 was delivering a signal to the cells. Significantly, both CD44<sup>high</sup> and CD44<sup>low</sup> cells were rescued from apoptosis at this dose of IL-15; although CD44<sup>high</sup> cells exhibited a faster rate of spontaneous apoptosis in medium alone, the addition of IL-15 to the culture reduced the percentage of apoptotic cells by >50% in both subsets (Fig. 3B). These results therefore show that in addition to inducing proliferation of memory phenotype CD8<sup>+</sup> T cells, IL-15 also rescues them from apoptosis. Furthermore, lower concentrations of IL-15 were required to signal for survival vs proliferation. Experiments using CFSE-labeled cells confirmed that IL-15 could protect cells from apoptosis without inducing proliferation; an example of this is shown in Fig. 3D (note that CFSE-labeled cells exhibited higher



**FIGURE 1.** IL-15 induces activation and proliferation of CD44<sup>high</sup> but not CD44<sup>low</sup>CD8<sup>+</sup> T cells. A, Expression of activation markers on CD44<sup>high</sup> (upper panels) or CD44<sup>low</sup> (lower panels) CD8<sup>+</sup> T cells cultured in the absence (dashed line) or presence (solid line) of IL-15 (50 ng/ml) for 48 h. B, Proliferation of CD44<sup>high</sup> (●) or CD44<sup>low</sup> (○) CD8<sup>+</sup> T cells cultured in the presence of different concentrations of IL-15 for 40 h. C, Expression of CD69 (■) or CD25 (□) by CD44<sup>high</sup>CD8<sup>+</sup> T cells cultured in the presence of different concentrations of IL-15 for 48 h. CD44<sup>high</sup> and CD44<sup>low</sup>CD8<sup>+</sup> cells were purified by cell sorting before culture. Proliferation was assessed by pulsing cells with [<sup>3</sup>H]thymidine for the final 16 h of culture; the mean cpm ± SD from triplicate wells is shown. Phenotype and proliferation data are representative of at least three independent experiments.



**FIGURE 2.** IL-15 reduces the spontaneous apoptosis of naive phenotype CD8<sup>+</sup> T in vitro. *A*, FSC and SSC properties of purified CD44<sup>high</sup> or CD44<sup>low</sup>CD8<sup>+</sup> T cells cultured in the presence or absence of IL-15 (50 ng/ml) for 48 h. *B*, Spontaneous apoptosis of purified CD44<sup>low</sup>CD8<sup>+</sup> T cells cultured in the absence or presence of IL-15 (50 ng/ml). The numbers on the histograms indicate the percentage of apoptotic cells, i.e., cells which stain as DiOC6<sup>low</sup> (upper panels) or annexin V<sup>+</sup> (middle panels), or which have sub-G1 DNA content (lower panels), measured after 48 h of culture. *C*, Spontaneous apoptosis, measured by annexin V staining, of CD44<sup>low</sup>CD8<sup>+</sup> T cells purified from F5RAG mice and cultured in the presence (●) or absence (○) of IL-15 (50 ng/ml) for different lengths of time. Results are representative of three independent experiments.



spontaneous apoptosis in culture due to toxic effects of the dye). In fact, very low doses of IL-15 were capable of rescuing both CD44<sup>high</sup> and CD44<sup>low</sup>CD8<sup>+</sup> T cells from apoptosis, with pro-survival effects apparent at concentrations as low as 50 pg/ml (Fig. 3C).

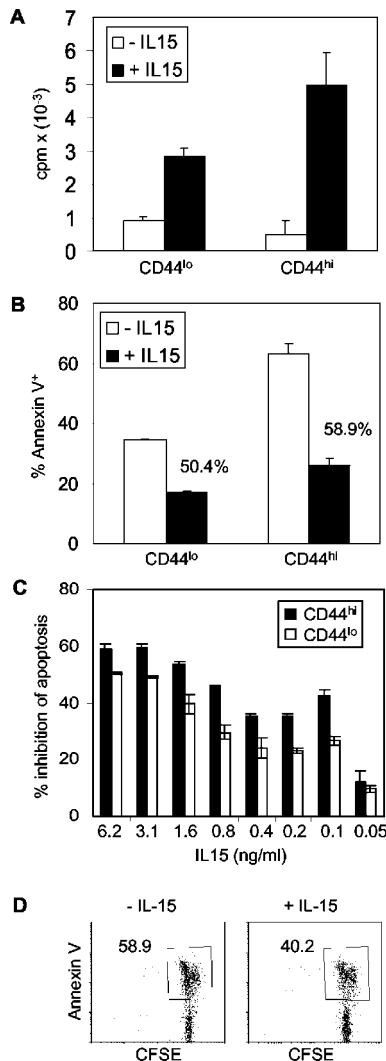
#### IL-15 induces increased expression of Bcl-2 and Bcl-X<sub>L</sub> in resting CD8<sup>+</sup> T cells

The observation that IL-15 treatment inhibited the loss of mitochondrial membrane potential in CD8 cells in culture (as shown by DiOC6 staining) suggested that Bcl-2 family proteins could be mediating the antiapoptotic effects of IL-15, since these molecules have been shown to target the permeability transition pore complex of the mitochondrial membrane (28). In addition, exposure to IL-15 has been shown to modify the expression of antiapoptotic Bcl-2 family members in different cell types, with the changes observed varying depending on the cell examined and its state of activation. For example, IL-15 has been reported to induce up-regulation of Bcl-2 but not Bcl-x<sub>L</sub> in activated  $\alpha\beta$  T cells (20, 22, 29, 30) or T cell lines (31), Bcl-x<sub>L</sub> but not Bcl-2 expression in activated intraepithelial  $\gamma\delta$  T cells (32), Bcl-x<sub>L</sub> but not Bcl-2 in mouse mast cells (33), and Bcl-2 in human NK cells (34, 35). Hence, it was of interest to examine the effects of IL-15 on Bcl-2 and Bcl-x<sub>L</sub> expression in resting naive and memory phenotype CD8<sup>+</sup> T cells.

Purified CD44<sup>low</sup> and CD44<sup>high</sup>CD8<sup>+</sup> T cells were cultured for 24 h in medium alone or in medium containing IL-15 (50 ng/ml), and Bcl-2 plus Bcl-x<sub>L</sub> expression were assessed by Western blotting. As shown in Fig. 4 (A and C), both CD44<sup>low</sup> and CD44<sup>high</sup> cells expressed markedly higher levels of Bcl-2 when cultured in the presence of IL-15 compared with medium alone. Interestingly, IL-15 induced elevated Bcl-x<sub>L</sub> expression in CD44<sup>high</sup> but not CD44<sup>low</sup>CD8<sup>+</sup> cells. This difference was not linked directly to the induction of proliferation, since treatment of CD44<sup>high</sup> cells with a dose of IL-15 that did not stimulate proliferation (6 ng/ml) also resulted in up-regulation of both Bcl-2 and Bcl-x<sub>L</sub> (Fig. 4, B and D). The data therefore show that IL-15 does induce expression of antiapoptotic Bcl-2 family proteins in resting CD8 cells, with overlapping but distinct effects on naive and memory phenotype cells.

#### Differential role of IL-15R $\alpha$ -, $\beta$ -, and $\gamma$ -chains in promoting CD8<sup>+</sup> T cell proliferation vs survival

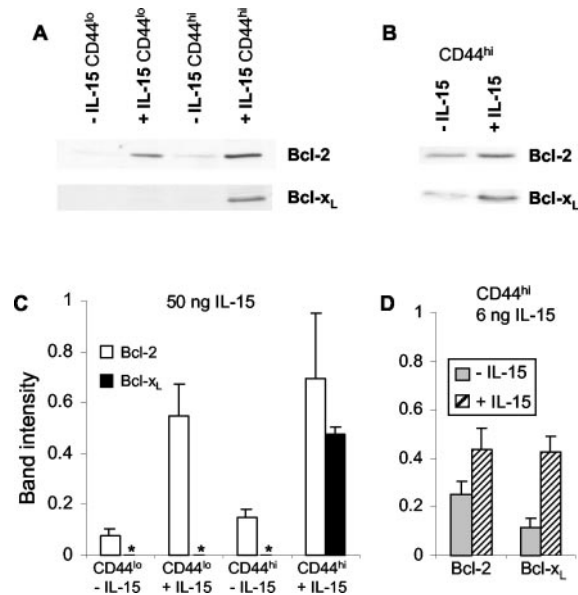
One explanation proposed to account for the selective proliferation of CD44<sup>high</sup> but not CD44<sup>low</sup>CD8<sup>+</sup> T cells in response to IL-15 is the higher expression of the IL-15R  $\beta$ -chain (CD122) on the former subset (7). Our data, by contrast, suggest that high surface levels of CD122 are not required for delivery of an antiapoptotic signal, since IL-15 promotes the survival of naive and memory phenotype CD8 cells to a similar extent. To examine the importance of CD122 and CD132 (IL-2R $\gamma$ ) in the response to IL-15, graded doses of blocking Abs against these molecules were added to cultures of CD44<sup>high</sup>CD8<sup>+</sup> T cells in the absence or presence of IL-15 and activation and survival were assessed. In the absence of IL-15, treatment of cells with these Abs, individually or in combination, had no effect on survival, proliferation, or phenotypic activation of CD8 cells at the concentrations used (data not shown). By contrast, addition of either anti-CD122 or anti-CD132 reduced proliferation and phenotypic activation in response to IL-15 (Fig. 5, A and B); both responses were abolished completely at an Ab concentration of 2.5  $\mu$ g/ml, while partial inhibition was observed at lower doses. Note that culture of cells in the presence of anti-CD122 did not affect cell surface expression of CD132, while treatment with anti-CD132 did not affect cell surface expression of CD122, ruling out cocapping of receptor chains as an explanation for why treatment with either Ab affects the response to IL-15 (data not shown). Therefore, binding of IL-15 to both of these chains is an absolute requirement for the overt activation of memory phenotype CD8<sup>+</sup> cells. In addition, both Abs inhibited substantially the pro-survival effects of IL-15, indicating that the  $\beta$ - and  $\gamma$ -chains of the receptor also play key roles in delivery of the antiapoptotic signal (Fig. 5A). Interestingly, however, a residual IL-15-induced rescue from apoptosis was observed when the Abs were added individually, even at high concentrations. This pro-survival effect of IL-15 disappeared only when both Abs were added to the same cultures (Fig. 5C), a treatment that also eliminated the up-regulation of Bcl-2 and Bcl-x<sub>L</sub> (Fig. 5D). The failure of anti-CD122 or anti-CD132 to block IL-15-induced rescue from apoptosis when added individually implied that IL-15 can deliver



**FIGURE 3.** Subproliferative concentrations of IL-15 inhibit the spontaneous apoptosis of CD44<sup>low</sup> and CD44<sup>high</sup>CD8<sup>+</sup> T cells. *A*, [<sup>3</sup>H]uridine incorporation by purified CD44<sup>low</sup> or CD44<sup>high</sup>CD8<sup>+</sup> T cells cultured for 40 h in the presence (■) or absence (□) of IL-15 (6 ng/ml). Cells were pulsed with [<sup>3</sup>H]uridine for the final 16 h of culture and the mean cpm ± SD for triplicate wells is shown. *B*, Spontaneous apoptosis of purified CD44<sup>low</sup> or CD44<sup>high</sup>CD8<sup>+</sup> T cells cultured in the presence (■) or absence (□) of IL-15 (6 ng/ml). *C*, Effect of different concentrations of IL-15 on the spontaneous apoptosis of purified CD44<sup>low</sup> or CD44<sup>high</sup>CD8<sup>+</sup> T cells. *D*, Apoptosis of CFSE-labeled CD44<sup>low</sup>CD8<sup>+</sup> T cells cultured in medium alone (*left*) or in medium plus 3 ng/ml IL-15 (*right*); numbers indicate percent apoptotic cells (gated population). Apoptosis was measured by annexin V staining after 48 h of culture. Percent inhibition of apoptosis was calculated as described in *Materials and Methods*. All results are representative of at least three independent experiments.

some level of pro-survival signal when binding to a receptor lacking either the  $\beta$ - or  $\gamma$ -chain (but not both).

To examine the role of the IL-15R  $\alpha$ -chain in the response of CD8 cells to IL-15, we used CD8<sup>+</sup> T cells from IL-15R $\alpha$ <sup>-/-</sup> mice. Although the number of CD44<sup>high</sup>CD8<sup>+</sup> T cells is reduced in these mice, a residual population of memory phenotype CD8 cells remains (14). Notably, in addition to lacking  $\alpha$ -chain expression, the CD44<sup>high</sup>CD8<sup>+</sup> T cells present in the IL-15R $\alpha$ <sup>-/-</sup> mice had lower expression of CD122 than CD44<sup>high</sup> cells in control mice, equivalent to the levels present on CD44<sup>low</sup>CD8<sup>+</sup> cells (Fig. 6A). This is similar to the phenotype of CD44<sup>high</sup>CD8<sup>+</sup> T cells present in IL-15<sup>-/-</sup> mice (21).

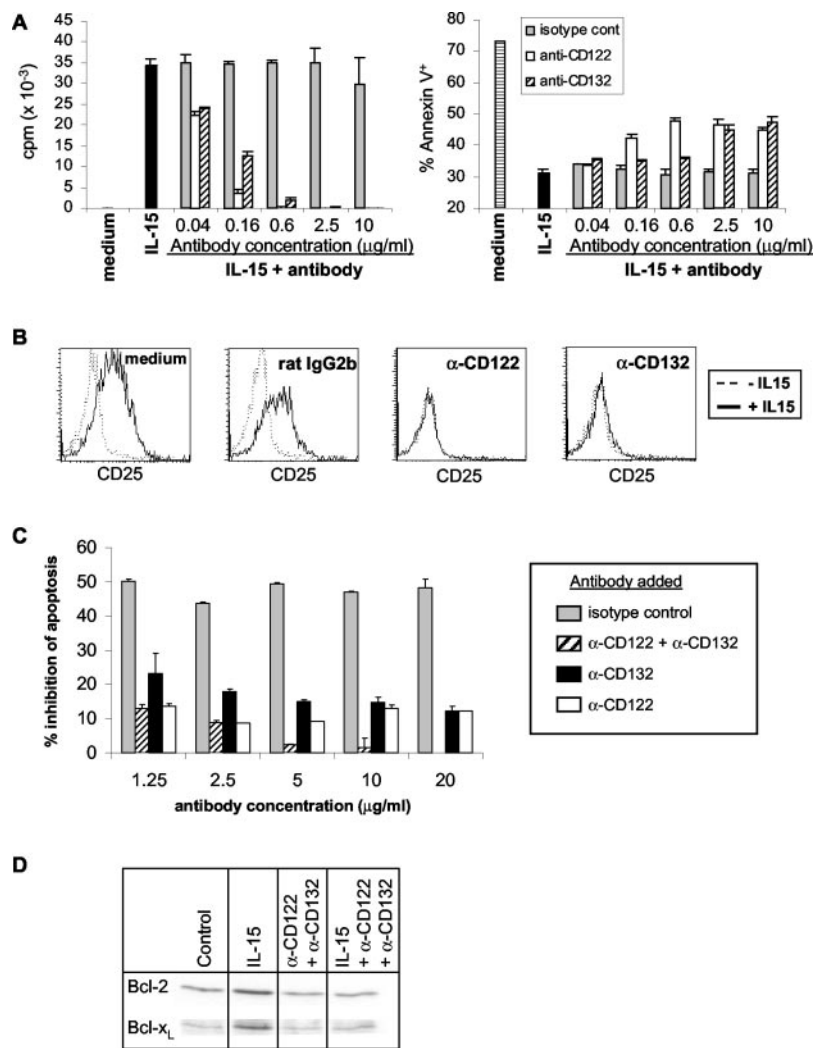


**FIGURE 4.** IL-15 induces up-regulation of Bcl-2 expression by CD44<sup>low</sup> and CD44<sup>high</sup>CD8<sup>+</sup> T cells and Bcl-x<sub>L</sub> expression by CD44<sup>high</sup> cells only. *A*, Western blots showing Bcl-2 and Bcl-x<sub>L</sub> protein expression in CD44<sup>low</sup> and CD44<sup>high</sup>CD8<sup>+</sup> T cells cultured for 24 h in medium alone or in medium plus 50 ng/ml IL-15. *B*, Western blots showing Bcl-2 and Bcl-x<sub>L</sub> protein expression in CD44<sup>high</sup>CD8<sup>+</sup> T cells cultured for 24 h in medium alone or in medium plus 6 ng/ml IL-15. *C* and *D*, Mean ± SD band intensities (in arbitrary units, as generated using densitometry software) for triplicate measurements of the blots shown in *A* and *B*, respectively. \*, Band intensity was below the level of detection.

Whether IL-15R $\alpha$  was required for IL-15-induced proliferation was assessed by adding IL-15 to CD8<sup>+</sup> T cells purified from either IL-15R $\alpha$ <sup>-/-</sup> mice or controls, culturing the cells in the presence of BrdU, and measuring the incorporation of BrdU into CD44<sup>high</sup> cells (7). Since IL-15 does not induce up-regulation of CD44 on naive phenotype cells (see above), labeling of CD44<sup>high</sup> cells with BrdU reflects proliferation of memory-phenotype cells present initially in the culture. As shown in Fig. 6B, IL-15 stimulated the proliferation of both IL-15R $\alpha$ <sup>-/-</sup> and IL-15R $\alpha$ <sup>+/+</sup> CD44<sup>high</sup>CD8<sup>+</sup> T cells, although some differences were apparent. In particular, the magnitude of the increase in proliferation among CD44<sup>high</sup> cells was reduced slightly for IL-15R $\alpha$ <sup>-/-</sup> cells vs controls. In addition, a prominent population of BrdU<sup>+</sup>CD44<sup>int</sup> cells was observed among IL-15R $\alpha$ <sup>-/-</sup> but not IL-15R $\alpha$ <sup>+/+</sup> cells after IL-15 treatment. Despite these differences, however, the key finding from these data is that neither IL-15R $\alpha$  expression nor high levels of CD122 were required for IL-15-induced proliferation of memory phenotype CD8<sup>+</sup> cells.

To assess the importance of IL-15R $\alpha$  in mediating pro-survival effects, various concentrations of IL-15 were added to CD8<sup>+</sup> T cells purified from IL-15R $\alpha$ <sup>-/-</sup> or IL-15R $\alpha$ <sup>+/+</sup> mice and apoptosis was measured by annexin V staining (Fig. 7). At a high dose (50 ng/ml), IL-15 reduced the percentage of apoptotic cells in WT and IL-15R $\alpha$ -deficient CD8 cell cultures to a similar extent. This was not due strictly to induction of proliferation, since both CD44<sup>high</sup> and CD44<sup>low</sup>CD8<sup>+</sup> T cells from IL-15R $\alpha$ <sup>-/-</sup> mice were rescued efficiently at this concentration of IL-15 (Fig. 7, *B* and *C*). Notably, however, IL-15 prevented apoptosis less well among IL-15R $\alpha$ <sup>-/-</sup> CD8<sup>+</sup> cells than IL-15R $\alpha$ <sup>+/+</sup>CD8<sup>+</sup> cells when added at low concentrations. Again, this decreased efficiency of rescue was evident among both CD44<sup>high</sup> and CD44<sup>low</sup>CD8<sup>+</sup> T cells (Fig. 7, *B* and *C*). Therefore, the inefficient rescue of IL-15R $\alpha$ <sup>-/-</sup> cells

**FIGURE 5.** CD122 and CD132 are required for induction of proliferation and promotion of survival by IL-15. *A*, Purified CD44<sup>high</sup>CD8<sup>+</sup> T cells were preincubated with the indicated concentration of anti-CD122 or anti-CD132 mAbs (or isotype control) and then cultured for 48 h in the absence or presence of IL-15; IL-15 was used at 50 ng/ml for proliferation assays (*left*) or at 6 ng/ml for survival assays (*right*). Proliferation was assessed by adding [<sup>3</sup>H]thymidine and then culturing cells for another 16 h, while apoptosis was measured by annexin V staining at 48 h. Mean cpm  $\pm$  SD for triplicate wells are shown. *B*, Purified CD44<sup>high</sup>CD8<sup>+</sup> T cells were treated with Abs (as in *A*) and cultured for 48 h in the presence of IL-15 (50 ng/ml), after which phenotypic markers were measured by flow cytometry. Ab treatment had equivalent effects on the expression of other activation markers (data not shown). *C*, Purified CD44<sup>high</sup>CD8<sup>+</sup> T cells were treated with Abs (as in *A*) and cultured in the absence or presence of IL-15 (6 ng/ml) for 48 h, after which apoptosis was measured by annexin V staining. *D*, Purified CD44<sup>high</sup>CD8<sup>+</sup> T cells were treated with Abs (10  $\mu$ g/ml; as in *A*) and cultured in the absence or presence of IL-15 (6 ng/ml) for 24 h. Expression of Bcl-2 and Bcl-x<sub>L</sub> was determined by Western blotting.



was likely not due to reduced CD122 expression. Rather, the results suggest that the IL-15R  $\alpha$ -chain, although not essential for delivery of the antiapoptotic signal from IL-15 to CD8 cells, is required for their efficient rescue from death at low concentrations of IL-15.

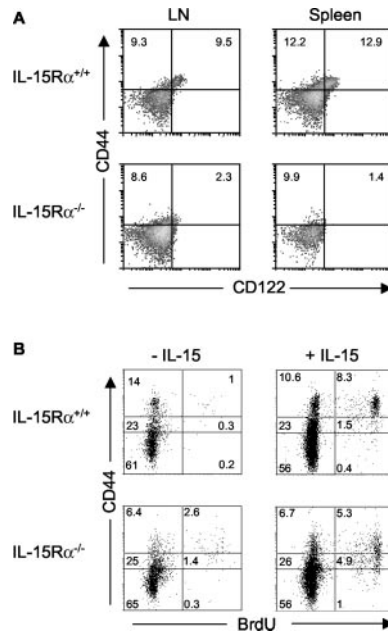
## Discussion

Previous studies have demonstrated that IL-15 is able to stimulate the proliferation of memory phenotype CD8<sup>+</sup> T cells and plays an important role in the maintenance of these cells in vivo. In this article, we have shown that IL-15 can also affect the life span of naive phenotype CD8<sup>+</sup> T cells. Although IL-15 did not stimulate overt activation or proliferation of CD44<sup>low</sup>CD8<sup>+</sup> cells, it inhibited the spontaneous apoptosis of these cells in vitro. Inhibition of apoptosis by IL-15 also applied to CD44<sup>low</sup>CD8<sup>+</sup> T cells isolated from TCR-transgenic mice (on a RAG-deficient background), providing strong evidence that truly naive CD8 cells were responsive to IL-15. These results imply that IL-15 may also act as a survival factor for naive CD8 T cells in vivo and provide an explanation for the reduced numbers of naive phenotype CD8<sup>+</sup> cells found in IL-15<sup>-/-</sup> and IL-15R $\alpha$ <sup>-/-</sup> mice (14, 15).

In addition to promoting the survival of naive CD8 cells, IL-15 prevented apoptosis of CD44<sup>high</sup>CD8<sup>+</sup> T cells in vitro, confirming a recent report by Judge et al. (21). IL-15-stimulated protection from apoptosis was evident at the level of the mitochondrion, i.e., IL-15 treatment inhibited the loss of mitochondrial membrane po-

tential in CD8 cells in culture (as shown by DiOC6 staining). This finding suggested that Bcl-2 family proteins might be involved in the antiapoptotic pathway triggered by IL-15, since these molecules have been shown to target the permeability transition pore complex of the mitochondrial membrane (28). Consistent with this idea, promotion of survival by IL-15 was associated with increased expression of Bcl-2 in both CD44<sup>low</sup> and CD44<sup>high</sup> cells. Interestingly, IL-15 treatment stimulated up-regulation of Bcl-x<sub>L</sub> in CD44<sup>high</sup> but not in CD44<sup>low</sup> cells; this was evident at doses of IL-15 that induced proliferation of CD44<sup>high</sup> cells, but also at doses that did not. These data imply that the IL-15-triggered signals delivered to CD44<sup>low</sup> and CD44<sup>high</sup> cells differ, even at low concentrations of IL-15, and leave open the possibility that IL-15 may inhibit apoptosis of these cells by different mechanisms.

IL-15 inhibited apoptosis at much lower concentrations than required for inducing proliferation of CD44<sup>high</sup>CD8<sup>+</sup> T cells. Hence, IL-15 may have a dual role in the maintenance of memory phenotype CD8 cells, promoting cell survival without division unless a threshold concentration of IL-15 is reached, at which point memory phenotype CD8 cells also divide. This is consistent with the observation that the background proliferation of CD44<sup>high</sup>CD8<sup>+</sup> T cells in normal mice, although higher than that of the naive phenotype cells, is relatively slow. Thus, under resting conditions, memory phenotype CD8<sup>+</sup> T cells divide approximately once every 1–3 wk (3, 6, 36). This rate of cell division is greatly increased after injection of IL-15 or inducers of IL-15, with up to 80% of

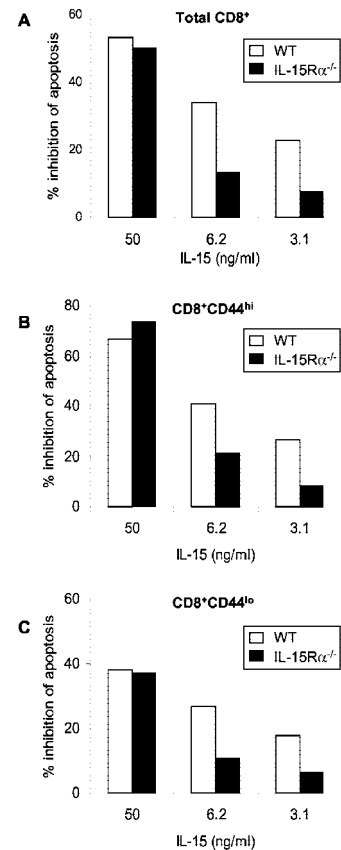


**FIGURE 6.** Neither IL-15R $\alpha$  nor high expression of CD122 is required for IL-15-induced proliferation of CD44<sup>high</sup>CD8<sup>+</sup> T cells. *A*, Expression of CD44 and CD122 on CD8<sup>+</sup> T cells from IL-15R $\alpha$ <sup>-/-</sup> mice (*lower panels*) vs WT controls (*upper panels*). *B*, Purified CD8<sup>+</sup> T cells (pooled spleen and LN) from IL-15R $\alpha$ <sup>-/-</sup> mice (*lower panels*) and WT controls (*upper panels*) were cultured for 36 h with or without added IL-15 (50 ng/ml) in the presence of BrdU. Regions on dot plots separate cells into BrdU positive or negative and CD44<sup>low</sup>, CD44<sup>int</sup>, or CD44<sup>high</sup>, while the numbers show the percentage of cells falling into each region.

CD44<sup>high</sup>CD8<sup>+</sup> T cells entering cell division in a 3-day time span (7, 37–39). Therefore, although direct measurements of IL-15 expression *in vivo* have yet to be reported, it can be speculated that basal expression of IL-15 is low in normal mice. These levels of IL-15 are adequate to promote survival, with memory phenotype CD8 cells only infrequently encountering microenvironments with sufficiently high concentrations of IL-15 to stimulate cell division. Upon injection of substances such as IFNs, poly(IC), or LPS, IL-15 expression by macrophages and dendritic cells (and perhaps other cells) increases (7, 40, 41), resulting in IL-15-dependent bystander proliferation of CD44<sup>high</sup>CD8<sup>+</sup> T cells (7, 21, 37–39).

Investigation into the role of IL-15R subunits in mediating the functional effects of IL-15 showed that expression of the IL-15R  $\alpha$ -chain was dispensable for IL-15-induced proliferation of memory phenotype CD8<sup>+</sup> T cells. Curiously, along with proliferation of CD44<sup>high</sup> cells, we detected substantial cell division among CD44<sup>int</sup>CD8<sup>+</sup> T cells from IL-15R $\alpha$ <sup>-/-</sup> but not WT mice in response to IL-15. Although the nature of the CD44<sup>int</sup> cells is unclear, it is interesting to note that naive CD8<sup>+</sup> T cells undergoing so-called “homeostatic” proliferation, which occurs in lymphopenic mice and is driven by contact with self-peptide-MHC complexes and IL-7, exhibit a CD44<sup>int</sup> phenotype (42). Furthermore, it has been shown that IL-15 can enhance the homeostatic proliferation of naive CD8 cells (43). Therefore, it is possible that the CD44<sup>int</sup>CD8<sup>+</sup> cells that proliferate in response to IL-15 *in vitro* represent the product of homeostatic proliferation; these cells may be more prominent in IL-15R $\alpha$ <sup>-/-</sup> mice because of their mild lymphopenia.

Expression of IL-15R $\alpha$  was also not essential for CD8 cells to respond to the antiapoptotic effects of IL-15. However, it was striking that the pro-survival effects of low concentrations of IL-15 were greatly reduced for IL-15R $\alpha$ <sup>-/-</sup> CD8 cells compared with



**FIGURE 7.** IL-15R $\alpha$  is not required for IL-15-mediated rescue from apoptosis but enhances the response to low concentrations of IL-15. Purified CD8<sup>+</sup> T cells from IL-15R $\alpha$ <sup>-/-</sup> mice and WT controls were cultured for 24 h in medium alone or in the indicated concentration of IL-15. Apoptosis among total (*A*), CD44<sup>high</sup> (*B*), and CD44<sup>low</sup> (*C*) CD8<sup>+</sup> cells was determined by annexin V staining and flow cytometry. Data are shown as percent inhibition of apoptosis, which was calculated as described in *Materials and Methods*.

controls. This result implies that the main role of the  $\alpha$ -chain is to increase the sensitivity of cells to IL-15, in keeping with the high affinity of this subunit for IL-15 ( $K_a$ ,  $10^{11}$ /M) (12). In fact, it was recently shown that IL-15 and IL-15R $\alpha$  can form stable complexes that persist on the surface of activated monocytes for >24 h and that IL-15 retained in this way could be presented *in trans* to CD8 T cells lacking IL-15R $\alpha$  expression (44). Whether such intercellular presentation occurs among purified CD8 cells is unclear. Nevertheless, a role for IL-15R $\alpha$  in enhancing the sensitivity of cells to IL-15 suggests that the main reason for the deficit in CD8 cells in IL-15R $\alpha$ <sup>-/-</sup> mice could be a failure of cells to survive in response to relatively low basal levels of IL-15. In this respect, it is notable that CD8<sup>+</sup> T cells in these mice have reduced expression of Bcl-2 (22). However, it is also possible that the threshold concentration of IL-15 required to induce proliferation of memory phenotype CD8 cells is increased in the absence of the  $\alpha$ -chain, leading also to a reduction in proliferative renewal (17).

Unlike IL-15R $\alpha$ , the  $\beta$ - and  $\gamma$ -chains of the IL-15R were required for induction of proliferation by IL-15. Furthermore, the data support the concept that there is a quantitative relationship between the extent of IL-15 binding to  $\beta\gamma$  and the amount of proliferation by CD44<sup>high</sup>CD8<sup>+</sup> T cells; this is suggested by three findings. First, both the expression of activation markers by CD44<sup>high</sup> cells and the magnitude of proliferation increased in a dose-dependent manner in response to IL-15. Second, decreased



proliferation of CD44<sup>high</sup>CD8<sup>+</sup> T cells was observed when low concentrations of anti-CD122 or anti-CD132 were added to cell cultures, indicating that a reduction in the number of available IL-15R sites diminished cell division. Third, the CD122<sup>low</sup>CD44<sup>high</sup>CD8<sup>+</sup> T cells from IL-15R $\alpha$ <sup>-/-</sup> mice exhibited reduced, yet significant IL-15-induced proliferation compared with WT cells; these data are in accordance with a recent report showing that CD122<sup>low</sup>CD44<sup>high</sup>CD8<sup>+</sup> cells in normal mice also proliferate less vigorously to IL-15 than CD122<sup>high</sup>CD44<sup>high</sup>CD8<sup>+</sup> cells (21). Significantly, however, the results also indicate that the failure of CD44<sup>low</sup>CD8<sup>+</sup> T cells to proliferate to IL-15 is not due to their relatively low expression of IL-15R $\beta$ , since CD44<sup>high</sup> cells expressing the same amount of this receptor chain divide in response to IL-15. A similar conclusion was reached by Gasser et al. (45), who generated transgenic mice expressing a chimeric receptor possessing the intracellular portion of the human IL-4R and the intracellular portion of the mouse CD122. The key finding in that study was that memory but not naive phenotype CD8<sup>+</sup> T cells proliferated in response to human IL-4 despite high expression of the chimeric receptor on both subsets. Taken together, these results imply that CD44<sup>high</sup>CD8<sup>+</sup> T cells possess characteristics independent of IL-15R expression that allow them to enter cell division upon exposure to IL-15; the same properties might account for their ability to up-regulate Bcl-x<sub>L</sub> in response to IL-15.

The  $\beta$ - and  $\gamma$ -chains of the IL-15R were also shown to play a key role in the delivery of an antiapoptotic signal to CD8 cells. This was clear from the fact that blocking both CD122 and CD132 abrogated completely the pro-survival effects of IL-15. Interestingly, however, a residual antiapoptotic effect remained when the  $\beta$ - or  $\gamma$ -chains were blocked individually, implying that functional IL-15 receptors lacking one or the other chain exist on the surface of CD8<sup>+</sup> T cells. Whether such receptors also include the IL-15R  $\alpha$ -chain is unclear, although it is worth noting that this  $\beta$ - or  $\gamma$ -chain-independent rescue was apparent at low concentrations of IL-15 (0.4 ng IL-15, data not shown). Therefore, given the importance of the  $\alpha$ -chain in responsiveness to low doses of IL-15, it seems likely that the receptors mediating the  $\beta$ - or  $\gamma$ -chain-independent response do include the  $\alpha$ -chain, i.e., are in the form of  $\alpha\beta$  or  $\alpha\gamma$ . This possibility then raises the question of how such receptors can deliver an antiapoptotic signal. In this respect, it has been reported that IL-15 can stimulate epithelial cells (46) or fibroblasts (47) lacking expression of CD122 or CD132, respectively. Furthermore, there is evidence that IL-15R $\alpha$  can deliver signals through association with TNFR-associated factor (TRAF) 2 in fibroblasts (47) and melanoma cells (48) or Syk in B cells (49). Significantly, it was shown that association of IL-15R $\alpha$  with TRAF2 contributed to IL-15-mediated inhibition of TNF- $\alpha$ -induced apoptosis in fibroblasts (47). It would therefore be of interest to determine whether IL-15R $\alpha$  associates with TRAF2 on CD8<sup>+</sup> T cells.

In conclusion, we have found that IL-15 can act as a pro-survival factor for naive CD8<sup>+</sup> T cells. This adds to an extensive body of literature providing evidence that IL-15 can inhibit the apoptosis of a wide range of cell types (19, 20, 22, 29–35, 50–59), although it is interesting to note that IL-15 does not rescue resting CD4<sup>+</sup> T cells from apoptosis (data not shown). It remains unclear to what extent IL-15 contributes to the maintenance of these cells in vivo, since IL-7 is considered to be the main cytokine required for survival of naive CD8<sup>+</sup> T cells; evidence supporting this idea has come from studies showing rapid disappearance of naive phenotype CD8<sup>+</sup> cells after adoptive transfer of IL-7R $\alpha$ <sup>-/-</sup> cells into normal mice or IL-7R $\alpha$ <sup>+/+</sup> cells into IL-7-deficient mice (43, 60). Nevertheless, it is worth noting that chronic treatment of thymectomized mice with anti-IL-7R Ab resulted in only a modest reduc-

tion in the long-term persistence of the naive phenotype CD8<sup>+</sup> T cells, implying that another factor(s) can support the survival of these cells (61). Furthermore, as stated above, naive phenotype CD8<sup>+</sup> T cell numbers are reduced in both IL-15<sup>-/-</sup> and IL-15R $\alpha$ <sup>-/-</sup> mice, supporting the idea that IL-15 is one such factor. Therefore, the accumulated data suggest that both IL-7 and IL-15 control naive CD8 T cell survival, with IL-7 playing the major role. This would be the reciprocal situation to what exists for memory phenotype CD8 cells, where IL-15 is the main survival factor but can be substituted for by high concentrations of IL-7 (62, 63).

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