

## T Cell-independent Interleukin 15R $\alpha$ Signals Are Required for Bystander Proliferation

James P. Lodolce, Patrick R. Burkett, David L. Boone, Marcia Chien, and Averil Ma

*Departments of Medicine and the Ben May Institute for Cancer Research, University of Chicago, Chicago, IL 60637*

### Abstract

Cytokine driven or “bystander” proliferation of T cells occurs in vivo independently of major histocompatibility complex–T cell receptor interactions. This process may be important for supporting T cell homeostasis and facilitating T cell responses to microbial antigens, and may involve the cytokine interleukin (IL)-15. In this study, we find that IL-15R $\alpha$ -deficient (IL-15R $\alpha$ <sup>-/-</sup>) mice fail to undergo poly I:C or IL-15 driven bystander proliferation of CD8<sup>+</sup> T cells. Surprisingly, IL-15R $\alpha$ <sup>-/-</sup> CD8<sup>+</sup> T cells proliferate in response to poly I:C when adoptively transferred into normal mice, and normal CD8<sup>+</sup> T cells fail to proliferate in IL-15R $\alpha$ <sup>-/-</sup> mice. Normal mice reconstituted with IL-15R $\alpha$ <sup>-/-</sup> bone marrow cells also fail to exhibit bystander responses. Thus, CD8<sup>+</sup> T cell independent IL-15R $\alpha$  signals from radiation sensitive hematopoietic cells are likely required for bystander responses. Moreover, normal CD8<sup>+</sup> T cells proliferate in IL-15R $\alpha$ <sup>-/-</sup> mice after treatment with IL-15. Therefore, IL-15R $\alpha$  signals may mediate a positive feedback loop involving the further physiological production of IL-15. These findings provide new insights into how IL-15R $\alpha$  supports memory phenotype CD8<sup>+</sup> T cell proliferation, and suggest novel mechanisms by which memory CD8<sup>+</sup> T cells are maintained in vivo.

Key words: IL-15 • lymphoid homeostasis • memory • CD8<sup>+</sup> T lymphocyte • poly I:C

### Introduction

Immune responses to microbial pathogens involve coordinated responses of innate and adaptive immune cells (1, 2). These responses are initiated when conserved microbial motifs (i.e., bacterial cell wall components or viral nucleic acids) are recognized by stromal and innate immune cells via pattern recognition receptors. Such stimuli lead to the elaboration of type I (IFN- $\alpha/\beta$ ) and type II (IFN- $\gamma$ ) interferons, IL-12, IL-15, and IL-18. These cytokines activate other innate immune cells, and are also able to stimulate adaptive T and B lymphocytes. Prior studies have demonstrated that such cytokine driven, or “bystander” T cell stimulation can occur in the absence of MHC-antigen recognition by TCR (3). Innate immune cytokines can also induce T cell death independently of TCR stimulation in a process referred to as “bystander suppression” (4). In these ways, innate immune cytokines can regulate adaptive immune responses.

The viral RNA mimic, polyinosine:cytosine (poly I:C), selectively induces proliferation of CD8<sup>+</sup> T cells expressing

high levels of activation markers such as CD44 (3), suggesting that bystander stimuli support preexisting memory CD8<sup>+</sup> T cells and/or recently activated CD8<sup>+</sup> T cells. Bystander activation of memory T cells may be an important mechanism for supporting reactivation and/or proliferation of these cells, as their persistence appears to be independent of TCR–MHC interactions (5, 6). Bystander activation of recently activated T cells may provide early amplification of adaptive immune responses (7). The net affect of bystander stimuli in either context may be to facilitate the selection and proliferation of T cells bearing appropriate T cell receptors and to support their differentiation into effector cells.

The precise sequence of events leading from microbial recognition by pattern receptors to T cell proliferation has not been well defined. Interferons are rapidly secreted by multiple cell types in response to poly I:C (8). Interferons do not directly stimulate T cell proliferation in vitro, but they lead to the production of IL-15, a cytokine which resembles IL-2 in its receptor binding capacity and its ability to directly support T cell proliferation in vitro (9–11). Macrophages synthesize IL-15 mRNA in response to poly I:C and LPS in vitro, and heterologous IL-15 induces proliferation of CD8<sup>+</sup> T cells in mice (12–14). These studies

Address correspondence to Averil Ma, Departments of Medicine and the Ben May Institute for Cancer Research, University of Chicago, MC6084, 5841 S. Maryland Ave., Chicago, IL 60637. Phone: 773-834-0687; Fax: 773-702-2281; E-mail: ama@medicine.bsd.uchicago.edu

suggest that poly I:C and/or interferons induce IL-15 secretion, which in turn stimulates CD8<sup>+</sup> T cell proliferation. However, IL-15 protein levels have been elusive to detect in vivo, and complicated posttranscriptional and posttranslational mechanisms regulate its activity (15). Deficiency of memory phenotype CD8<sup>+</sup> T cells in IL-15 receptor  $\alpha$  (IL-15R $\alpha$ ) and IL-15-deficient mice indicate that endogenous IL-15 signals are essential for homeostatic maintenance of these cells (16, 17), but it is unknown whether this homeostatic defect is related to bystander activation signals. Therefore, the role of endogenous IL-15 in bystander activation is not established.

IL-15 binds with high affinity to the IL-15R $\alpha$  chain of the IL-15 receptor, which also consists of the IL-2R $\beta$  and common  $\gamma$  chains used by the IL-2 receptor (18). Given the similarity of in vitro responses of T cells to IL-2 and IL-15, IL-15 binding to IL-15R $\alpha$  likely leads to recruitment of IL-2R $\beta$  and common  $\gamma$  chain, and initiation of signaling cascades from these shared chains (19). Alternatively, IL-15 may bind to IL-2R $\beta$ /common  $\gamma$  chain dimeric complexes and induce signaling in the absence of IL-15R $\alpha$ . It has also been suggested that IL-15R $\alpha$  may directly transduce signals via its 37 residue cytoplasmic domain (20). Finally, IL-15 may initiate signals by binding to an additional IL-15 receptor, IL-15RX (21). Hence, the question of which IL-15 receptors are essential for bystander responses remains unresolved.

Understanding the roles of IL-15 and IL-15R $\alpha$  in mediating bystander T cell activation is complicated by the pleiotropic expression of both IL-15 and IL-15R $\alpha$  by multiple hematopoietic and nonhematopoietic cell types (10, 22). The absence of IL-15 signals results in deficiencies in several innate immune cell populations as well as CD8<sup>+</sup>CD44<sup>Hi</sup> T cells in both IL-15R $\alpha$  (IL-15R $\alpha$ <sup>-/-</sup>) and IL-15-deficient (IL-15<sup>-/-</sup>) mice (16, 17). Thus, IL-15 signals through IL-15R $\alpha$  receptors play nonredundant and essential roles in the development and/or homeostasis of multiple cell lineages. Accordingly, while the paucity of memory phenotype CD8<sup>+</sup> T cells in IL-15R $\alpha$ <sup>-/-</sup> mice may suggest a critical role for IL-15R $\alpha$  signals on T cells for supporting homeostatic maintenance of CD8<sup>+</sup>CD44<sup>Hi</sup> T cells, it is also possible that these CD8<sup>+</sup> T cell defects are due to defective IL-15R signals on innate immune cells or even nonhematopoietic stromal cells. To investigate the role of IL-15R $\alpha$  signals in bystander T cell responses, we have studied poly I:C responses in IL-15R $\alpha$ <sup>-/-</sup> mice.

## Materials and Methods

**Mice.** The generation and preliminary characterization of IL-15R $\alpha$ <sup>-/-</sup> mice were described previously (16). All mice were housed in specific pathogen free facilities according to University of Chicago IACUC guidelines. IL-15R $\alpha$ <sup>-/-</sup> mice used for adoptive transfer experiments were backcrossed to C57BL/6J mice (The Jackson Laboratory) for five generations. C57BL/6J Ly-5.2<sup>+</sup> congenic mice were purchased from The Jackson Laboratory. For poly I:C responses, mice were injected intraperitoneally with either 150  $\mu$ l PBS, or 150  $\mu$ g poly I:C (Amersham Pharma-

cia Biotech). For proliferation studies, mice were fed 0.8 mg/ml 5-bromo-2'-deoxyuridine (BrdU; Sigma-Aldrich) in drinking water, changed daily, and proliferation responses were measured 4 d after poly I:C injection. For IL-15 responses, mice were injected with either 150  $\mu$ l PBS, or 2 or 10  $\mu$ g recombinant human IL-15 (R&D Systems). For adoptive transfer experiments, 5–10  $\times$  10<sup>6</sup> purified CD8<sup>+</sup> T cells were transferred intravenously into the lateral tail vein. CD8<sup>+</sup> T cells were purified by depleting freshly isolated axillary, posterior, inguinal, and mesenteric peripheral lymph node cells (PLNs) of CD4<sup>+</sup> T cells and B cells by magnetic depletion, using purified anti-mouse-CD4 (BD PharMingen) and anti-mouse-Ig magnetic beads (Dyna). NK cells were depleted from normal mice by injection of 200  $\mu$ g of anti-mouse NK1.1 (azide free, clone PK136; BD PharMingen) at days -2 at +1 relative to poly I:C injection. For bone marrow reconstitution experiments, femur bone marrow cells were depleted of mature T cells using anti-Thy1.2 magnetic beads (Dyna) and transferred into tail veins of lethally irradiated (950 rads) C57BL/6J/SJL (Ly5.2<sup>+</sup>) congenic mice.

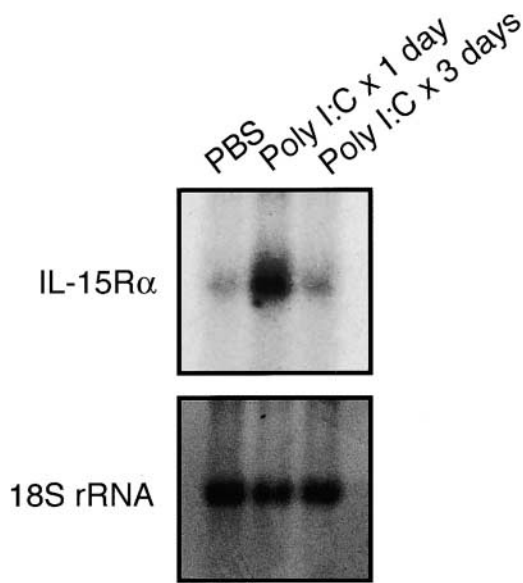
**Flow Cytometric Analysis.** Single cell suspensions of lymphocytes were prepared from PLNs, mesenteric lymph node (MLN), or spleens as described previously (16). Detection of lymphocyte surface antigens was performed using biotin and fluorochrome conjugated monoclonal antibodies specific for murine CD4, CD8, CD44, CD69, Ly-6C, Ly-5.1, or Ly-5.2 proteins. Biotinylated antibodies were detected using fluorochrome-conjugated streptavidin (SA). Cells were analyzed with a FACSCalibur<sup>TM</sup> flow cytometer equipped with CELLQuest<sup>TM</sup> software (Becton Dickinson). Intracellular BrdU incorporation was detected using a BrdU Flow Kit according to the manufacturer's instructions (BD PharMingen).

**RNA Analyses.** RNA was extracted using Trizol reagent according to the manufacturer's protocol (GIBCO BRL). For Northern analysis of IL-15R $\alpha$  mRNA expression, 10  $\mu$ g total RNA from each sample was separated on 1% agarose gels, transferred to nylon membranes, and hybridized with a <sup>32</sup>P-labeled probe representing the coding region of the IL-15R $\alpha$  cDNA, as described previously (16). RNase protection assays were performed with a Riboquant kit according to manufacturer's instructions (BD PharMingen), using 20  $\mu$ g of total RNA from the indicated tissues and probes mCK1 and mCK2B.

## Results and Discussion

**IL-15R $\alpha$  mRNA Is Induced during Poly I:C Bystander Responses In Vivo.** IL-15 binds to the IL-15R $\alpha$  chain with high affinity (18) and no other cytokines are known to bind to IL-15R $\alpha$ . Thus, IL-15R $\alpha$  expression levels should reflect the capacity of cells to respond to IL-15 in vivo. To investigate the role of IL-15R $\alpha$  signals in mediating bystander proliferation responses, we injected poly I:C into normal mice and measured the expression of IL-15R $\alpha$ -specific mRNA on purified CD8<sup>+</sup> T cells from these mice. CD8<sup>+</sup> T cells from poly I:C-treated mice expressed markedly more IL-15R $\alpha$  mRNA 24 h after poly I:C treatment, and returned to baseline levels by 72 h (Fig. 1). Thus, IL-15R $\alpha$  may be particularly important for mediating early IL-15 signals during poly I:C bystander responses.

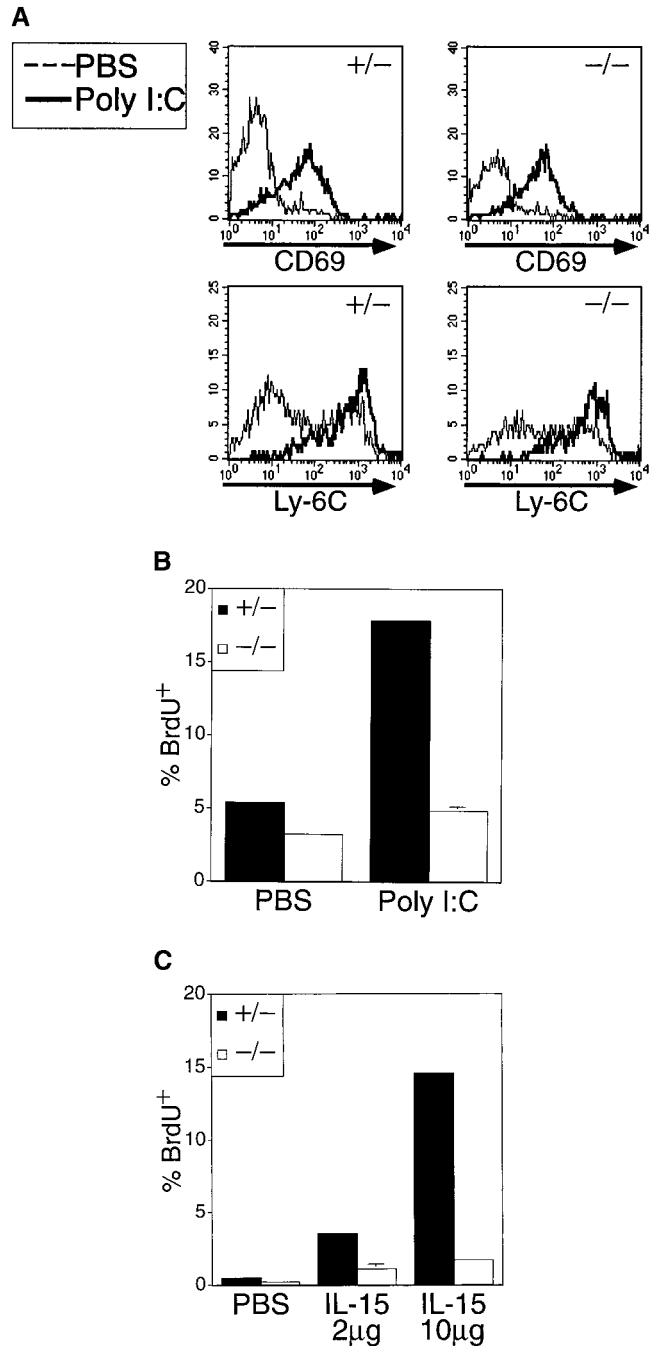
**IL-15R $\alpha$ <sup>-/-</sup> CD8<sup>+</sup> T Cells Fail to Proliferate in Response to Poly I:C.** As IL-15R $\alpha$  mRNA is induced by poly I:C, we sought to examine the requirement for IL-15R $\alpha$ -



**Figure 1.** IL-15R $\alpha$  mRNA is selectively induced upon poly I:C treatment in normal mice. Northern analysis of IL-15R $\alpha$ -specific mRNA. RNA was made from purified CD8 $^{+}$  T cells pooled from the PLN, MLN, and spleen of B6 mice injected intraperitoneally with PBS or poly I:C (1 and 3 d after injection). The intensity of the ethidium stained 18S rRNA is shown for normalization of gel loading. Data are representative of three experiments.

mediated signals during poly I:C bystander responses. Accordingly, IL-15R $\alpha^{+/-}$  and IL-15R $\alpha^{-/-}$  mice were injected with poly I:C and fed BrdU containing water for 4 d. Flow cytometric analysis of splenic (data not shown) and lymph node lymphocytes harvested after poly I:C treatment revealed that CD8 $^{+}$  T cells in both IL-15R $\alpha^{+/-}$  and IL-15R $\alpha^{-/-}$  mice acquired the early surface activation markers CD69 and Ly-6C within 24 h after poly I:C injection, indicating that both IL-15R $\alpha^{+/-}$  and IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells receive an activation signal in response to poly I:C (Fig. 2 A). As has been previously observed, most proliferating CD8 $^{+}$  T cells expressed high levels of the activation marker CD44 (reference 3, and data not shown). While significant induction of CD8 $^{+}$  T cell proliferation was observed in poly I:C-treated IL-15R $\alpha^{+/-}$  mice, only a minimal increase in BrdU $^{+}$  CD8 $^{+}$  T cells was observed in poly I:C-treated IL-15R $\alpha^{-/-}$  mice (Fig. 2 B). In addition, no significant difference in the number of annexin-positive cells was identified in IL-15R $\alpha^{-/-}$  versus IL-15R $\alpha^{+/-}$  mice (data not shown). Thus, IL-15R $\alpha$  appears to be required for poly I:C-induced proliferation but not for the induction of early T cell activation markers. These findings are consistent with the idea that poly I:C-induced secretion of cytokines such as IFN $\alpha/\beta$  leads to T cell activation independently of IL-15R $\alpha$  signals, but that proliferative stimuli to these cells depends on IL-15R $\alpha$  and is not compensated for by other common  $\gamma$  chain receptors in vivo.

Poly I:C-induced bystander responses are associated with the elaboration of several cytokines, including IL-15. As IL-15 can induce CD8 $^{+}$  T cell proliferation in vivo and in vitro



**Figure 2.** IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells fail to proliferate in response to poly I:C or IL-15 in vivo. (A) Activation of IL-15R $\alpha^{+/-}$  CD8 $^{+}$  T cells (left histograms) or IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells (right histograms) in response to poly I:C. 1 d after intraperitoneal injection with poly I:C, PLNs were analyzed for acute activation using antibodies against CD69 or Ly-6C. Events displayed in histograms are gated on the CD8 $^{+}$  T cells in PBS (dashed line) versus poly I:C (solid line) treated mice. Data are representative of one mouse out of five mice analyzed for each genotype. (B) Proliferation of IL-15R $\alpha^{+/-}$  CD8 $^{+}$  T cells (black bars) or IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells (white bars) in response to poly I:C. “% BrdU $^{+}$ ” values are calculated as the percentage of total CD8 $^{+}$  T cells which have incorporated BrdU. Data are one representative experiment of five performed. (C) Proliferation of IL-15R $\alpha^{+/-}$  CD8 $^{+}$  T cells (black bars) or IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells (white bars) in response to IL-15. Data are one representative experiment of three performed.

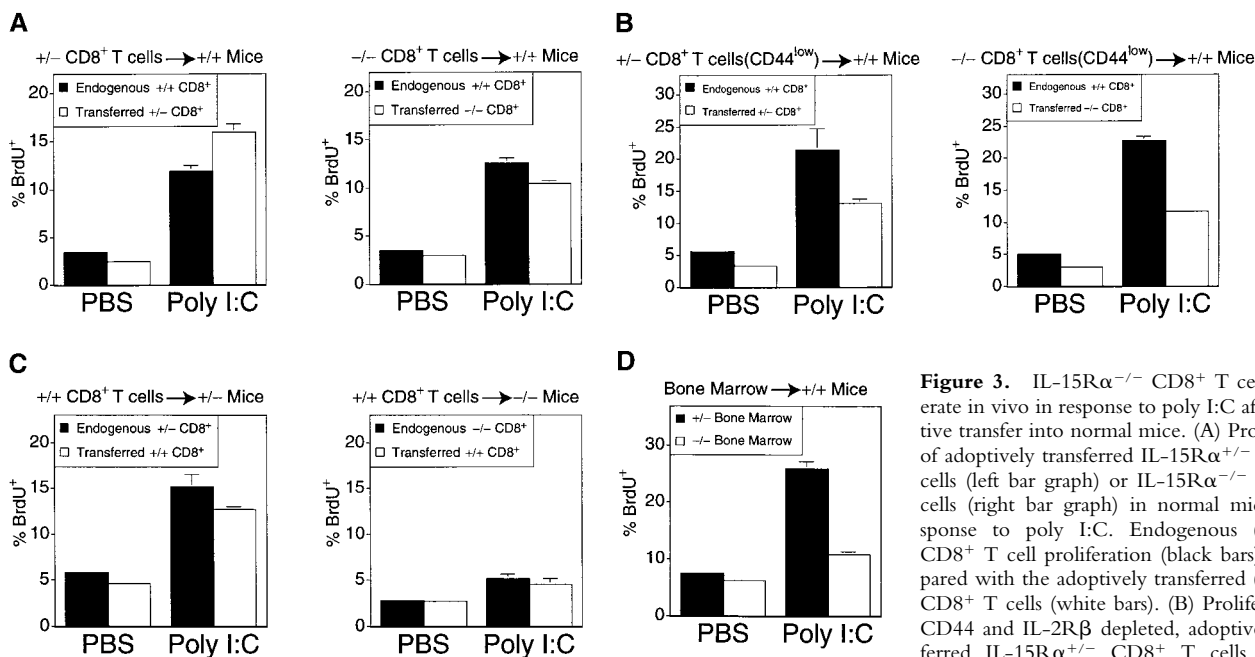
(12), we sought to determine the response of IL-15R $\alpha^{-/-}$  mice to IL-15. Injection of purified IL-15 into normal mice induced the selective proliferation of CD8 $^{+}$  T cells. By contrast, IL-15 induced only minimal CD8 $^{+}$  T cell proliferation in IL-15R $\alpha^{-/-}$  mice (Fig. 2 C). Taken together, these data suggest that both poly I:C and IL-15-induced bystander proliferation signals are delivered largely through IL-15 receptors containing IL-15R $\alpha$ , most likely heterotrimeric IL-15R $\alpha$ /IL-2R $\beta$ /common  $\gamma$  chain complexes.

**IL-15R $\alpha^{-/-}$  T Cells Proliferate in Response to Poly I:C in a Normal Host.** As IL-15R $\alpha$  is expressed in multiple cell types, we sought to determine whether IL-15R $\alpha$  signals on CD8 $^{+}$  T cells (versus other cells) were essential for mediating poly I:C-induced bystander T cell responses. Accordingly, we purified CD8 $^{+}$  T cells from either IL-15R $\alpha^{+/+}$  or IL-15R $\alpha^{-/-}$  mice that were backcrossed five generations onto a C57BL/6J (Ly-5.1) background, and adoptively transferred these cells into Ly-5.2 C57BL/6J mice. Recipient mice were then treated with poly I:C, and fed BrdU water as described previously. Analysis of Ly-5.1 $^{+}$  donor lymphocytes 4 d after poly I:C treatment revealed that IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells were now able to proliferate in response to the same dose of poly I:C that failed to induce proliferation of IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells in intact IL-15R $\alpha^{-/-}$  mice (Fig. 3 A). Donor IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells from the same pool failed to proliferate significantly when transferred into PBS-injected normal mice, indicating that IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells proliferated in response

to poly I:C (Fig. 3 A). Thus, IL-15R $\alpha$  signals on T cells do not appear to be essential for bystander responses.

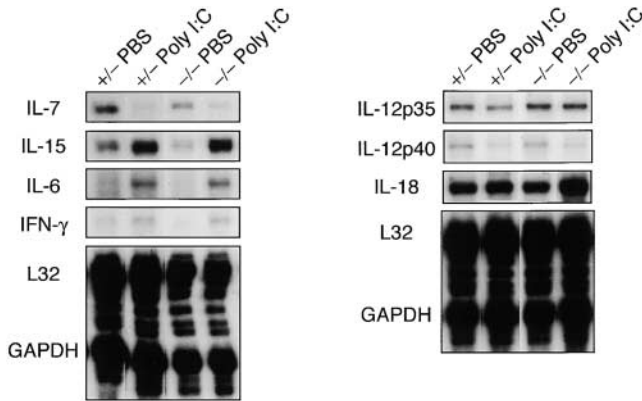
The percentage of donor IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells that proliferated in response to poly I:C was lower than that of donor IL-15R $\alpha^{+/+}$  CD8 $^{+}$  T cells (Fig. 3 A). This difference could be due to the reduced number of memory phenotype CD44 $^{Hi}$ CD8 $^{+}$  donor T cells present in IL-15R $\alpha^{-/-}$  mice (16). Depletion of CD44 $^{Hi}$ CD8 $^{+}$  donor T cells with anti-CD44 and anti-IL-2R $\beta$  antibodies before transfer reduced the proliferative response of both IL-15R $\alpha^{-/-}$  and normal donor cells to comparable levels (Fig. 3 B). Thus, IL-15R $\alpha$  signals on CD44 $^{Lo}$ CD8 $^{+}$  T cells do not appear to support bystander responses. It remains possible that IL-15R $\alpha$  signals on CD44 $^{Hi}$ CD8 $^{+}$  T cells support bystander responses. Nevertheless, the significant proliferation of IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells in normal mice demonstrates that CD8 $^{+}$  T cell-independent IL-15R $\alpha$  signals are essential for mediating poly I:C bystander responses.

The ability of IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells to proliferate in a normal host treated with poly I:C suggests that factors other than IL-15 may also directly stimulate CD8 $^{+}$  T cell proliferation, perhaps in conjunction with IL-15. It is also possible that IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells proliferate in normal mice partly due to IL-15 binding to IL-2R $\beta$ /common  $\gamma$  receptor complexes on T cells. Indeed, we have observed that IL-15R $\alpha^{-/-}$  T cells can proliferate in response to high doses of IL-15 in vitro (data not shown). Conversely, the similar phenotypes of unperturbed IL-15R $\alpha^{-/-}$  and IL-15 $^{-/-}$



**Figure 3.** IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells proliferate in vivo in response to poly I:C after adoptive transfer into normal mice. (A) Proliferation of adoptively transferred IL-15R $\alpha^{+/+}$  CD8 $^{+}$  T cells (left bar graph) or IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells (right bar graph) in normal mice in response to poly I:C. Endogenous (Ly-5.2 $^{+}$ ) CD8 $^{+}$  T cell proliferation (black bars) is compared with the adoptively transferred (Ly-5.1 $^{+}$ ) CD8 $^{+}$  T cells (white bars). (B) Proliferation of CD44 and IL-2R $\beta$  depleted, adoptively transferred IL-15R $\alpha^{+/+}$  CD8 $^{+}$  T cells (left bar graph) or IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells (right bar

graph) in normal mice in response to poly I:C. Endogenous (Ly-5.2 $^{+}$ ) CD8 $^{+}$  T cell proliferation (black bars) is compared with the adoptively transferred (Ly-5.1 $^{+}$ ) CD8 $^{+}$  T cells (white bars). (C) Proliferation of adoptively transferred normal CD8 $^{+}$  T cells in IL-15R $\alpha^{+/+}$  (left bar graph) or IL-15R $\alpha^{-/-}$  (right bar graph) mice in response to poly I:C. Endogenous (Ly-5.1 $^{+}$ ) CD8 $^{+}$  T cell proliferation (black bars) is compared with the adoptively transferred (Ly-5.2 $^{+}$ ) CD8 $^{+}$  T cells (white bars). (D) Bystander proliferation response of lethally irradiated normal mice reconstituted with bone marrow cells from IL-15R $\alpha^{+/+}$  or IL-15R $\alpha^{-/-}$  mice. Proliferation of IL-15R $\alpha^{+/+}$  donor CD8 $^{+}$  T cells (black bars) is compared with IL-15R $\alpha^{-/-}$  donor CD8 $^{+}$  T cells (white bars). The "% BrdU $^{+}$ " values were calculated as the percentage of total CD8 $^{+}$  T cells that have incorporated BrdU. Data are representative of three experiments. The total number of total CD8 $^{+}$  T cells recovered was similar in all adoptive transfer experiments.



**Figure 4.** Comparable cytokine mRNA expression levels from spleens of poly I:C-treated IL-15R $\alpha^{+/+}$  or IL-15R $\alpha^{-/-}$  mice. RNase protection analysis of cytokine mRNA expression levels from poly I:C-treated spleens. Data are representative of mRNA from at least three sets of mice.

mice (16, 17), and the high binding affinity of IL-15R $\alpha$  alone for IL-15 (18) suggest that most physiological IL-15 signals are transduced by IL-15R $\alpha$  bearing receptors in vivo.

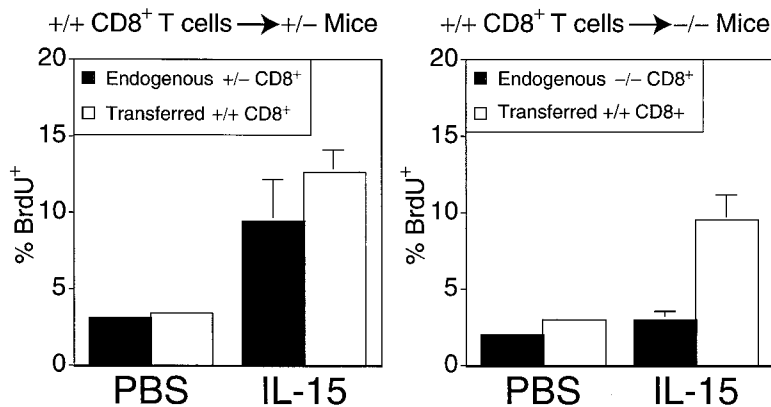
Significant poly I:C-induced proliferation of IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells in normal mice also raises the novel concept that the failure of IL-15R $\alpha^{-/-}$  CD8 $^{+}$  T cells to proliferate in intact IL-15R $\alpha^{-/-}$  mice may largely be due to defects in cells other than CD8 $^{+}$  T cells. Given the expression of IL-15R $\alpha$  in multiple hematopoietic and nonhematopoietic cells, this requirement for IL-15R $\alpha$  signals may involve many cell lineages. For example, IL-15 can induce CD40 expression on macrophages, and may synergize with IL-12 to induce IFN- $\gamma$  secretion from NK cells (23). As IL-15R $\alpha^{-/-}$  mice exhibit developmental deficiencies in multiple innate immune cell types, it is also possible that the absence of any of these cell types may explain the inability of IL-15R $\alpha^{-/-}$  mice to support bystander responses (16). Thus, there are several mechanisms by which CD8 $^{+}$  T cell independent IL-15R $\alpha$  signals may mediate bystander responses.

*Normal T Cells Fail to Proliferate in Response to Poly I:C in IL-15R $\alpha^{-/-}$  Mice.* To further examine whether CD8 $^{+}$  T cell-independent IL-15R $\alpha$  signals are essential for poly I:C

bystander responses, purified CD8 $^{+}$  T cells from normal mice were adoptively transferred into either IL-15R $\alpha^{+/+}$  or IL-15R $\alpha^{-/-}$  mice that were subsequently injected with poly I:C. Examination of the proliferation of donor CD8 $^{+}$  T cells revealed that these cells proliferated comparably with host CD8 $^{+}$  T cells in IL-15R $\alpha^{+/+}$  mice (Fig. 3 C). However, donor CD8 $^{+}$  T cells from the same pool failed to proliferate in IL-15R $\alpha^{-/-}$  mice after poly I:C treatment. The Ly-5.2 $^{+}$  donor cells in IL-15R $\alpha^{-/-}$  mice expressed increased levels of the Ly-6C activation marker after poly I:C injection, indicating that they had been activated by an innate immune stimulus (perhaps from IFN- $\alpha/\beta$ ; data not shown). However, despite the transmission of such activation signals to the normal donor Ly-5.2 $^{+}$  CD8 $^{+}$  T cells, these cells failed to receive sufficient stimuli to proliferate. This finding confirms that IL-15R $\alpha$  supports poly I:C responses through T cell independent mechanisms.

*Radiation-sensitive Hematopoietic Cells May Be Essential for Poly I:C-induced Bystander Activation of CD8 $^{+}$  T Cells.* Given the evidence above that T cell-independent IL-15R $\alpha$  signals may mediate critical steps in poly I:C responses, we tested whether hematopoietic versus nonhematopoietic cells might mediate such signals. Bone marrow cells from either IL-15R $\alpha^{+/+}$  or IL-15R $\alpha^{-/-}$  mice were depleted of T cells and transferred into lethally irradiated congenic Ly-5.2 $^{+}$  hosts. After 8 wk, poly I:C responses were tested in these mice, and these studies revealed that mice reconstituted with IL-15R $\alpha^{-/-}$  cells exhibited deficient poly I:C responses when compared with mice reconstituted with IL-15R $\alpha^{+/+}$  cells (Fig. 3 D). These experiments argue against a critical role for radiation-resistant stromal cells in mediating IL-15R $\alpha$ -dependent bystander response signals. Rather, it is likely that radiation-sensitive, bone marrow-derived hematopoietic cells contribute these essential IL-15R $\alpha$  signals.

*Neither NK nor NKT Cells Are Essential for Poly I:C-induced Bystander Activation of CD8 $^{+}$  T Cells.* As IL-15R $\alpha^{-/-}$  mice are deficient in both NK and NKT cells (16), we tested the possibility that these hematopoietic cell lineages might contribute essential signals during poly I:C responses in vivo. For example, NK cells can elaborate IFN- $\gamma$ , a factor that may contribute to bystander T cell pro-



**Figure 5.** Normal CD8 $^{+}$  T cells proliferate in vivo in response to IL-15 after adoptive transfer into IL-15R $\alpha^{-/-}$  mice. Proliferation of adoptively transferred normal CD8 $^{+}$  T cells in IL-15R $\alpha^{+/+}$  (left bar graph) or IL-15R $\alpha^{-/-}$  (right bar graph) mice in response to IL-15. 4 d after intraperitoneal injection with 10  $\mu$ g IL-15, CD8 $^{+}$  T cells from PLNs were analyzed for incorporation of BrdU. Endogenous (Ly-5.1 $^{+}$ ) CD8 $^{+}$  T cell proliferation (black bars) is compared with the adoptively transferred (Ly-5.2 $^{+}$ ) CD8 $^{+}$  T cells (white bars). In this experiment one PBS-injected mouse and three IL-15-injected mice for each genotype were analyzed.

liferation (24); and NKT cells can elaborate both IL-4 and IFN- $\gamma$  in response to stimuli such as  $\alpha$ -GalCer (25). We thus investigated the role of these cells in poly I:C responses by depleting normal mice of NK1.1<sup>+</sup> cells with anti-NK1.1 antibody. After elimination of >90% of NK1.1<sup>+</sup> and DX5<sup>+</sup> cells from normal mice, poly I:C treatment caused 21% of CD8<sup>+</sup> T cells to proliferate, compared with 16% in nondepleted mice. Moreover, poly I:C induced CD8<sup>+</sup> T cell proliferation was readily observed in CD1-deficient mice, which lack CD1-restricted NK T cells (26) (data not shown). Thus, neither NK nor NKT cells appear to be required for poly I:C-induced bystander proliferation.

*Normal T Cells Proliferate in Response to IL-15 in IL-15R $\alpha$ <sup>-/-</sup> Mice.* Innate immune hematopoietic cells elaborate multiple cytokines that can support T cell activation and proliferation, including IL-2, IL-4, IL-7, IL-12, IL-15, IL-18, and others (27). To determine whether IL-15R $\alpha$  might support bystander T cell proliferation by inducing the elaboration of such cytokines, we studied their mRNA expression levels in spleens from normal and IL-15R $\alpha$ <sup>-/-</sup> mice 16 h after poly I:C treatment. These assays revealed that mRNA for IL-6, IL-15, and IFN- $\gamma$  were clearly induced by poly I:C treatment, while others were unchanged (IL-12, IL-18), reduced (IL-7), or not detected at significant levels (IL-2 and IL-4; Fig. 4). No cytokine mRNA was differentially expressed between spleens of normal and IL-15R $\alpha$ <sup>-/-</sup> mice. However, an important caveat to this result is that complicated posttranslational regulation of IL-15 could cause bioavailable IL-15 levels to diverge from steady-state mRNA levels (15).

As IL-15 has the capacity to directly stimulate CD8<sup>+</sup> T cell proliferation, one possible explanation for the inability of normal CD8<sup>+</sup> T cells to proliferate in response to poly I:C when transferred into IL-15R $\alpha$ <sup>-/-</sup> mice could be that IL-15 production or secretion is compromised in these mice. We investigated this possibility by testing whether normal CD8<sup>+</sup> T cells would undergo bystander proliferation in IL-15R $\alpha$ <sup>-/-</sup> mice in response to heterologous IL-15. CD8<sup>+</sup> T cells were purified from normal mice and transferred into IL-15R $\alpha$ <sup>+/-</sup> and IL-15R $\alpha$ <sup>-/-</sup> mice as before, except that recipient mice were injected with recombinant IL-15 instead of poly I:C. Measurement of BrdU incorporation into donor cells revealed that these cells proliferated comparably in IL-15R $\alpha$ <sup>+/-</sup> and IL-15R $\alpha$ <sup>-/-</sup> mice (Fig. 5). Endogenous IL-15R $\alpha$ <sup>-/-</sup> CD8<sup>+</sup> T cells in the same mice failed to proliferate in response to IL-15, indicating that this dose of IL-15 does not directly mediate CD8<sup>+</sup> T cell proliferation through IL-2R $\beta$ /common  $\gamma$  receptors on CD8<sup>+</sup> T cells. Thus, bystander proliferation of normal CD8<sup>+</sup> T cells in IL-15R $\alpha$ <sup>-/-</sup> mice can be stimulated by exogenous IL-15, and this response is restricted to T cells bearing IL-15R $\alpha$ . This finding suggests that deficient IL-15 production or secretion may partly explain the inability of T cells to respond to poly I:C in IL-15R $\alpha$ <sup>-/-</sup> mice.

Taken together, our studies establish that IL-15R $\alpha$  is induced on T cells during bystander responses to poly I:C and that these responses require IL-15R $\alpha$ . While some of our findings are consistent with the idea that IL-15 is the fi-

nal cytokine in an innate immune cytokine cascade which directly stimulates CD8<sup>+</sup> T cell proliferation (12), the bulk of our data demonstrate that CD8<sup>+</sup> T cell-independent IL-15R $\alpha$  signals are critical for mediating poly I:C bystander responses. As normal T cells proliferate in response to IL-15 but not poly I:C after adoptive transfer into IL-15R $\alpha$ <sup>-/-</sup> mice, insufficient IL-15 production may be a consequence of IL-15R $\alpha$  deficiency. Thus, while multiple IL-15R $\alpha$ -dependent pathways may regulate bystander proliferation, one intriguing possibility is that a novel positive feedback loop may exist by which IL-15R $\alpha$  signals might lead to further IL-15 production during bystander responses. This putative feedback loop, might involve macrophages and/or dendritic cells that produce and respond to IL-15 (12, 23).

Our studies have revealed essential novel roles for IL-15R $\alpha$  signals during bystander T cell responses, and these functions may be critical for supporting memory T cells. IL-15 is expressed constitutively by multiple nonlymphoid tissues which preferentially house memory T cells (28, 29), and IL-15 may support memory T cell survival and/or proliferation in vivo (30). Thus, our discovery that T cell-independent hematopoietic IL-15R $\alpha$  signals are critical for poly I:C-induced bystander responses may suggest that similar mechanisms could support the homeostasis of memory T cells. Further studies with IL-15R $\alpha$ <sup>-/-</sup> cells and mice will likely reveal novel mechanisms by which IL-15 supports T cell proliferation, homeostasis, and memory.

We thank Barbara Malynn for critically reading the manuscript. We thank Chyung-Ru Wang for CD1-deficient mice. We thank Suneeta Fernandes for excellent technical assistance.

This work was supported by grants from the National Institutes of Health (AI45860, DK52751) and the Crohn's Colitis Foundation of America (CCFA) (A. Ma), Digestive Diseases Research Center (DDRC) DK42086, training grants GM07183 (J.P. Lodolce) and GM07281 (P.R. Burkett), the Mr. and Mrs. Arthur Edelstein FACS<sup>®</sup> facility, and the Martin Boyer Laboratories. A. Ma is a Cancer Research Institute Scholar.

Submitted: 29 May 2001

Revised: 30 July 2001

Accepted: 27 August 2001

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