Differential Responsiveness of Innate-like IL-17– and IFN-γ–Producing γδ T Cells to Homeostatic Cytokines

Theresa M. Corpuz, Jessica Stolp, Hee-Ok Kim, Gabriela V. Pinget, Daniel H. D. Gray, Jae-Ho Cho, Jonathan Sprent and Kylie E. Webster

J Immunol 2016; 196:645-654; Prepublished online 16 December 2015;
doi: 10.4049/jimmunol.1502082
http://www.jimmunol.org/content/196/2/645

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Differential Responsiveness of Innate-like IL-17– and IFN-γ–Producing γδ T Cells to Homeostatic Cytokines

Theresa M. Corpuz,* Jessica Stolp,* Hee-Ok Kim,† Gabriela V. Pinget,* Daniel H. D. Gray,‡,§ Jae-Ho Cho,† Jonathan Sprent,*,¶,[1] and Kylie E. Webster*,¶,[1]

γδ T cells respond to molecules upregulated following infection or cellular stress using both TCR and non-TCR molecules. The importance of innate signals versus TCR ligation varies greatly. Both innate-like IL-17–producing γδ T (γδT-17) and IFN-γ–producing γδ T (γδT-IFNγ) subsets tune the sensitivity of their TCR following thymic development, allowing robust responses to inflammatory cytokines in the periphery. The remaining conventional γδ T cells retain high TCR responsiveness. We determined homeostatic mechanisms that govern these various subsets in the peripheral lymphoid tissues. We found that, although innate-like γδT-17 and γδT-IFNγ cells share elements of thymic development, they diverge when it comes to homeostasis. Both exhibit acute sensitivity to cytokines compared with conventional γδ T cells, but they do not monopolize the same cytokine. γδT-17 cells rely exclusively on IL-7 for turnover and survival, aligning them with NKT17 cells; IL-7 ligation triggers proliferation, as well as promotes survival, upregulating Bcl-2 and Bcl-x_L. γδT-IFNγ cells instead depend heavily on IL-15. They display traits analogous to memory $CD8^+$ T cells and upregulate Bcl-x_L and Mcl-1 upon cytokine stimulation. The conventional γδ T cells display low sensitivity to cytokine-alone stimulation and favor IL-7 for their turnover, characteristics reminiscent of naive αβ T cells, suggesting that they may also require tonic TCR signaling for population maintenance. These survival constraints suggest that γδ T cell subsets do not directly compete with each other for cytokines, but instead fall into resource niches with other functionally similar lymphocytes. *The Journal of Immunology*, **2016, 196: 645–654.**

The thymus produces two distinct lineages of T cells: the larger population expresses a αβ TCR, whereas the smaller population express a γδ TCR. Although αβ T cells dominate Ag-specific effector and memory stages, γδ T cells have fused adaptive and innate-like qualities to be at the forefront of immune responses. In this capacity, γδ T cells can directly kill infected cells, produce molecules required for pathogen clearance, and release immunomodulatory cytokines (1).

Becoming active imminently after pathogen encounter requires unique properties not inherent to naive αβ T cells. Generally, γδ T cells respond to molecules upregulated or released following infection or cellular stress. This response can be elicited by ligation of their TCR or non-TCR molecules. The part played by the TCR appears to be diverse, with some γδ T cells recognizing the inducible MHC-like molecules T22 and T10, ligands that do not present Ag (2, 3), whereas others respond to host- and microbe-derived phosphorylated prenyl metabolites (phosphoantigens) (4, 5), microbial protein Ags (6), or glycolipid Ags presented by nonclassical MHC molecules (7). However, the role of non-TCR molecules appears to be equally diverse, with the binding of pathogen pattern recognition receptors and cytokine receptors triggering robust responses (8, 9).

Rather than all γδ T cells having the potential to be stimulated equally via both TCR and non-TCR molecules, it appears that there are γδ T cells for which innate stimuli are more important than TCR ligation. IL-17–producing γδ T (γδT-17) cells are one such subset. Although they require a TCR signal for development in the thymus, this is then attenuated to a state of TCR hyporesponsiveness, thus allowing their peripheral activity to be directed primarily by innate signals, such as IL-1β and IL-23 (9, 10). Likewise, an innate-like IFN-γ–producing γδ T (γδT-IFNγ) cell subset was recently described (10). Delineated by CD45RB, these cells produce IFN-γ in response to IL-18 and IL-12 (10). Although there seems to be a role for the TCR in the activation of these innate-like cells, they clearly differ from the remaining conventional γδ T cells that display a phenotype more similar to naive αβ T cells (6, 10). These γδ T cells appear to comprise cells with TCR specificities as mentioned above (e.g., to the algae protein PE) (6). Interestingly, they still preserve the capacity to respond rapidly, not requiring clonal expansion to produce cytokine after Ag encounter.

For αβ T cells, the requirement for TCR specificity normally acts as a "safety switch," preventing inappropriate activation to other inflammatory stimuli. For innate-like T cells, relegation of this checkpoint suggests the existence of other mechanisms to prevent unwanted inflammation. Although it was recently found

*Immunology Division, Garvan Institute of Medical Research, Darlinghurst, New South Wales 2010, Australia; †Academy of Immunology and Microbiology, Institute for Basic Science, Pohang 790-784, Republic of Korea; ‡Molecular Genetics of Cancer Division and Immunology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia; §Department of Medical Biology, University of Melbourne, Parkville, Victoria 3010, Australia; and ¶St. Vincent's Clinical School, University of New South Wales, Sydney, New South Wales 2010, Australia

[1]J. Sprent and K.E.W. are joint senior authors.

Received for publication September 22, 2015. Accepted for publication November 17, 2015.

This work was supported by National Health and Medical Research Council (Australia) Grants 1045647 and 1045630 (to K.E.W.), 1011388 (to J.-H.C.), 1016953 (to J. Sprent), and 1078763 and 1049724 (to D.H.D.G.); National Health and Medical Research Council (Australia) Fellowship 1078243 (to J. Sprent) and Career Development Fellowship-2 (to D.H.D.G.); and the Institute for Basic Science (Republic of Korea) Grant IBS-R005-G1 (to J.-H.C.).

Address correspondence and reprint requests to Dr. Kylie Webster, Immunology Division, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia. E-mail address: k.webster@garvan.org.au

Abbreviations used in this article: APM, Ag-presenting molecule; BH3-only, Bcl-2–homology domain 3 only; B6.Ly5.1, B6.SJL-$Ptprc^aPepc^b$/BoyJ; CTV, CellTrace Violet; LIP, lymphopenia-induced proliferation; pLN, peripheral lymph node; γδT-17, IL-17–producing γδ T; γδT-IFNγ, IFN-γ–producing γδ T; WT, wild-type.

that the inhibitory receptor BTLA plays a key role in restraining cytokine production by γδT-17 cells (11), a complete understanding of how γδ T cells maintain homeostasis and the capacity for rapid response is still lacking.

Homeostasis of γδ T cells, in general, was shown to be dependent on a combination of the cytokines IL-15 and IL-7 (12, 13). However, these studies predate the delineation of the innate-like versus conventional subsets of γδ T cells, and it would be surprising if their mechanisms of survival were uniform. IL-7 treatment was found to enrich for IL-17–producing CD27$^-$ γδ T cells in in vitro cultures and in the lymph nodes of mice (14). However, it is not clear whether they can respond to IL-15 in the absence of IL-7 or what combination of homeostatic cytokines supports the other subsets.

In this study, we find that, although innate-like γδT-17 and γδT-IFNγ cells share elements of thymic development, they differ greatly when it comes to homeostasis in the periphery. Both exhibit acute sensitivity to cytokines compared with the remaining conventional γδ T cells but do not monopolize the same cytokine. γδT-17 cells rely exclusively on IL-7 for turnover and survival, with IL-7 ligation upregulating Bcl-2 and Bcl-x_L but not Mcl-1. γδT-IFNγ cells instead depend heavily on IL-15, with the ability to also use IL-7 if it is in excess. Cytokine stimulation results in an upregulation of Bcl-x_L and Mcl-1. The conventional γδ T cells display much lower sensitivity to cytokines and instead exhibit characteristics reminiscent of naive αβ T cells. These survival constraints suggest that γδ T cell subsets do not directly compete with each other for cytokines, but instead fall into resource niches with other functionally similar lymphocytes.

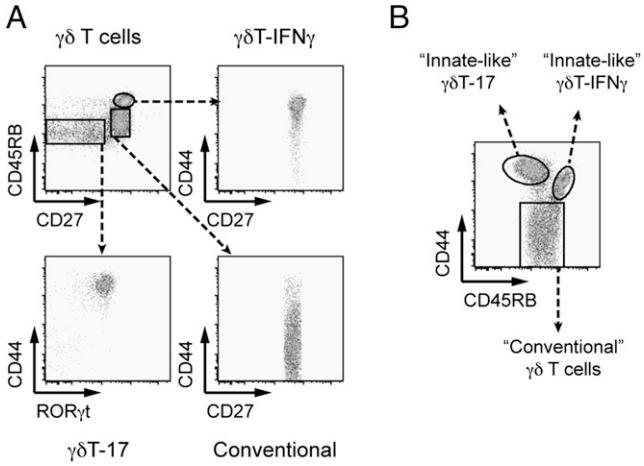

FIGURE 1. Innate-like γδT-17 and γδT-IFNγ cells and conventional γδ T cells are defined by CD44, CD45RB, CD27, and RORγt. (**A**) pLN γδ T cells (CD3$^+$γδTCR$^+$), subdivided by CD45RB and CD27 by flow cytometry, result in two CD27$^+$ populations (CD44^{hi} [γδT-IFNγ] and CD44^{lo} [conventional]) and one CD27$^-$ population, which is RORγt$^+$ and CD44^{hi} (γδT-17). (**B**) pLN γδ T cells (CD3$^+$γδTCR$^+$), subdivided by CD45RB and CD44 by flow cytometry, also consist of the three subsets, innate-like γδT-17 and γδT-IFNγ cells and conventional γδ T cells. Data are representative of three experiments, with two mice per experiment.

Materials and Methods

Mice

C57BL/6 and B6.SJL-Ptprc^{a}Pepc^b/BoyJ (B6.Ly5.1) mice were obtained from the Animal Resources Centre (Perth, Australia) and the Australian Bioresources Centre (Moss Vale, Australia). IL-7$^{-/-}$ and IL-15$^{-/-}$ colonies were maintained at the Australian Bioresources Centre. Mice were used at 6–12 wk of age. Animals were housed under conventional barrier protection and handled in accordance with the Garvan Institute of Medical Research and St Vincent's Hospital Animal Experimentation and Ethics Committee, which comply with the Australian code of practice for the care and use of animals for scientific purposes.

Flow cytometry

Single-cell suspensions were prepared from spleens, peripheral lymph nodes (pLNs) (inguinal, axillary, brachial, cervical), and thymus by mechanical disruption. Lymphocytes from single-cell suspensions of perfused livers were separated by a 33% isotonic Percoll density gradient (GE Healthcare). Cell suspensions were stained for FACS analysis using the following Abs (from eBioscience unless stated otherwise): anti-CD122 (TM-β1, BD Biosciences), anti-CD27 (LG.7F9), anti-CD45RB (C363.16A), anti-CD3 (17A2), anti-CD3 (145-2C11, BD Biosciences), anti-Ki67 (SolA15), anti-CD127 (A7R34), anti–ROR-γ(t) (B2D), anti–IFN-γ (XMG1.2), anti-CD45RB (C363.16A), anti-CD44 (IM7), anti–IL-17A (eBio17B7), anti-CD45.1 (A20), anti–γδ-TCR (GL3), anti–Bcl-2 (BCL/10C4,BioLegend), anti–Bcl-xL (54H6), and anti-rabbit IgG–Alexa Fluor 647 (Cell Signaling Technology). The isotype controls used were rat IgG2a and IgG2b, mouseIgG1, κ isotype control (MOPC-21, BioLegend) and rabbit (DA1E) IgG isotype control (Cell Signaling Technology). Anti-mouse Mcl-1 (clone 19C4.15) was a kind gift from Dr. David Huang (The Walter and Eliza Hall Institute). The Ab was conjugated to Alexa Fluor 647, according to the manufacturer's instructions (Invitrogen). Samples were analyzed on a FACSCanto II or sorted using a FACSAria (BD Biosciences). Data were analyzed using FlowJo software (TreeStar).

Isolation of γδ T cells

Single-cell suspensions of spleen and pLNs were negatively depleted by staining with anti-B220–biotin, anti-CD8–biotin, and anti-CD4–biotin, followed by incubation with Dynabeads Biotin Binder (Invitrogen) and magnetic separation. The depleted fraction was stained with anti-γδTCR and anti-CD3 and, in some cases, with anti-CD27 and anti-CD44 as well. Cell sorting was performed on a FACSAria (BD Biosciences) to obtain a pure population of γδ T cells or γδ T cell subsets.

Cytokine/mAb complexes

Recombinant mouse IL-7 or IL-15 (PeproTech) was mixed with anti–IL-7 mAb (M25; Bio X Cell) or IL-15 Rα/Fc Chimera (R&D Systems) and incubated at 37°C for 30 min, as described (15). Mice were injected i.p. with 1 μg cytokine plus 5 μg mAb on three consecutive days.

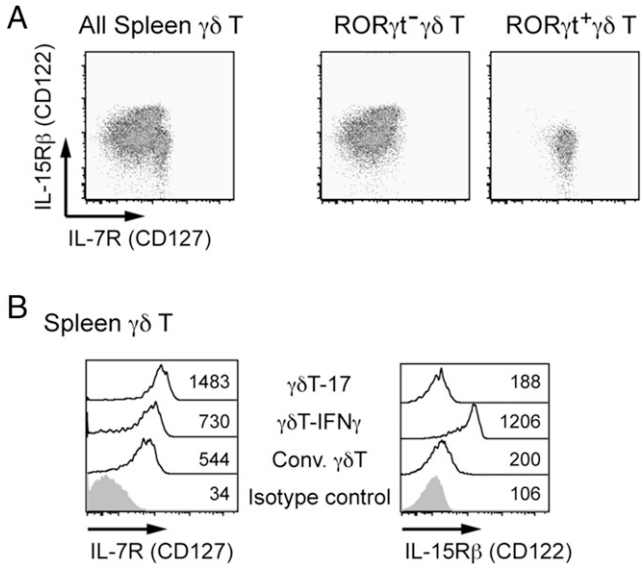

FIGURE 2. Innate-like and conventional γδ T subsets differentially express IL-7R and IL-15Rβ. (**A**) Expression of IL-7R (CD127) and IL-15Rβ (CD122) on spleen γδ T cells (CD3$^+$γδTCR$^+$) (*left panel*) and on gated RORγt$^-$ and RORγt$^+$ γδ T cells (*middle* and *right panels*), as shown by representative flow cytometry plots. (**B**) Expression of IL-7R (CD127) (*left panel*) and IL-15Rβ (CD122) (*right panel*) on spleen γδ T cells, gated into γδT-17 (RORγt$^+$), γδT-IFNγ (RORγt$^-$CD45RB^{hi}CD44^{hi}), and conventional γδ T (RORγt$^-$CD45RB^{lo}CD44^{lo}) cell subsets by flow cytometry. Numbers within the graphs are mean fluorescence intensity. Data are representative of four experiments, with two mice per experiment.

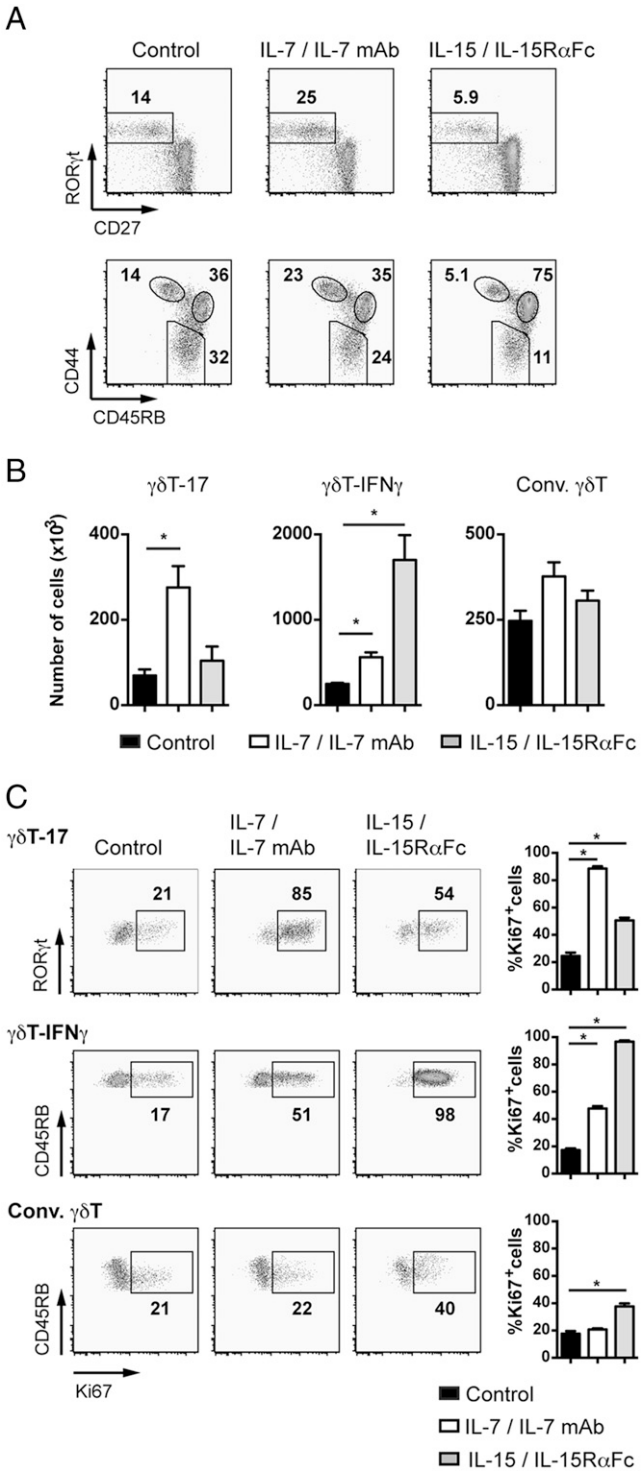

FIGURE 3. IL-7 and IL-15 preferentially expand γδT-17 and γδT-IFNγ cells, respectively. Mice were injected i.p. with control PBS, IL-7/IL-7 mAb (M25), or IL-15/IL-15RαFc (1 μg/5 μg) on days 0, 1, and 2 and analyzed on day 5. (**A**) Proportion of RORγt$^+$ γδ T cells (CD3$^+$γδTCR$^+$) (*upper panels*) and proportion of each of the three γδ T cell subsets, as subdivided by CD45RB and CD44 (*lower panels*) in the spleen of treated mice. (**B**) Absolute number of γδ T cell subsets (γδT-17 [RORγt$^+$], γδT-IFNγ [RORγt$^-$CD45RB^{hi}CD44^{hi}], and conventional γδ T [RORγt$^-$CD45RB^{lo}CD44^{lo}]) in the spleen of treated mice. (**C**) Proportion of Ki67$^+$ cells in each of the γδ T cell subsets (γδT-17 [RORγt$^+$], γδT-IFNγ [RORγt$^-$CD45RB^{hi}CD44^{hi}], and conventional γδ T [RORγt$^-$CD45RB^{lo} CD44^{lo}]) in the spleen of treated mice, shown as representative flow cytometry plots (*left panels*) and a bar graph of the average percentage of Ki67$^+$ cells (*far right panel*). Data are representative of two or three experiments with two mice per treatment per experiment. *$p \leq 0.05$, Mann–Whitney *U* test.

Cytokine measurement

For intracellular cytokine staining, spleen or pLN single-cell suspensions were incubated in 24-well plates at 37°C in complete RPMI 1640 medium with PMA (10 ng/ml) and ionomycin (1 μg/ml) for 4 h with monensin. Cells were surface stained, treated with Cytofix/Cytoperm, according to the manufacturer's protocol (BD Biosciences), and stained with IL-17 and IFN-γ.

CFSE and CellTrace Violet labeling

Lymphocytes were washed once in 0.1% BSA in PBS and labeled with 5 μM CFSE or 10 μM CellTrace Violet (CTV; both from Molecular Probes) at a density of 1×10^7 cells/ml 0.1% BSA in PBS for 10 min at 37°C (CFSE) or 20 min at 22°C (CTV). The reaction was stopped with RPMI 1640 10% FCS and washed twice.

Adoptive cell transfers

B6.Ly5.1$^+$ γδ T cells or γδ T cell subsets (CD27$^+$CD44^{hi} and CD27$^+$ CD44^{lo}) were purified from pooled spleens and pLNs by cell sorting, labeled with CFSE or CTV, and injected i.v. into C57BL/6, IL-15$^{-/-}$, or IL-7$^{-/-}$ recipient mice that were sublethally irradiated (550 rad) 1 d before cell transfer. For analysis of homeostatic expansion, spleens and livers were harvested on day 7, and the B6.Ly5.1$^+$ donor cells were analyzed using flow cytometry.

Statistical analysis

Results are presented as mean ± SEM. Statistical significance was assessed using the Mann–Whitney *U* test, one-way ANOVA, or two-way ANOVA with the Tukey multiple-comparison test (Prism; GraphPad), as detailed in the figure legends.

Results

CD45RB and CD44 delineate innate-like and conventional γδ T cell subsets

The TNFR family member CD27 has been used to differentiate IL-17– and IFN-γ–producing γδ T cells (16). γδT-17 cells are CD27$^-$ and express the transcription factor RORγt. γδT-IFNγ cells reside within the CD27$^+$ subset and can be delineated using CD45RB (10). Reflecting their similarities in thymic development, these two innate-like populations exit the thymus in a preactivated state, expressing high levels of CD44 (Fig. 1). The remaining conventional CD27$^+$ γδ T cells express low levels of CD44 and CD45RB. As such, CD44 and CD45RB, along with CD27 and RORγt, can be used to subdivide lymph node and spleen γδ T cells into two innate-like populations (γδT-17 and γδT-IFNγ) and one conventional CD44^{lo}CD45RB^{lo} population (Fig. 1).

Receptors for homeostatic cytokines segregate with innate-like and conventional γδ T cell subsets

Considering the shared characteristics of the innate-like γδ T cell subsets, we questioned whether they would also share dependence on the same homeostatic cytokines for population maintenance. To answer this question, we first analyzed the density of cell surface cytokine receptors. Upon examination of the receptors for IL-7 (CD127) and IL-15 (CD122, Rβ), we found that γδ T cells neatly divide into three populations, as observed by flow cytometry (Fig. 2A). RORγt$^+$ γδT-17 cells express the highest levels of IL-7R and very low levels of IL-15Rβ (Fig. 2), which is in agreement with previous reports (14, 17, 18). Interestingly, all three subsets express IL-7R to some degree, but only the innate-like γδT-IFNγ cells express significant levels of IL-15Rβ (Fig. 2B).

Cytokine-driven proliferation of innate-like γδ T cells exceeds that of conventional γδ T cells

To test whether these receptor levels directly conferred enhanced sensitivity to their respective cytokines, we treated mice with high-dose IL-7 or IL-15. The combination of IL-7 and anti–IL-7 mAb was found to enrich for RORγt$^+$ NKT cells (19), and in this study we observed a corresponding enrichment of RORγt$^+$ γδT-17 cells (Fig. 3A, 3B). This is in agreement with studies that found that addition of exogenous IL-7 to lymph node cultures or to mice enriched for CD27$^-$ γδT-17 cells (14). Conversely, treatment with IL-15/IL-15RαFc favored γδT-IFNγ (CD45RB^{hi}) cells (Fig. 3A, 3B). Such increases were a direct result of increased proliferation, as measured by Ki67 (Fig. 3C). Interestingly, although IL-7 and IL-15 induced proliferation in >80% of innate-like γδT-17 and γδT-IFNγ cells, respectively, moderate proliferation of each subset (~50%) was observed with the other homeostatic cytokine, suggesting that more than one cytokine may support their homeostasis. Although the conventional γδ T (CD44^{lo}) population exhibited similar baseline proliferation to the innate-like subsets, it did not proliferate extensively to either cytokine, with only a mild response to high-dose IL-15 observed.

Innate-like γδ T cell subsets exhibit faster lymphopenia-induced proliferation than conventional γδ T cells

Upon transfer into sublethally irradiated host animals, naive CD44^{lo} αβ T cells slowly proliferate and gradually acquire memory cell characteristics (20). This lymphopenia-induced proliferation (LIP) is driven by increased availability of homeostatic cytokines, especially IL-7, plus contact with self-peptide+MHC ligands. We found that conventional γδ T cells exhibited slower LIP than innate-like γδT-17 and γδT-IFNγ subsets, both of which had undergone extensive proliferation 1 wk after transfer (Fig. 4). This was particularly evident in the spleen but also was noted in the liver, where the conventional γδ T cell subset display increased proliferation. The slower proliferation of conventional γδ T cells compared with the innate-like subsets suggests that these cells, which retain TCR responsiveness after thymic export (6, 10), may align more with naive αβ T cells in their requirements for homeostatic maintenance. The rapid proliferation of γδT-17 and γδT-IFNγ cells is more reminiscent of memory CD8$^+$ T cells; turnover of these cells is MHC independent and is moderate in normal hosts but enhanced in irradiated hosts, reflecting the increased levels of γc cytokines under lymphopenic conditions (20).

IL-15 is dispensable for γδT-17 cells but obligatory for γδT-IFNγ cells

Considering the acute sensitivity to IL-7 and IL-15 displayed by innate-like γδ T cells, we next wished to examine these cells in cytokine-deficient mice. However, because IL-7R signaling is necessary for TCRγ locus accessibility in thymocytes and, thus, mature γδ T cells, we could not directly analyze IL-7–deficient animals (21, 22). γδ T cells are present in IL-15–deficient mice, but cytokine production is notably skewed away from IFN-γ and toward IL-17 (Fig. 5A), reflecting an almost complete loss of the innate-like γδT-IFNγ cells (Fig. 5B–D). In contrast, RORγt$^+$ γδT-17 cells are not adversely affected by the loss of IL-15 and even increase in number, particularly in the spleen (Fig. 5C). We also observed reduced numbers of the γδT-IFNγ subset in the thymus of IL-15$^{-/-}$ mice, suggesting that IL-15 plays a key role in their thymic development, as well as their postthymic homeostasis (Fig. 5E).

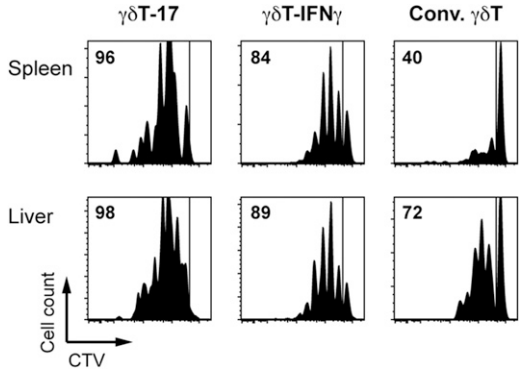

FIGURE 4. Innate-like γδ T cell subsets exhibit fast LIP. CTV profiles of congenically marked γδ T cells recovered from the spleen and livers on day 7 posttransfer into sublethally irradiated C57BL/6 mice. γδ T subsets were electronically gated from recovered cells into γδT-17 (CD27$^-$), γδT-IFNγ (CD27$^+$CD45RB^{hi}), and conventional γδ T (CD27$^+$CD45RB^{lo}) subsets and depicted by CTV cell divisions. Data are representative of two experiments with two mice per experiment.

Homeostasis of RORγt$^+$ γδT cells depends on IL-7 alone, whereas RORγt$^-$ γδ T cells use a mixture of IL-7 and IL-15

To explore homeostatic mechanisms without the confounding issue of thymic development, we examined LIP in cytokine-deficient mice. First, whole γδ T cells were purified by cell sorting, labeled with CFSE, and adoptively transferred into sublethally irradiated wild-type (WT), IL-7$^{-/-}$, and IL-15$^{-/-}$ recipients. RORγt$^+$ γδT-17 cells proliferated rapidly in both WT and IL-15$^{-/-}$ mice, confirming their dependence on IL-7 and indifference to IL-15 (Fig. 6A). In contrast, these cells were very difficult to detect in IL-7$^{-/-}$ hosts. The cells did not undergo significant proliferation and, compared with WT hosts, only 10% of cells were recovered (Fig. 6B). These data suggest that γδT-17 cells depend wholly on IL-7 for their homeostatic proliferation and survival, and although they can proliferate to excess IL-15 (Fig. 3C), baseline levels of IL-15 are insufficient to support them in the absence of IL-7.

We then questioned whether the other innate-like population, γδT-IFNγ cells, also depends on a single cytokine for its homeostasis. The robust proliferation of γδT-IFNγ cells to IL-15/IL-15RαFc and their absence from IL-15$^{-/-}$ mice suggest that they may exclusively use this cytokine. We found that γδT-IFNγ cells proliferated equally well in IL-7$^{-/-}$ mice as in WT recipients, indicating little dependence on this cytokine (Fig. 6C, 6D). However, we observed only a minor reduction in the proliferation of γδT-IFNγ cells in IL-15–deficient animals, from an average of 89–69% in the spleen and 97–84% in the liver from experiments with sorted donor cells (Fig. 6D). This proliferation was due to IL-7, because it was completely abolished by Ab blockade of IL-7R in IL-15$^{-/-}$ mice (Fig. 6C). As such, although IL-15 is most likely the main determinant of γδT-IFNγ homeostasis, the moderate levels of IL-7R expressed by these cells allow them to take advantage of excess IL-7 when IL-15 is absent. This is demonstrated by both transfer into irradiated recipients, where competition for available cytokine is minimal, and by the response to high concentrations of IL-7 after IL-7/M25 injections (Fig. 3C).

Proliferation of the conventional γδ T cell subset was markedly reduced in the absence of IL-7 (revealing strong IL-7 dependency as with naive αβ T cells) but was also mildly reduced in the absence of IL-15 (Fig. 6C, 6D). Notably, proliferation was completely blocked in the absence of both cytokines (Fig. 6C), ruling out any contribution from other γc cytokines. As mentioned earlier (Fig. 4), the conventional γδ T cell subset alone displayed

decidedly increased LIP in the liver compared with the spleen (Fig. 6D), perhaps reflecting increased availability of IL-7 in the liver (23).

Expression of antiapoptotic molecules in innate-like and conventional γδ T subsets

Homeostatic cytokines stimulate both proliferation and enhanced survival of lymphocytes. How homeostatic cytokines transmit survival signals to γδ T cells is largely unexplored. We found that the three subsets of γδ T cells differed significantly in their expression of antiapoptotic molecules. Innate-like subsets γδT-17 and γδT-IFNγ expressed high basal levels of Bcl-x_L compared with conventional γδ T cells, whereas γδT-IFNγ cells were further distinguished by their very high Bcl-2 expression (Fig. 7). Reflecting their selective dependence on IL-7, γδT-17 cells showed enhanced expression of Bcl-2 and Bcl-x_L, but not Mcl-1, in response to IL-7 but were unresponsive to IL-15 (Fig. 8A). For γδT-IFNγ cells, exposure to either IL-15 or IL-7 caused increased expression of Bcl-x_L and Mcl-1, although little change in Bcl-2 was observed (Fig. 8B). Mcl-1 upregulation was unexpectedly higher with IL-15 than IL-7, consistent with the preferential dependence of γδT-IFNγ cells on IL-15. Conventional γδ T cells upregulated Bcl-2, and to a lesser extent Bcl-x_L, upon exposure to IL-7 and IL-15, consistent with responsiveness to both cytokines (Fig. 8C).

Discussion

γδ T cells are a minor lymphocyte population, yet they encompass great diversity in effector function. Although they all share the ability to respond quickly to stimuli without the need for clonal expansion, the stimuli to which they respond and the manner in which they respond vary greatly. The observation that γδ T cells can tune the sensitivity of their TCR following thymic export (10) revealed that a sliding scale of antigenic versus innate stimuli exists. Those for which innate signals play a dominant role can be subdivided into IL-17 and IFN-γ producers. We found that γδT-17 cells are completely reliant on IL-7 for their survival and turnover. IL-7 alone can upregulate antiapoptotic molecules Bcl-2 and Bcl-x_L,

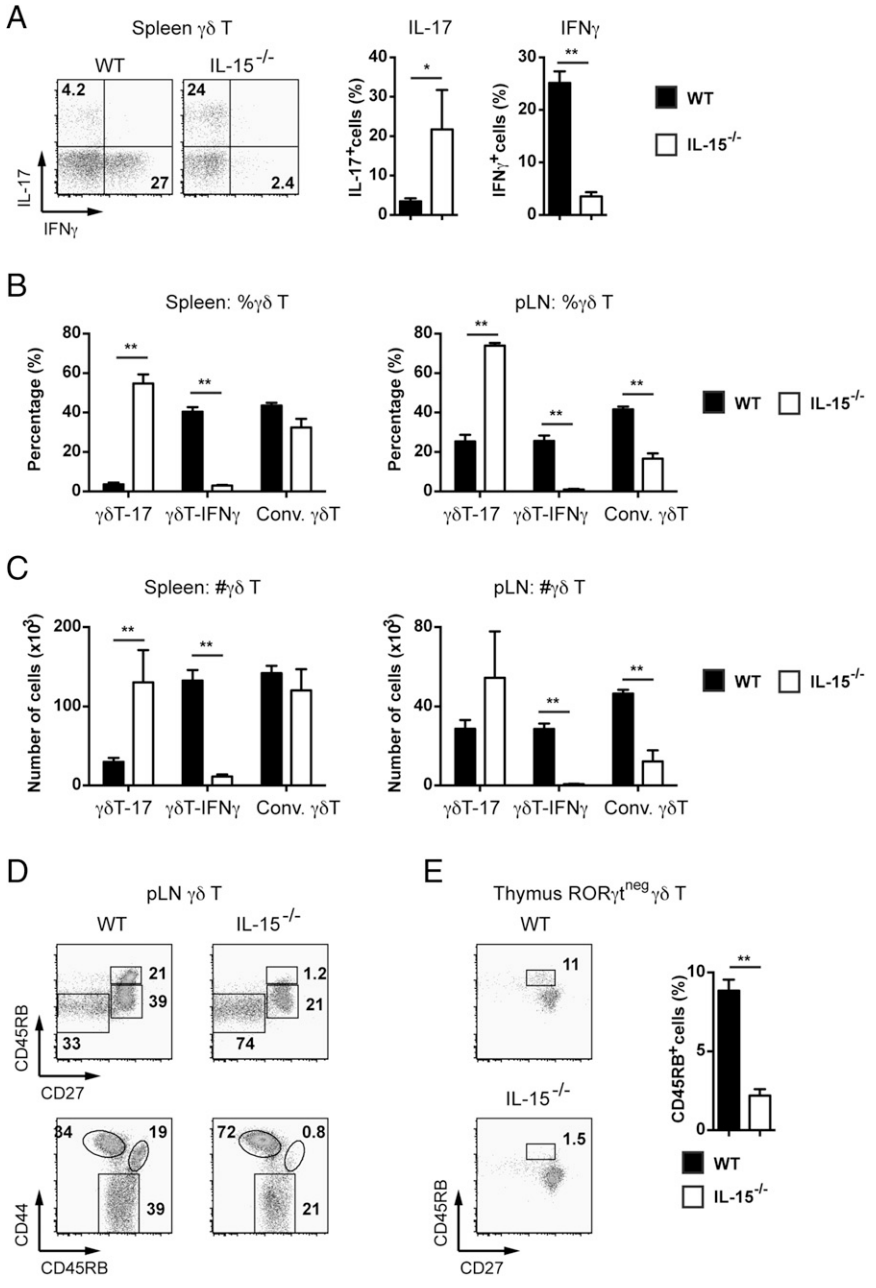

FIGURE 5. IL-15–deficient mice lack γδT-IFNγ cells. (**A**) Proportion of IL-17$^+$ and IFNγ$^+$ γδ T cells (CD3$^+$γδTCR$^+$) in the spleen of WT and IL-15$^{-/-}$ mice, as determined by intracellular cytokine staining. Data are presented as representative flow cytometry plots (*left panels*) and bar graphs of the average percentage of cytokine-producing cells (*middle* and *right panels*). (**B**) Proportion of γδ T cell subsets (γδT-17 [RORγt$^+$], γδT-IFNγ [RORγt$^-$CD45RB^{hi}CD44^{hi}], and conventional γδ T [RORγt$^-$CD45RB^{lo}CD44^{lo}]) of total γδ T cells in the spleen (*left panel*) and pLN (*right panel*) of WT and IL-15$^{-/-}$ mice. (**C**) Absolute number of γδ T cell subsets (γδT-17 [RORγt$^+$], γδT-IFNγ [RORγt$^-$CD45RB^{hi}CD44^{hi}], and conventional γδ T [RORγt$^-$CD45RB^{lo}CD44^{lo}]) in the spleen (*left panel*) and pLN (*right panel*) of WT and IL-15$^{-/-}$ mice. (**D**) Representative flow cytometry plots of the proportion of the three γδ T cell subsets, as subdivided by CD45RB and CD27 (*upper panels*) and CD45RB and CD44 (*lower panels*), in the pLN of WT and IL-15$^{-/-}$ mice. (**E**) Proportion of CD45RB$^+$ cells of RORγt$^-$ γδ T cells (CD3$^+$γδTCR$^+$) in the thymus of WT and IL-15$^{-/-}$ mice, depicted by flow cytometry dot plots (*left panels*) and a bar graph of the average percentage of CD45RB$^+$ cells (*right panel*). Flow cytometry data are representative of two or three independent experiments with two or three mice per group per experiment. Data in (A)–(C) and (E) are mean ± SEM for WT ($n = 5$) and IL-15$^{-/-}$ ($n = 5$) mice. $^*p \leq 0.05$, $^{**}p \leq 0.01$, Mann–Whitney U test.

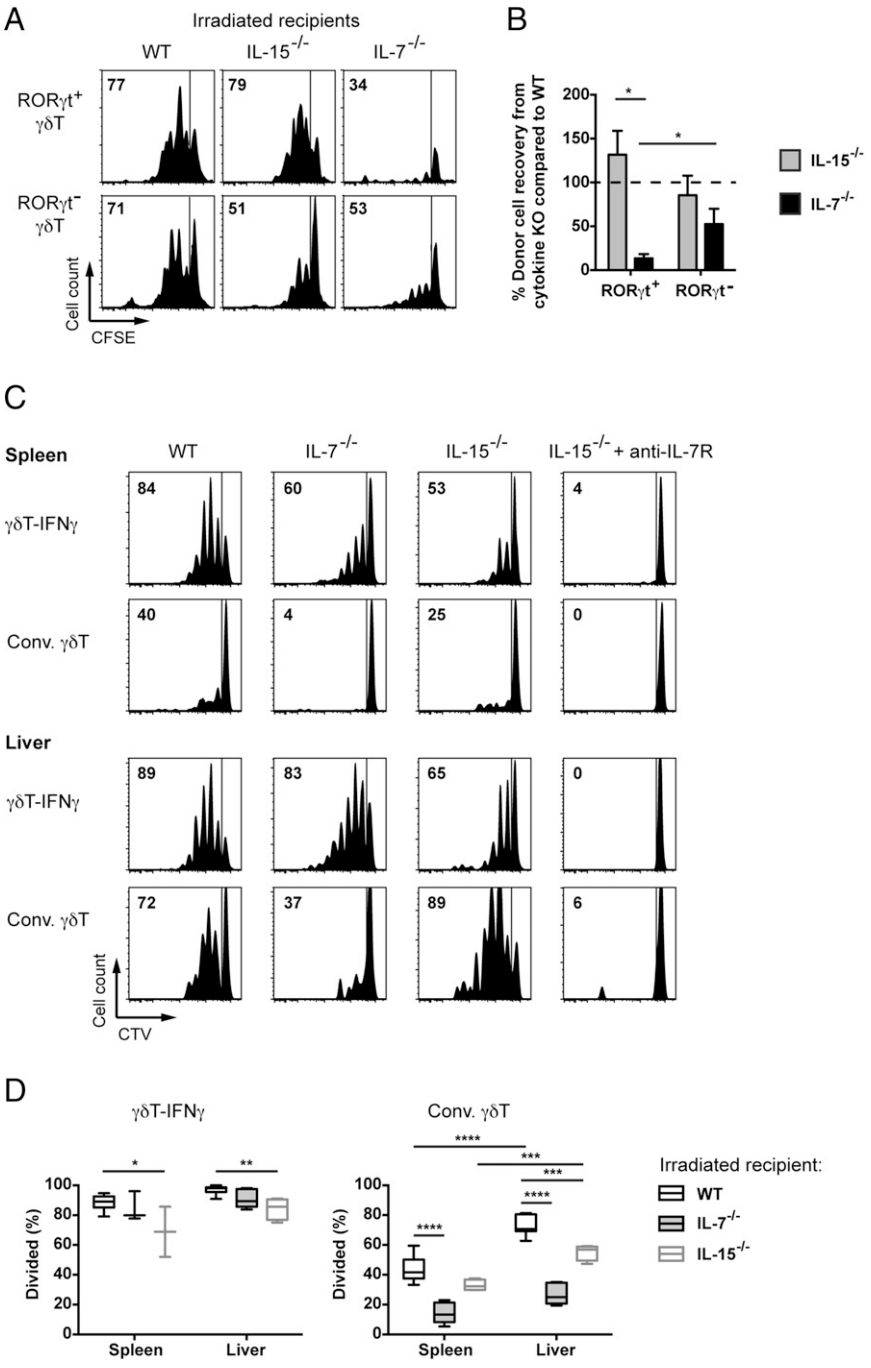

FIGURE 6. LIP in cytokine-deficient mice reveals cytokine dependency of γδ T cell subsets. (**A**) CFSE profiles of congenically marked γδ T cells recovered from spleens on day 7 posttransfer into sublethally irradiated WT, IL-$15^{-/-}$, and IL-$7^{-/-}$ mice. RORγt subsets were electronically gated and are depicted with CFSE cell divisions. (**B**) Recovery of cells from (A) in IL-$15^{-/-}$ and IL-$7^{-/-}$ recipients depicted as a percentage of cells recovered from WT recipients (dashed line at 100%). (**C**) CTV profiles of congenically marked γδ T cells recovered from spleens and livers at day 7 posttransfer into sublethally irradiated WT, IL-$7^{-/-}$, IL-$15^{-/-}$, and IL-$15^{-/-}$ mice treated with anti-IL-7R mAb. Donor γδ T cell subsets, γδT-IFNγ cells ($CD27^{+}CD45RB^{hi}CD44^{hi}$) and conventional γδ T cells ($CD27^{+}CD45RB^{lo}CD44^{lo}$), were electronically gated and are depicted with CTV divisions. (**D**) Average proportion of divided cells from congenically marked γδ T cell purified subsets, γδT-IFNγ ($CD27^{+}CD44^{hi}$) and conventional γδ T cells ($CD27^{+}CD44^{lo}$), recovered from spleens and livers at day 7 posttransfer into sublethally irradiated WT, IL-$7^{-/-}$, and IL-$15^{-/-}$ mice. Data are representative of two to five independent experiments with one or two recipient mice per group per experiment. Data in (B) are mean ± SEM and were analyzed by the Mann–Whitney *U* test. (D) The box-and-whisker plots represent 25–75% (box) and minimum to maximum (whiskers). The horizontal line in the box represents the median value. Data in (D) were analyzed by two-way ANOVA with the Tukey posttest. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$.

but not Mcl-1, and initiate entry into the cell cycle. γδT-IFNγ cells exhibit higher dependency on IL-15, but they can also use IL-7 via moderate expression of IL-7R. Although they express very high basal levels of Bcl-2, both IL-15 and IL-7 appear to transmit survival signals via upregulation of Bcl-x_L and Mcl-1. The γδ T cells that retain TCR responsiveness in the periphery exhibit homeostatic requirements similar to naive αβ T cells. They display lower sensitivity to cytokine stimulation and a greater dependence on IL-7 for turnover, as well as upregulate Bcl-2 after cytokine stimulation.

The pursuit of γδ T cell Ags has been long and is still far from complete. It was found that γδ T cells can recognize self-molecules upregulated on stressed or cancerous cells, in addition to foreign Ags, although the majority of γδ T cell ligands are still unaccounted for. The importance and identity of the Ag-presenting molecule (APM) have been equally difficult to determine. γδ T cells were found that bind MHC and MHC class I–like molecules, as well as the recently discovered butyrophilin, an Ig-like molecule (2, 3, 5, 7, 24). Even the innate-like γδ T cell subsets appear to use their TCR, although the nature of the interaction is not clear (6, 10). Considering the difficulties in ascertaining the identities of Ags and APMs for γδ T cell activation, it is not surprising that little is known about the role of the TCR in γδ T cell homeostasis. Early studies into γδ T cell homeostasis found that MHC class I and II molecules do not play a role (12, 13). However, because γδ T cells tend to use nonclassical APMs or directly recognize their ligand without an APM, these studies do not exclude the possibility that some TCR signaling is required for homeostatic maintenance. Because the innate-like subsets, γδT-17 and γδT-IFNγ, exhibit acute sensitivity to homeostatic cytokines, we expect that their homeostasis is similar to memory $CD8^{+}$ T cells, which is heavily reliant on cytokines and

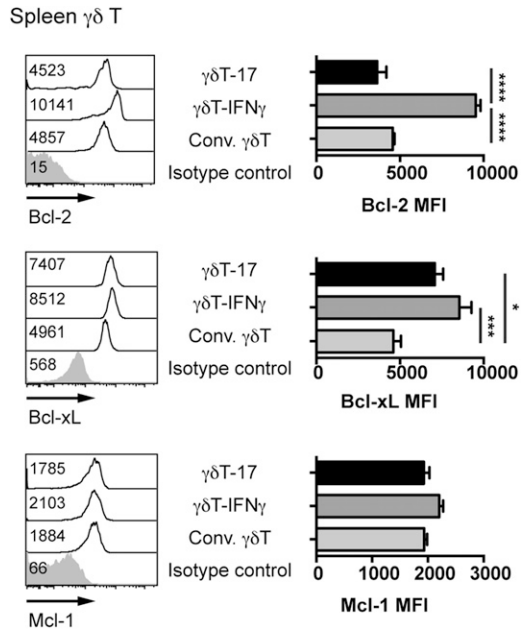

FIGURE 7. Steady-state levels of prosurvival molecules vary between innate-like and conventional γδ T cell subsets. Expression of intracellular Bcl-2, Bcl-x_L, and Mcl-1 in γδ T cell subsets, shown as representative flow cytometry graphs (*left panels*) and mean fluorescence intensity (*right panels*). γδ T cells ($CD3^+γδTCR^+$) were electronically gated into subsets: γδT-17 cells ($ROR\gamma t^+$), γδT-IFNγ cells ($ROR\gamma t^-CD45RB^{hi}CD44^{hi}$), and conventional γδ T cells ($ROR\gamma t^-CD45RB^{lo}CD44^{lo}$). Data in the bar graphs are mean ± SEM. Data are representative of two or three independent experiments with two or three mice per group per experiment. Data were analyzed by one-way ANOVA with the Tukey multiple-comparison test. $*p \leq 0.05$, $***p \leq 0.001$, $****p \leq 0.0001$.

largely independent of TCR ligation. Because $CD44^{lo}$ conventional γδ T cells exhibit many similarities to naive αβ T cells, we predict that they may require a combination of cytokines and tonic TCR signaling for homeostatic maintenance. This supposition is supported by a recent study that found similarities between γδ T subsets and naive and memory $CD8^+$ T cells (25). Using CD44 and Ly6C to divide γδ T cells into subsets, the investigators found that the $CD44^{lo}$ cells turn over slowly in the steady-state but convert to $CD44^{hi}$ cells in a lymphopenic environment, much like naive $CD8^+$ T cells.

The maintenance of the $ROR\gamma t^+$ γδT-17 subset is an interesting case. Unlike IFN-γ–producing γδ T cells, cells that are precommitted to IL-17 production can only develop from embryonic or neonatal thymocytes (26, 27). Without the option of developing from adult thymocytes, their persistence throughout adulthood depends on their capacity for self-renewal and, correspondingly, their ability to compete for resources. That they are the only γδ T cell subset that depends exclusively on one cytokine, and a cytokine that is in high demand, seems like a precarious existence. Apart from maintaining naive αβ T cells, IL-7 is critical for several IL-17–producing populations. $ROR\gamma t^+$ innate lymphoid cells and $ROR\gamma t^+$ NKT17 cells use IL-7 for their survival and expansion (19, 28). Although IL-7 produced in the bone marrow, thymus, and lymph nodes clearly supports lymphocyte development and survival (29), more recent studies found that it can also be produced in nonlymphoid tissues, including intestine, skin, liver, and lung (30, 31), and that it can even be induced in these sites by microbial signals (23, 30, 32). Because γδT-17 cells, NKT cells, and innate lymphoid cells are enriched in barrier tissues and their draining lymph nodes, we suggest that they are kept in balance, both in the steady-state and during an immune response, by competition for locally produced IL-7. γδT-17 cells, along with NKT17 cells (19), express very high levels of IL-7R compared with naive αβ T cells and, thus, would be able to effectively compete for this in-demand cytokine.

A recent article also suggests a role for IL-15Rα in restraining γδT-17 development and peripheral homeostasis (18). The investigators observed an increase in the number of γδT-17 cells in the lymph node of IL-15Rα–deficient, but not IL-15–deficient, mice, which led to the conclusion that signals received via IL-15Rα in *cis* can constrain γδT-17 cells. The difference between receptor and cytokine knockout led them to suggest that IL-15Rα was interacting with an unidentified cytokine or receptor chain. We observed an increase in the proportion and number of γδT-17 cells in our $IL\text{-}15^{-/-}$ mice, particularly in the spleen (Fig. 5B, 5C). These findings may indicate that IL-15 signaling via IL-15Rα also plays a role in constraining γδT-17 cells.

Lymphocyte survival is known to depend on the expression of Bcl-2 family members. Prosurvival proteins, such as Bcl-2, Bcl-x_L, and Mcl-1, bind and sequester Bcl-2–Homology Domain 3 only (BH3-only) molecules, preventing them from activating death effectors Bax and Bak and the intrinsic death pathway (33). Therefore, the survival of cells is dependent on the balance of prosurvival molecules and their partner BH3-only proteins (34, 35). The relative importance of different Bcl-2 family members varies among lymphocyte populations, and our studies show that γδ T cell subsets are no exception. Upregulation of prosurvival molecules varied with subset and cytokine stimulation. These data begin to explain how homeostatic cytokines transmit survival signals to γδ T cells; however, there is still much to understand. Although it was not surprising that only IL-7 could increase prosurvival molecules in γδT-17 cells, it was surprising that Mcl-1 was not one of these molecules. Culture of αβ T cells with IL-7 was found to significantly increase Mcl-1 at both the mRNA and protein levels (36). That such an IL-7–dependent population as γδT-17 cells does not upregulate Mcl-1 is unexpected. Instead, we found that γδT-IFNγ cells upregulate Mcl-1 after IL-7 and IL-15 stimulation, but particularly the latter. Although IL-15 can also increase Mcl-1 in αβ T cells, it is never as marked as the effect of IL-7 (36, 37). This increase in Mcl-1 by IL-15 is instead reminiscent of the response of NK cells to IL-15 (38), suggesting that γδ T cells blend aspects of innate and adaptive cell behavior, not only for their activation, but also for their survival.

Considering that more than one prosurvival molecule was upregulated with cytokine stimulation, we also would like to discern whether particular molecules are critical or redundant for the survival of each subset; these experiments will require gene-knockout mice to answer unequivocally. Furthermore, we still need to explore the BH3-only proteins. Bim, in particular, appears to be an important antagonist of Bcl-2 and Mcl-1 in lymphocytes (34–36, 39). Levels of BH3-only proteins will provide a more complete picture of the mechanisms of homeostatic survival of γδ T cells.

Along with promoting host defense, it is becoming apparent that γδ T cells play a role in disease pathogenesis. γδT-17 cells enhance autoimmunity in the mouse model of multiple sclerosis (9, 40), and an enrichment of these cells was observed in active disease in patients with ankylosing spondylitis (41). Furthermore, γδ T cells are the predominant dermal cell producing IL-17 in murine psoriasis and are greatly increased in the skin lesions of psoriasis patients (42). In addition to inflammatory pathologies, dysregulation of γδ T cells occurs in the context of cancer. γδ T cell lymphoma is rare, accounting for only 1% of lymphoid neoplasms, but it is highly aggressive. One classification, hepatosplenic T cell lymphoma, predominantly affects young adults and is refractory to conventional therapies. Up to 20% of cases

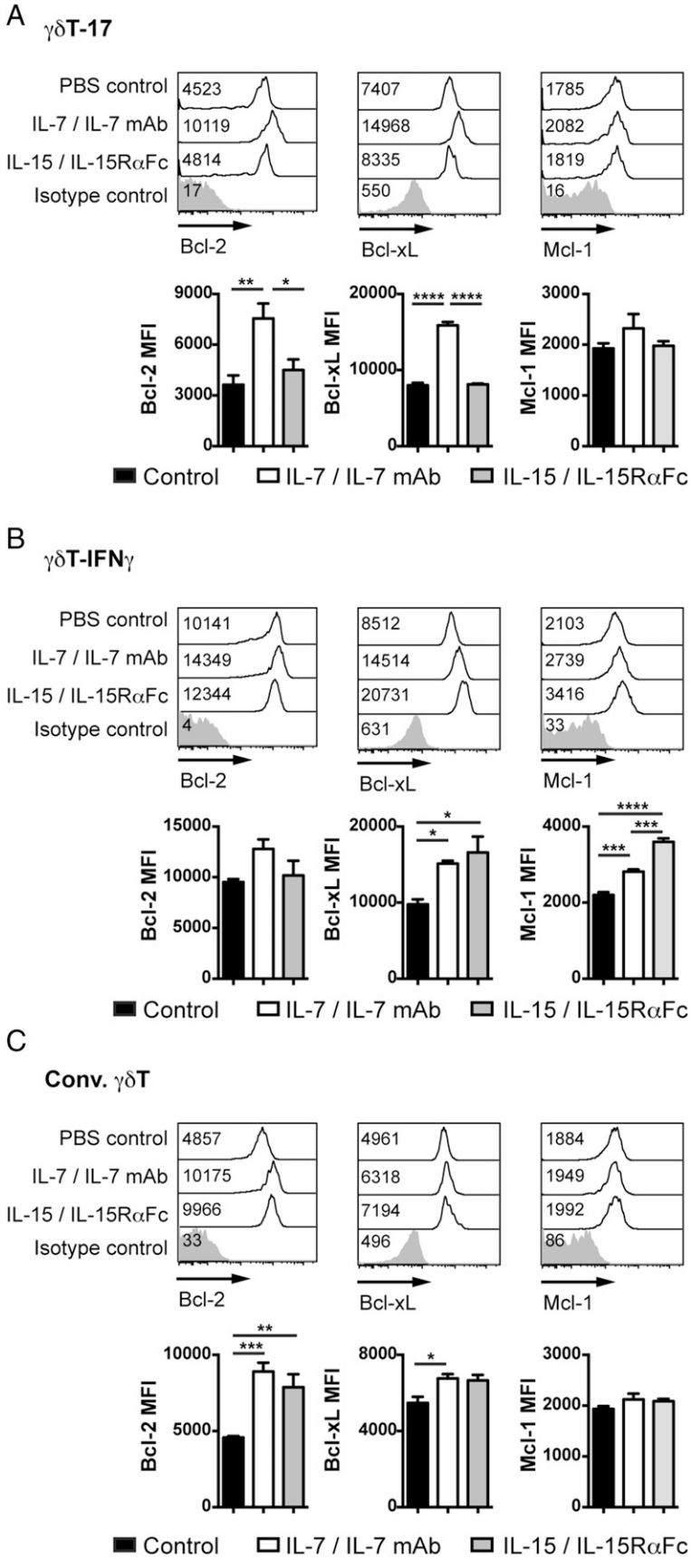

FIGURE 8. Homeostatic cytokines IL-7 and IL-15 differentially transmit survival signals to γδ T cell subsets. Mice were injected i.p. with control PBS, IL-7/IL-7 mAb (M25), or IL-15/IL-15RαFc (1 μg/5 μg) on days 0, 1, and 2, and spleens were analyzed on day 3. γδ T cells ($CD3^+$ $γδTCR^+$) were electronically gated into subsets: γδT-17 cells ($ROR\gamma t^+$), γδT-IFNγ cells ($ROR\gamma t^-CD45RB^{hi}CD44^{hi}$), and conventional γδ T cells ($ROR\gamma t^-CD45RB^{lo}CD44^{lo}$). Expression of intracellular Bcl-2, Bcl-x_L, and Mcl-1 in γδ T cell subsets was measured by flow cytometry, with the data shown as representative flow cytometry graphs (*upper panels*) and mean fluorescence intensity (*lower panels*) for γδT-17 cells (**A**), γδT-IFNγ cells (**B**), and conventional γδ T cells (**C**). Data in bar graphs are mean ± SEM. Data are representative of two or three independent experiments, with three mice per group per experiment. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$, one-way ANOVA with the Tukey multiple-comparison test.

arise in the context of chronic immune suppression, either post-transplant (cyclosporine) or in patients treated with azathioprine and infliximab (anti-TNF) for Crohn's disease (43). Together, these disorders emphasize the importance of understanding the conditions that support and corrupt homeostasis of different γδ T cells to appropriately direct potential therapies.

In summary, we showed that, within the γδ T cell pool, subsets of innate-like and conventional cells exist with differing requirements for survival and homeostasis. Although they are clearly different from αβ T cells, their TCR responsiveness and sensitivity to cytokines align them with specific αβ T cell subsets. γδT-17 cells, with their dependence on IL-7, align with NKT17 cells. In contrast, γδT-IFNγ cells, which depend heavily on IL-15 and upregulate Bcl-x_L upon cytokine stimulation, align more closely with memory $CD8^+$ T cells. They also increase Mcl-1 expression, particularly after IL-15 ligation, a response similar to NK cells. Conventional γδ T cells, which retain TCR responsiveness, proliferate slowly to cytokine, and favor IL-7 for turnover while being able to use both IL-7 and IL-15 for survival, more closely resemble naive αβ T cells (44, 45). As such, the differing dependence on homeostatic cytokines ensures that γδ T cell subsets fill different resource niches and, instead, compete for survival with other lymphocytes of similar function.

Acknowledgments

We thank the staff of the Biological Testing Facility (Garvan Institute) for help with animal breeding and care; Dr. Robert Salomon and Eric Lam for cell sorting and Amanda Hong for genotyping (Garvan Institute); Stephanie Grabow and Alex Delbridge for technical assistance (The Walter and Eliza Hall Institute); Dr. David Huang for the gift of anti-mouse Mcl-1; and Dr. Tri Phan (Garvan Institute) for critical reading of the manuscript.

Disclosures

The authors have no financial conflicts of interest.

References

1. Bonneville, M., R. L. O'Brien, and W. K. Born. 2010. Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat. Rev. Immunol.* 10: 467–478.
2. Crowley, M. P., A. M. Fahrer, N. Baumgarth, J. Hampl, I. Gutgemann, L. Teyton, and Y. Chien. 2000. A population of murine gammadelta T cells that recognize an inducible MHC class Ib molecule. *Science* 287: 314–316.
3. Wingren, C., M. P. Crowley, M. Degano, Y. Chien, and I. A. Wilson. 2000. Crystal structure of a gammadelta T cell receptor ligand T22: a truncated MHC-like fold. *Science* 287: 310–314.
4. Constant, P., F. Davodeau, M. A. Peyrat, Y. Poquet, G. Puzo, M. Bonneville, and J. J. Fournié. 1994. Stimulation of human gamma delta T cells by nonpeptidic mycobacterial ligands. *Science* 264: 267–270.
5. Vavassori, S., A. Kumar, G. S. Wan, G. S. Ramanjaneyulu, M. Cavallari, S. El Daker, T. Beddoe, A. Theodossis, N. K. Williams, E. Gostick, et al. 2013. Butyrophilin 3A1 binds phosphorylated antigens and stimulates human γδ T cells. *Nat. Immunol.* 14: 908–916.
6. Zeng, X., Y. L. Wei, J. Huang, E. W. Newell, H. Yu, B. A. Kidd, M. S. Kuhns, R. W. Waters, M. M. Davis, C. T. Weaver, and Y. H. Chien. 2012. γδ T cells recognize a microbial encoded B cell antigen to initiate a rapid antigen-specific interleukin-17 response. *Immunity* 37: 524–534.
7. Uldrich, A. P., J. Le Nours, D. G. Pellicci, N. A. Gherardin, K. G. McPherson, R. T. Lim, O. Patel, T. Beddoe, S. Gras, J. Rossjohn, and D. I. Godfrey. 2013. CD1d-lipid antigen recognition by the γδ TCR. *Nat. Immunol.* 14: 1137–1145.
8. Martin, B., K. Hirota, D. J. Cua, B. Stockinger, and M. Veldhoen. 2009. Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. *Immunity* 31: 321–330.
9. Sutton, C. E., S. J. Lalor, C. M. Sweeney, C. F. Brereton, E. C. Lavelle, and K. H. Mills. 2009. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* 31: 331–341.
10. Wencker, M., G. Turchinovich, R. Di Marco Barros, L. Deban, A. Jandke, A. Cope, and A. C. Hayday. 2014. Innate-like T cells straddle innate and adaptive immunity by altering antigen-receptor responsiveness. *Nat. Immunol.* 15: 80–87.
11. Bekiaris, V., J. R. Sedý, M. G. Macauley, A. Rhode-Kurnow, and C. F. Ware. 2013. The inhibitory receptor BTLA controls γδ T cell homeostasis and inflammatory responses. *Immunity* 39: 1082–1094.
12. French, J. D., C. L. Roark, W. K. Born, and R. L. O'Brien. 2005. {gamma}{delta} T cell homeostasis is established in competition with {alpha}{beta} T cells and NK cells. *Proc. Natl. Acad. Sci. USA* 102: 14741–14746.
13. Baccala, R., D. Witherden, R. Gonzalez-Quintial, W. Dummer, C. D. Surh, W. L. Havran, and A. N. Theofilopoulos. 2005. Gamma delta T cell homeostasis is controlled by IL-7 and IL-15 together with subset-specific factors. *J. Immunol.* 174: 4606–4612.
14. Michel, M. L., D. J. Pang, S. F. Haque, A. J. Potocnik, D. J. Pennington, and A. C. Hayday. 2012. Interleukin 7 (IL-7) selectively promotes mouse and human IL-17-producing γδ cells. *Proc. Natl. Acad. Sci. USA* 109: 17549–17554.
15. Webster, K. E., S. Walters, R. E. Kohler, T. Mrkvan, O. Boyman, C. D. Surh, S. T. Grey, and J. Sprent. 2009. In vivo expansion of T reg cells with IL-2-mAb complexes: induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression. *J. Exp. Med.* 206: 751–760.
16. Ribot, J. C., A. deBarros, D. J. Pang, J. F. Neves, V. Peperzak, S. J. Roberts, M. Girardi, J. Borst, A. C. Hayday, D. J. Pennington, and B. Silva-Santos. 2009. CD27 is a thymic determinant of the balance between interferon-gamma- and interleukin 17-producing gammadelta T cell subsets. *Nat. Immunol.* 10: 427–436.
17. Paget, C., M. T. Chow, N. A. Gherardin, P. A. Beavis, A. P. Uldrich, H. Duret, M. Hassane, F. Souza-Fonseca-Guimaraes, D. A. Mogilenko, D. Staumont-Sallé, et al. 2015. CD3bright signals on γδ T cells identify IL-17A-producing Vγ6Vδ1 + T cells. *Immunol. Cell Biol.* 93: 198–212.
18. Colpitts, S. L., L. Puddington, and L. Lefrançois. 2015. IL-15 receptor α signaling constrains the development of IL-17-producing γδ T cells. *Proc. Natl. Acad. Sci. USA* 112: 9692–9697.
19. Webster, K. E., H. O. Kim, K. Kyparissoudis, T. M. Corpuz, G. V. Pinget, A. P. Uldrich, R. Brink, G. T. Belz, J. H. Cho, D. I. Godfrey, and J. Sprent. 2014. IL-17-producing NKT cells depend exclusively on IL-7 for homeostasis and survival. *Mucosal Immunol.* 7: 1058–1067.
20. Surh, C. D., and J. Sprent. 2008. Homeostasis of naive and memory T cells. *Immunity* 29: 848–862.
21. Maki, K., S. Sunaga, Y. Komagata, Y. Kodaira, A. Mabuchi, H. Karasuyama, K. Yokomuro, J. I. Miyazaki, and K. Ikuta. 1996. Interleukin 7 receptor-deficient mice lack gammadelta T cells. *Proc. Natl. Acad. Sci. USA* 93: 7172–7177.
22. Ye, S. K., Y. Agata, H. C. Lee, H. Kurooka, T. Kitamura, A. Shimizu, T. Honjo, and K. Ikuta. 2001. The IL-7 receptor controls the accessibility of the TCRgamma locus by Stat5 and histone acetylation. *Immunity* 15: 813–823.
23. Sawa, Y., Y. Arima, H. Ogura, C. Kitabayashi, J. J. Jiang, T. Fukushima, D. Kamimura, T. Hirano, and M. Murakami. 2009. Hepatic interleukin-7 expression regulates T cell responses. *Immunity* 30: 447–457.
24. Schild, H., N. Mavaddat, C. Litzenberger, E. W. Ehrich, M. M. Davis, J. A. Bluestone, L. Matis, R. K. Draper, and Y. H. Chien. 1994. The nature of major histocompatibility complex recognition by gamma delta T cells. *Cell* 76: 29–37.
25. Lombes, A., A. Durand, C. Charvet, M. Rivière, N. Bonilla, C. Auffray, B. Lucas, and B. Martin. 2015. Adaptive Immune-like γ/δ T Lymphocytes Share Many Common Features with Their α/β T Cell Counterparts. *J. Immunol.* 195: 1449–1458.
26. Gray, E. E., K. Suzuki, and J. G. Cyster. 2011. Cutting edge: Identification of a motile IL-17-producing gammadelta T cell population in the dermis. *J. Immunol.* 186: 6091–6095.
27. Haas, J. D., S. Ravens, S. Düber, I. Sandrock, L. Oberdörfer, E. Kashani, V. Chennupati, L. Föhse, R. Naumann, S. Weiss, et al. 2012. Development of interleukin-17-producing γδ T cells is restricted to a functional embryonic wave. *Immunity* 37: 48–59.
28. Satoh-Takayama, N., S. Lesjean-Pottier, P. Vieira, S. Sawa, G. Eberl, C. A. Vosshenrich, and J. P. Di Santo. 2010. IL-7 and IL-15 independently program the differentiation of intestinal CD3-NKp46+ cell subsets from Id2-dependent precursors. *J. Exp. Med.* 207: 273–280.
29. Link, A., T. K. Vogt, S. Favre, M. R. Britschgi, H. Acha-Orbea, B. Hinz, J. G. Cyster, and S. A. Luther. 2007. Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat. Immunol.* 8: 1255–1265.
30. Shalapour, S., K. Deiser, O. Sercan, J. Tuckermann, K. Minnich, G. Willimsky, T. Blankenstein, G. J. Hämmerling, B. Arnold, and T. Schüler. 2010. Commensal microflora and interferon-gamma promote steady-state interleukin-7 production in vivo. *Eur. J. Immunol.* 40: 2391–2400.
31. Repass, J. F., M. N. Laurent, C. Carter, B. Reizis, M. T. Bedford, K. Cardenas, P. Narang, M. Coles, and E. R. Richie. 2009. IL7-hCD25 and IL7-Cre BAC transgenic mouse lines: new tools for analysis of IL-7 expressing cells. *Genesis* 47: 281–287.
32. Roye, O., N. Delhem, F. Trottein, F. Remoué, S. Nutten, J. P. Decavel, M. Delacre, V. Martinot, J. Y. Cesbron, C. Auriault, and I. Wolowczuk. 1998. Dermal endothelial cells and keratinocytes produce IL-7 in vivo after human *Schistosoma mansoni* percutaneous infection. *J. Immunol.* 161: 4161–4168.
33. Strasser, A. 2005. The role of BH3-only proteins in the immune system. *Nat. Rev. Immunol.* 5: 189–200.
34. Bouillet, P., S. Cory, L. C. Zhang, A. Strasser, and J. M. Adams. 2001. Degenerative disorders caused by Bcl-2 deficiency prevented by loss of its BH3-only antagonist Bim. *Dev. Cell* 1: 645–653.
35. Wojciechowski, S., P. Tripathi, T. Bourdeau, L. Acero, H. L. Grimes, J. D. Katz, F. D. Finkelman, and D. A. Hildeman. 2007. Bim/Bcl-2 balance is critical for maintaining naive and memory T cell homeostasis. *J. Exp. Med.* 204: 1665–1675.
36. Opferman, J. T., A. Letai, C. Beard, M. D. Sorcinelli, C. C. Ong, and S. J. Korsmeyer. 2003. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. *Nature* 426: 671–676.
37. Shenoy, A. R., S. Kirschnek, and G. Häcker. 2014. IL-15 regulates Bcl-2 family members Bim and Mcl-1 through JAK/STAT and PI3K/AKT pathways in T cells. *Eur. J. Immunol.* 44: 2500–2507.
38. Sathe, P., R. B. Delconte, F. Souza-Fonseca-Guimaraes, C. Seillet, M. Chopin, C. J. Vandenberg, L. C. Rankin, L. A. Mielke, I. Vikstrom, T. B. Kolesnik, et al. 2014. Innate immunodeficiency following genetic ablation of Mcl1 in natural killer cells. *Nat. Commun.* 5: 4539.

39. Pierson, W., B. Cauwe, A. Policheni, S. M. Schlenner, D. Franckaert, J. Berges, S. Humblet-Baron, S. Schönefeldt, M. J. Herold, D. Hildeman, et al. 2013. Antiapoptotic Mcl-1 is critical for the survival and niche-filling capacity of Foxp3^{+} regulatory T cells. *Nat. Immunol.* 14: 959–965.
40. Petermann, F., V. Rothhammer, M. C. Claussen, J. D. Haas, L. R. Blanco, S. Heink, I. Prinz, B. Hemmer, V. K. Kuchroo, M. Oukka, and T. Korn. 2010. γδ T cells enhance autoimmunity by restraining regulatory T cell responses via an interleukin-23-dependent mechanism. *Immunity* 33: 351–363.
41. Kenna, T. J., S. I. Davidson, R. Duan, L. A. Bradbury, J. McFarlane, M. Smith, H. Weedon, S. Street, R. Thomas, G. P. Thomas, and M. A. Brown. 2012. Enrichment of circulating interleukin-17-secreting interleukin-23 receptor-positive γ/δ T cells in patients with active ankylosing spondylitis. *Arthritis Rheum.* 64: 1420–1429.
42. Cai, Y., X. Shen, C. Ding, C. Qi, K. Li, X. Li, V. R. Jala, H. G. Zhang, T. Wang, J. Zheng, and J. Yan. 2011. Pivotal role of dermal IL-17-producing γδ T cells in skin inflammation. *Immunity* 35: 596–610.
43. Tripodo, C., E. Iannitto, A. M. Florena, C. E. Pucillo, P. P. Piccaluga, V. Franco, and S. A. Pileri. 2009. Gamma-delta T-cell lymphomas. *Nat. Rev. Clin. Oncol.* 6: 707–717.
44. Rathmell, J. C., E. A. Farkash, W. Gao, and C. B. Thompson. 2001. IL-7 enhances the survival and maintains the size of naive T cells. *J. Immunol.* 167: 6869–6876.
45. Berard, M., K. Brandt, S. Bulfone-Paus, and D. F. Tough. 2003. IL-15 promotes the survival of naive and memory phenotype CD8+ T cells. *J. Immunol.* 170: 5018–5026.