Effects of Increasing IL-7 Availability on Lymphocytes during and after Lymphopenia-Induced Proliferation

Nabil Bosco, Fabien Agenès and Rhodri Ceredig

*J Immunol* 2005; 175:162-170; doi: 10.4049/jimmunol.175.1.162
http://www.jimmunol.org/content/175/1/162

Why The *JI*?

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

References

This article cites 55 articles, 26 of which you can access for free at: http://www.jimmunol.org/content/175/1/162.full#ref-list-1

Subscription

Information about subscribing to The *Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Effects of Increasing IL-7 Availability on Lymphocytes during and after Lymphopenia-Induced Proliferation

Nabil Bosco,* Fabien Agenès,* and Rhodri Ceredig†

IL-7 is critically involved in regulating peripheral T cell homeostasis. To investigate the role of IL-7 on lymphopenia-induced proliferation of polyclonal lymphocytes, we have transferred CFSE-labeled cells into a novel T-lymphopenic, IL-7-transgenic mouse line. Results obtained indicate that T and B cells do not respond in the same way to IL-7-homeostatic signals. Overexpression of IL-7 enhances proliferation of both CD4+ and CD8+ T cells but with distinctly temporal effects. Expansion of naturally arising CD4+ regulatory T cells was like that of conventional CD4+ T cells. IL-7 had no effect on B cell proliferation. By immunohistology, transferred T cells homed to T cell areas of spleen lymphoid folicles. Increasing IL-7 availability enhanced T cell recovery by promoting cell proliferation and reducing apoptosis during early stages of lymphopenia-induced proliferation. Taken together, these results provide new insights into the pleiotropic effects of IL-7 on lymphopenia-induced T cell proliferation. The Journal of Immunology, 2005, 175: 162–170.

Despite declining thymic output with age, the peripheral T cell pool of an adult animal remains remarkably stable (1, 2). How the T cell pool is maintained remains a central question in immunology. Compelling data have been provided indicating that long term survival and homeostatic proliferation of T lymphocytes is dependent on a combination of low level TCR and cytokine stimulation. After transfer into a lymphopenic environment, T cells sense the absence of T cells and proliferate slowly, a process that has been termed lymphopenia-induced proliferation (LIP) (3). Cytokines, such as IL-7 and IL-15, have been shown to play a major role in both LIP and T cell survival in mice (1, 4). IL-7 is also a crucial cytokine for lymphocyte development providing survival-promoting signals for immature and mature T cells as well as for immature B cells (5).

Different experimental procedures have been designed to study the role of IL-7 in LIP. IL-7 enhances survival of mature T cells in vitro (6, 7), and exogenous IL-7 administration increases both the pool size of peripheral T cells (8) and the rate of hemopoietic reconstitution after bone marrow (BM) transplantation in vivo (9). Nevertheless, when IL-7 is injected into normal mice, it has been difficult to distinguish between its effects on thymic output and that on peripheral T cells. Syngenic adoptive transfer experiments into either IL-7-deficient or wild-type mice treated with anti-IL-7 or anti-IL-7Ra mAb show that perturbation of IL-7 signals prevents the expansion of transferred T cells (1, 10). Although several groups have shown that IL-7 is crucial for T cell survival and LIP, the mechanisms by which IL-7 levels regulate the size and diversity of the peripheral T cell pool are still not well understood.

Differences in experimental systems used to investigate the role of IL-7 in LIP may account for some apparently conflicting data. These differences concern either the nature of the recipient mouse or that of the transferred cells. Preconditioning the recipient mouse by irradiation or other lymphocyte-depleting regimens probably alters lymphoid organ architecture, Ag-presenting cell function, and cytokine milieu (11, 12). For instance, after irradiation, IL-7 and TGFβ levels may change, thereby affecting LIP (12, 13). Furthermore, in sublethally irradiated recipients, residual bystander T cells persist and compete with transferred T cells for cytokine and or self peptide-MHC complexes, thereby influencing reconstitution of the T cell compartment (12). Transferred cells are frequently derived from TCR-transgenic, recombinase-activating gene-deficient (RAG−/−) mice. Such monoclonal T cell populations express a TCR of predefined specificity and affinity, two parameters that correlate with CD5 expression and that may dictate whether a T cell undergoes LIP (14–16). Second, such monoclonal T cell populations lack naturally arising CD4+CD25+ regulatory T cells (17, 18), known to alter the behavior of cells during LIP (19, 20). In such experiments, long term survival is rarely monitored because for various reasons, including the absence of CD4+CD25+ regulatory T cells, recipients frequently develop autoimmune diseases (21, 22).

Under normal T cell-replete, nonlymphopenic conditions, IL-7 probably acts as a survival factor for T cells (4). In normal mice, the net level of IL-7 availability is low, resulting both from limited production by stromal cells and simultaneous consumption by T cells (23). In T cell-lymphopenic conditions, the net IL-7 level increases by a poorly defined mechanism, thereby allowing residual T cells to sense lymphopenia, augment TCR signaling, and consequently triggering LIP. This notion is consistent with available data and particularly with the previously reported phenotype of IL-7-transgenic (IL-7TG) mice (24) that contain a stable expanded (~20-fold) T cell pool (25, 26). It could be postulated that net IL-7 availability provides the main clue whereby T cells sense...
lymphopenia. Thus, in IL-7Tg mice, T cells increase in number until capable of absorbing the increased available IL-7. Recently, Li et al. (27) predicted that in lymphopenic recipients, where IL-7 availability was increased, T cell LIP would be triggered. However, to date, this hypothesis has not been directly demonstrated, and no study has systematically examined the behavior of polyclonal T and B cells transferred together into mice differing in IL-7 availability.

For the studies reported herein, we have developed a novel T cell lymphopenic mouse strain that overexpresses IL-7. The IL-7 transgene was introduced into C57BL/6.CD3e gene-deficient (CD3e−/−) mice (28). IL-7Tg.CD3e−/− and nontransgenic CD3e−/− littermate controls provide a pair of T-lymphopenic, B lymphocyte-containing mice differing only in net IL-7 availability and not requiring further conditioning before use as recipients. To investigate how IL-7 availability influences the behavior of cells undergoing LIP in T-lymphopenic animals, we transferred CFSE-labeled polyclonal T and B cells into recipient mice. Parameters investigated included the kinetics of both CD4+ and CD8+ T cell as well as B cell growth and division, anatomical localization, the kinetics of Bcl-2 expression and cell loss by apoptosis, as well as long term cell recovery and turnover. Taken together, our results provide new insights into the dynamics of IL-7-dependent LIP and the pleiotropic effects of IL-7 on T and B cell homeostasis.

Materials and Methods

Mice

IL-7-transgenic mice on a C57BL/6 (B6) genetic background (IL-7Tg.B6) have been described previously (24); they were maintained in a heterozygous state by breeding transgenic males with B6 females (Iffa-Credo). IL-7Tg.CD3−/− and nontransgenic littermates (3- to 5-fold) where B cells are absent (not shown). The IL-7 transgene was introduced into C57BL/6.CD3e gene-deficient (CD3e−/−) mice (28). IL-7Tg.CD3e−/− and nontransgenic CD3e−/− littermate controls provide a pair of T-lymphopenic, B lymphocyte-containing mice differing only in net IL-7 availability and not requiring further conditioning before use as recipients. To investigate how IL-7 availability influences the behavior of cells undergoing LIP in T-lymphopenic animals, we transferred CFSE-labeled polyclonal T and B cells into recipient mice. Parameters investigated included the kinetics of both CD4+ and CD8+ T cell as well as B cell growth and division, anatomical localization, the kinetics of Bcl-2 expression and cell loss by apoptosis, as well as long term cell recovery and turnover. Taken together, our results provide new insights into the dynamics of IL-7-dependent LIP and the pleiotropic effects of IL-7 on T and B cell homeostasis.

Flow cytometry

The following mAbs, obtained from BD Pharmingen or e-Bioscience, were used for staining: anti-CD4 (GK1.5); anti-CD8 (53-6.7); anti-CD19 (1D3); anti-CD20 (L243); anti-CD21 (7G6); anti-CD23 (B64); anti-CD45R (RA3-6B2); anti-CD25 (7D4); anti-CD44 (IM7); anti-CD62L (MEL-14); anti-CD69 (H1-2F3); anti-CD117 or c-KIT (2B8); anti-CD122 (TM-β1); anti-CD127 (A7R34); anti-CD132 (4G3); anti-CD11 (PK136); and anti-CD14 (R6-60.2). Cells were three- or four-color stained with appropriate combinations of FITC-, PE-, biotin-labeled anti-CD45R (R73), anti-CD21 (7G6); anti-CD23 (B3B4); and anti-IgM (R6-60.2). Cells were prepared and purified over a Ficoll gradient before counting viable cells by trypan blue exclusion. For naive T cell transfer experiments, naive CD4+CD45RBhigh and CD8+CD44− T cells were sorted from spleen of 8-wk-old B6 mice with a MoFlo (Cytomation) with a purity of 97%, then labeled with CFSE, and cotransferred as described above.

Apoptosis assay

Externalization of phosphatidylserine was detected by PE-conjugated annexin V mAb using the apoptosis detection kit (BD Pharmingen) according to the instructions of the manufacturer. In brief, 106 splenocytes from adoptively transferred mice at day 3 or 28 were surface stained and then washed with binding buffer. Cells were incubated for 15 min at room temperature in the dark with annexin V + 7-aminoactinomycin D in binding buffer. Then, 106 cells were analyzed by FACS as described above.

In vivo BrdU labeling

At 23 days after T cell transfer, mice were given 1 mg of BrdU (Sigma-Aldrich) i.p. injection and 1 mg BrdU in their drinking water for 5 days. Recipient mice were sacrificed at day 28 after T cell transfer, and spleen cell suspensions were prepared, surface stained as described above, washed, fixed, and permeabilized before labeling with anti-BrdU mAb as described previously (32, 33) using the BrdU flow kit (BD Pharmingen). Then, 106 cells were analyzed by FACS.

Intracellular staining for Bcl-2

As described above, 106 total spleen cells from unmanipulated B6 or adoptively transferred mice were prepared and surface stained. After unbound mAb were washed, cells were subjected to intracellular staining for Bcl-2 using the Cytofix/Cytoperm kit (BD Pharmingen). For intracellular staining, FITC-conjugated hamster anti-mouse Bcl-2 mAb (clone 3F11) or an isotype control mAb (hamster IgG) was used.

Quantitative RT-PCR for IL-7 expression

Quantification of IL-7 transcript was performed on 6- to 8-wk-old mice. RNA was extracted from 2 to 5 × 107 thymus or spleen cells of IL-7 transgenic mice or their negative littermate controls. RNA was extracted (Trizol; Invitrogen), DNase digested (DNase I; Invitrogen) and cDNA synthesized using SuperScriptII reverse transcriptase (Invitrogen). Real time quantitative PCR was performed using a LightCycler Instrument using SYBR Green FastStart kit, 1:reaction (Roche Diagnostics). The real time PCR conditions were as follows: 95°C for 8 min; then 45 cycles with 95°C for 15 s at 60°C for 1 min. Mouse elongation factor 1α (mEif1α) mRNA was used as a positive control and to quantitate total amplified RNA. Analysis was conducted with Light Cycler 3.5 software (Roche Diagnostics). Results were expressed as the ratio of IL-7 mRNA to mEif1α mRNA and arbitrarily set at 1 for reference mice. The primer (Euorgenetics) sequences were as follows: mIL7 sense, 5′-CAGACAGCTGTTGTCATGTTGTTTTTTA-3′; mIL7 antisense, 5′-CTTTCCTCGTCTTTAATGGTGCACCTAGA-3′; mEif1α sense, 5′-CTGACACCTCAGGGCAAAAT-3′; and mEif1α antisense, 5′-TCTTTTCTTAAAGCTCAGCAACCTG-3′.

Immunohistochemistry

Spleens were harvested from recipient mice at different times after adoptive transfer, embedded in Cryo-M-Bed compound (Bright Instrument), and frozen at ~80°C. Frozen sections 5–7 µm thick were fixed for 10 min in cold acetone and dried extensively. Sections were stained with PE-labeled anti-B220 mAb (RA3-6B2) for B cells and, when CFSE staining had become negative, FITC-labeled anti-Cd90 mAb (T24) to detect T cells. Cells were imaged using a fluorescent confocal microscope (Leica TCS-SP3).

Results

Characterization of lymphopenic recipient mice

To study the effect of increased IL-7 availability on LIP, two models of lymphopenic mice overexpressing IL-7 were generated. For most of the experiments reported herein, we used only IL-7Tg.CD3e−/− and CD3e−/− littermates. The expression of the mouse IL-7 cDNA transgene is controlled by the mouse MHC class II promoter (24), and MHC class II genes are actively transcribed in mouse B cells (34). Because B cells are present in CD3e−/− littermates (10- to 30-fold) than between IL-7Tg.CD3e−/− and CD3e−/− littersmates (10- to 30-fold) than between IL-7Tg.RAG-2−/− and RAG-2−/− littersmates (3- to 5-fold) where B cells are absent (not shown).
Overexpression of IL-7 perturbs B cell development, primarily by expanding the progenitor pool in the BM (35, 36). To demonstrate this in the IL-7Tg.CD3ε−/− mouse line and because no reliable assay exists to quantitate available IL-7 protein in organs, a series of phenotypic analyses was performed. The total number of CD19+ B cells was increased ~6-fold in the BM of IL-7Tg.CD3ε−/− mice compared with littermate controls (Fig. 1A). Both CD117+ pro-B/pre-BI and CD25+ B220+ IgM+ pre-BII cells were increased ~10-fold; the numbers of B220+ IgMlow immature B and IgM++ or CD23+ mature B cells remained unchanged. Thus, the known IL-7-dependent stages of BM B cell development were the most affected by MHC class II promoter-driven IL-7 overexpression (32, 35, 36).

Two major changes were noted in the spleens of adult IL-7Tg.CD3ε−/− mice. First, the spleen contained large numbers of IL-7-dependent CD117+ pro/pre-BI cells (Fig. 1B, bar graph) and B220+ IgM+ immature B cells (Fig. 1B, cytograms); such cells are only normally found in neonatal mice (37). Second, although CD23+ CD21+ follicular B cell numbers were similar, there were fewer CD23low/CD21+ marginal zone B cells in IL-7Tg.CD3ε−/− mice (Fig. 1B, lower cytograms and bar graph). Importantly, confocal microscopy indicated that spleen architecture was largely preserved (see below). Qualitatively, Ab responses to the T-independent type II Ag trinitrophenyl-Ficoll were normal in IL-7Tg.CD3ε−/− mice (data not shown). In the peritoneal cavity, CD23+ CD21+ follicular B cells were increased 4-fold whereas in the peripheral blood, B220+ IgM+ pre-BII and B220+ IgMlow immature cells were present (Ref. 32 and data not shown).

Analysis of thymocytes as reviewed by Ceredig and Rolink (33) indicated that the numbers of IL-7-responsive CD117+ CD44+CD25− DN1 and CD44+, CD25+ DN2 cells were increased ~2-fold but that the number of CD44+ CD25− DN3 thymocytes remained unchanged (Fig. 1C) (38). Significantly, the proportion of total CD44+ CD25− DN1 cells was increased with many cells staining weakly for CD44 (Fig. 1C, upper cytograms). As expected (37), additional analysis indicated that these CD44high/CD25− cells were CD19+ B cells comprising a mixture of B220+ IgM+ pre-BII and B220+ IgMlow immature B cells. CD19+ B cells in the thymus of CD3ε−/− mice were mature IgM+ cells (Fig. 1C, lower cytograms). Taken together, these phenotypic and quantitative PCR data indicate that in IL-7Tg.CD3ε−/− mice, BM B cell development was drastically altered, and immature B cells were present in the blood, spleen, and even the thymus. These changes reflect the increased IL-7 availability in these mice.

Increase of IL-7 availability promotes polyclonal T cell recovery in vivo

To carefully characterize the contribution of IL-7 to T cell homeostasis, unirradiated IL-7Tg.CD3ε−/− and their CD3ε−/− littermates received i.v. 10^7 pooled LN and spleen lymphocytes, containing ~2–3 × 10^6 CD4+ and CD8+ T cells. Recipients were sacrificed between 1 and 28 days after transfer. Fig. 2A shows that after adoptive transfer, both CD4+ and CD8+ T cells persisted in the spleen of IL-7-responsive recipient mice. Cell numbers increased slowly up to day 3 and thereafter more rapidly. At day 28 post-transfer, the CD3ε−/− spleen contained ~15 × 10^6 CD4+ and 5 × 10^6 CD8+ T cells, respectively, or a >10- to 15-fold expansion of T cells.

**FIGURE 1.** IL-7 overexpression perturbs B cell lymphopoiesis. A, BM cells were three-color stained as described in Materials and Methods. Bars show the average (n = 3) cell number (left ordinates) of the indicated subpopulation. III, Ratio of each subpopulation in IL-7Tg.CD3ε−/− vs CD3ε−/− mice (right ordinates). B, Spleen cells were three-color stained with anti-CD19, anti-B220, and anti-IgM or anti-CD19, anti-CD21, and anti-CD23. **Upper cytograms,** B220 vs IgM; **lower cytograms,** CD23 vs CD21 profiles of gated CD19+ cells. **Right bar graph,** Mean number and ratios of the indicated subpopulations. Imm.B, Immature B cells; FB, follicular B; MZB, marginal zone B. C. Thymus cells were four-color stained with anti-CD25, anti-CD44, anti-B220, and anti-IgM. Upper cytograms show CD25 vs CD44 profiles of all live cells; lower cytograms show the B220 vs IgM staining of cells gated for intermediate CD44-expressing cells (round gate in upper cytograms). Right bar graph shows mean number and ratios of the indicated subpopulations. In each cytogram display, numbers in quadrants represent the percentage of positive cells. Results are representative of at least five experiments.
the transferred cells between days 1 and 28. Thus, numbers are almost similar to those in the spleen of a normal B6 mouse.

Overexpression of IL-7 in CD3ε−/− mice had a significant effect on the behavior of transferred T cells. Thus, we observed a greater rate of expansion of both CD4+ and CD8+ T cells in IL-7Tg.CD3ε−/− recipient mice and also an increase in long term cell recovery. Therefore, from day 3 and up to day 28, IL-7Tg.CD3ε−/− contained more CD4+ and CD8+ T cells than their CD3ε−/− controls with lymphocyte numbers reaching a plateau at about day 14. At day 28, the spleen of IL-7Tg.CD3ε−/− recipients contained ∼52 × 10^6 CD4+ and 17 × 10^6 CD8+ T cells, representing a 3- to 4-fold higher expansion compared with control CD3ε−/− recipients (Fig. 2A). A similar pattern of growth and recovery was obtained in the LN (not shown). Thus, increased availability of IL-7 in vivo increases the yield of T cells after adoptive transfer.

Next, we investigated in more detail the in vivo impact of IL-7 overexpression on transferred T cells. To rule out a possible preferential and exacerbated monoclonal expansion of T cells, we analyzed their TCRα and TCRβ repertoire by flow cytometry. The repertoire of transferred T cells in both recipient mice was large, polyclonal, and comparable with that of T cells from unmanipulated B6 mice (not shown) with no obvious bias after expansion.

Then, we determined the phenotype of transferred T cells by four-color flow cytometry. Analysis of T cells during early phases of proliferation (days 3, 5, and 7 posttransfer) showed changes similar to those in other published reports (3); namely, cells became larger, as judged by forward light scatter, remained CD25− and CD69− and CD44+ (not shown). One month after transfer, CD4+ and CD8+ T cells had acquired a memory-like phenotype (3, 39–42) (Fig. 2B, left and middle cytograms) with most CD4+ T cells being CD44hiCD62Llow and many CD8+ T cells being CD44hiCD122hi. This phenotypic conversion that accompanies expansion of polyclonal T cells in both recipient mice was only slightly more pronounced in IL-7 Tg recipients. Additionally, we observed that ∼10% of the injected CD4+ T cells spontaneously express CD25. Therefore, among CD4+ cells, the proportion of CD25+ cells in both types of recipient mice remained the same with ∼10% of CD4+ T cells expressing CD25 at 28 days after transfer (Fig. 2B, right cytograms).

Localization of transferred cells in vivo

It has been shown that transferred T cells migrate to the periarteriolar lymphocyte sheaths (PALS) of the spleen where they presumably receive division and survival signals (43). To exclude that altered T cell recovery was due to a difference in spleen architecture, a confocal immunohistology study was conducted. Despite IL-7-dependent alteration of BM B cell development (Fig. 1B) and the presence of immature B cells, no difference in B cell follicle size or organization was observed in the spleen of IL-7Tg.CD3ε−/− mice (Fig. 3). At day 1 posttransfer (Fig. 3, a and b), CFSE+ cells were found in PALS of both recipients, with slightly more in the follicles of IL-7Tg.CD3ε−/− mice. At day 28 posttransfer, T cells had lost CFSE labeling but were identified using anti-CD90 (Thy-1) staining. Results indicated that transferred T cells were localized to T cell areas of follicles but that restoration of these areas in both recipients was only partial compared with B6 controls (Fig. 3, c–e). These findings suggest that T cells undergo LIPEC within a dedicated area inside B cell follicles (43) that presumably provides specific factors, including IL-7, necessary for maintaining T cell survival and proliferation.

IL-7 overexpression has differential proliferative effects on CD8+ and CD4+ subpopulations

Using CFSE labeling, we compared the proliferation of T cells in lymphopoenic hosts differing in IL-7 availability. Three days after transfer into CD3ε−/− recipients, few CD4+ cells had divided (Fig. 4A, left panels) and the average number of divisions was below 1. Over the same time period, CD8+ T cells had proliferated more, with an average of almost two divisions. In contrast, in IL-7Tg.CD3ε−/− recipients, a greater proportion of both CD4+ and CD8+ T cells had reduced CFSE fluorescence and had undergone between one and four rounds of division, respectively. That increasing IL-7 availability promoted LIPEC of CD8+ T cells was somewhat expected, but in contrast to other published reports (6, 7), there was also a clear effect of IL-7 on CD4+ T cells. At 5 days after transfer (Fig. 4A, right panels), T cells had undergone more rounds of division. Again CD8+ cells had divided more than CD4+ cells. This difference between CD8+ and CD4+ cells was maintained regardless of differences in IL-7 availability. Both T cell subpopulations proliferated more in IL-7Tg.CD3ε−/− vs CD3ε−/− littermates. Similar differences between CD8+ and CD4+ cell division were also seen when the cells were transferred to IL-7Tg.RAG-2−/− or RAG-2−/− littermates where the difference in IL-7 transcript levels was 3- to 5-fold (not shown). Again, because there was no up-regulation of CD69 or CD25 expression on either CD8+ or CD4+ T cells (not shown) this, together with the relatively slow kinetics of proliferation, indicated that LIPEC rather than activation-induced proliferation was being measured (39).
After 14 days, the number of transferred T cells had reached a plateau, and they became mostly CFSE negative. BrdU labeling experiments showed that after 28 days, the turnover of transferred T cells was similar in both recipients with ~30 and 20% of CD4+ and CD8+ T cells, respectively, incorporating BrdU (Fig. 4B). However, due to the overall increase in T cell recovery in IL-7Tg/Cd3−/− mice (3, 41, 42). As shown in Fig. 6, the thymus of both CD3ε−/− and IL-7Tg/Cd3ε−/− murine recipients contained neither CD4+CD8+ double-positive cells (Fig. 6, left panels) nor CD4+CD25+ (DN4) thymocytes (Fig. 6, right panels). This shows the absence of thymus reconstitution by T cell progenitors in unirradiated recipients and that the observed BrdU incorporation into peripheral T cells (Fig. 4C) was not the result of de novo

FIGURE 3. Transferred CFSE+ cells homed to PALS following transfer. Frozen spleen sections were stained as described in Materials and Methods. Recipient mice were killed 1 day (a and b) or 4 wk (c and d) after transfer of CFSE-labeled pooled LN + spleen cells. Sections were stained for B cells (red) with PE-conjugated anti-B220 mAb (a–e). CFSE+ donor cells (green) are situated almost exclusively in the periarteriolar lymphocyte sheath (PALS) (a and b). Four weeks after transfer (c and d), transferred T cells had lost CFSE staining, and T cells were stained with FITC-conjugated anti-CD90.2 (Thy1.2) mAb. The T area is not completely restored (c and d) compared with normal B6 mice (e). Sections a and c are from CD3ε−/− recipients, and sections b and d are from IL-7Tg/Cd3ε−/− recipients. Data are representative of one of three mice analyzed individually. Scale bar, 80 μm.

FIGURE 4. IL-7 promotes proliferation of polyclonal CD4+ and CD8+ cells in lymphopenic mice. A. CFSE profiles of gated CD4+ or CD8+ spleen cells 3 days (left panels) or 5 days (right panels) after transfer of 10^6 pooled labeled LN and spleen cells into CD3ε−/− (upper panels) or IL-7Tg/Cd3ε−/− (bottom panels) recipients. Similar results were obtained with LN cells (not shown). B. Cytoplasmic BrdU profiles of gated CD4+ (left histograms) or CD8+ (right histograms) cells 28 days after transfer and labeled for the last 5 days with BrdU as outlined in Materials and Methods. Within each panel, the percent of BrdU+ cells is indicated. Negative controls were <1% BrdU+. FL, Fluorescence.
Data are representative of two independent experiments. FL, Fluorescence.

In contrast, the early antiapoptotic action of IL-7 on transferred CD8$^+$ T cells was less pronounced (Fig. 7, A and C). In IL-7Tg.CD3e$^{-/-}$ recipients, 3 days posttransfer, there was only a 5–10% reduction in apoptosis for each round of division (Fig. 7A) and no increase in cytoplasmic Bcl-2 expression (Fig. 7C). However, by day 28, annexin V$^+$ cells had decreased (Fig. 7B), and Bcl-2 levels had increased in CD8$^+$ T cells and were even higher than in CD4$^+$ T cells (Fig. 7). Thus, the effects of increasing IL-7 availability have temporally independent effects on CD4$^+$ vs CD8$^+$ T cells. All the changes described above in polyclonal populations of CD8$^+$ lymphocytes were also observed with TCR-transgenic cells. Thus, when CD8$^+$ T cells from P14.RAG-2$^{-/-}$ mice were transferred to IL-7Tg.CD3e$^{-/-}$ or CD3e$^{-/-}$ recipients, proliferation was more rapid, apoptosis was decreased, expression of cytoplasmic Bcl-2 was increased, and cell recovery at 28 days increased 10-fold in IL-7Tg.CD3e$^{-/-}$ compared with CD3e$^{-/-}$ recipients (not shown).

The transferred CFSE-labeled cells contained B cells, but there did not appear to be an effect of IL-7 overexpression on their proliferation, survival, or Bcl-2 expression (Fig. 7C and data not shown) in either IL-7Tg.CD3e$^{-/-}$ or CD3e$^{-/-}$ recipients. In contrast, in completely lymphopenic Rag-2$^{-/-}$ or IL-7Tg.RAG-2$^{-/-}$ recipients, transferred B cells proliferated slowly but proliferation was similar in IL-7 transgenic and nontransgenic recipients (N. Bosco, unpublished observation).

Discussion

In this report, we have studied the behavior of polyclonal populations of lymphocytes transferred into novel T-lymphopenic IL-7-transgenic recipient mice (Figs. 1 and 2). Results obtained indicate that increasing IL-7 enhanced recovery of both CD4$^+$ and CD8$^+$ polyclonal T cells but had markedly different effects on proliferation (Figs. 4 and 5) and apoptosis (Fig. 7) of transferred cells depending on whether cells were actively undergoing LIP or surviving in a replenished peripheral compartment. The prediction had been made that in lymphopenic recipients, increasing IL-7 availability in vivo should increase the rate of T cell proliferation (27), but this hypothesis has not been directly addressed. Our in vivo results clearly demonstrate that during LIP, increasing IL-7 availability increases the rate of division of both CD4$^+$ and CD8$^+$ T cells but not of B cells.

The rate of proliferation of CD8$^+$ T cells, as measured by CFSE fluorescence, was enhanced by increasing IL-7 availability. This was true for both polyclonal and P14 TCR-transgenic T cells. The proliferation of CD4$^+$ cells, although intrinsically slower than that
of CD8+ cells, was also increased in IL-7Tg.CD3ε−/− recipients (Figs. 4 and 5). Although initial results had indicated that proliferation of CD4+ SP neonatal thymocytes in vitro (44) could be maintained in the presence of IL-7, subsequent reports had indicated that IL-7 had no effect on CD4+ T cell proliferation (6, 7), but this notion has been recently revised (27, 45, 46).

In IL-7Tg.CD3ε−/− recipients, Bcl-2 was up-regulated in both CD4+ and CD8+ T cells but with different kinetics (Fig. 7). Early after transfer, there was a distinct effect of IL-7 availability on Bcl-2 expression in CD4+ cells whereas CD8+ cells were not affected. Later on, Bcl-2 expression and apoptosis resistance were observed and were more pronounced in CD8+ T cells. In contrast, Bcl-2 levels in T cells transferred into CD3ε−/− recipients were similar to those of unmanipulated B6 controls. It has been shown that CD8+ but not CD4+ memory cells overexpress Bcl-2 protein (47). Phenotypic analysis indicated that after LIP, in both transgenic and nontransgenic recipients, T cells were enriched in so-called memory-like cells (3). Differences in the proportion of memory-like cells within surviving cells were minor, yet there was a clear increase in cytoplasmic Bcl-2 expression (Figs. 2 and 7). This indicates that changes in Bcl-2 levels were IL-7 dependent and not the result of an altered cellular composition of CD4+ or CD8+ T cell subsets. Even so-called naive CD4+ CD45RBhigh and CD8+ CD44low T cells respond to increased IL-7 availability (Fig. 5A) and undergo naive to memory-like phenotypic conversion after transfer into lymphopenic hosts (Fig. 5B and Refs. 3 and 48). Although sharing certain properties with true memory cells, it is generally agreed that the slower kinetics of induction of effector functions by memory-like cells clearly distinguishes them from true memory cells (48–50). It could be either that IL-7 sustains naive T cell differentiation into memory cells or that IL-7 promotes selection of memory CD8+ subset among the transferred cells that then outcompete naive T cells in LIP. The latter hypothesis would be in agreement with our previous report describing an increase of naturally arising CD8+ memory cells in nonimmunized IL-7Tg.B6 mice (26). Importantly, the combined effects of decreasing annexin V staining and increasing cytoplasmic Bcl-2 expression could account for the increased T cell yield in IL-7-transgenic recipients. This reproducible difference in the degree of apoptosis is important if we consider the exponential growth of T cells during LIP. Decreasing cell loss by 10 or 15% at each division can lead to a 50% increase in cell recovery after eight or five divisions, respectively. Indeed, cell recovery is a balance between the rate of cell proliferation and cell loss. The inoculum used in most of our experiments contained twice as many CD4 as CD8 T cells, and this difference may partially explain the increased recovery of CD4+ vs CD8+ T cells 1 month after transfer. In addition, as shown in Fig. 7, there is a major effect of IL-7 overexpression on Bcl-2 expression by CD4+ but not CD8+ T cells early after transfer. This is likely to have major consequences on overall cell recovery. This could explain why CD4+ T cell recovery is higher than CD8+ cells despite their slower proliferation rate.

One month after transfer, when the T cell compartment had reached equilibrium, there was no difference in overall turnover of CD4+ or CD8+ T cells, whereas the total number of T cells was 4 times higher in IL-7Tg.CD3ε−/− recipients (Figs. 2 and 4). That increasing IL-7 availability per se does not increase turnover of the established T cell pool is consistent with studies in IL-7Tg.B6 mice (26) (N. Bosco, unpublished observation) in which the proportion of dividing CD4+ or CD8+ T cells is similar to that of
nontransgenic controls. However, given the increased pool size of T cells, the number of dividing cells is proportionately higher, and equilibrium at this elevated cell number is maintained by balanced proliferation and simultaneous cell loss.

The IL-7R signal transduction cascade has been shown to activate expression of Bcl-2 family genes (51). We showed a temporal difference in Bcl-2 expression between CD4+ and CD8+ T cells in response to increased availability of IL-7 (Fig. 7). As recently reported (52), the signaling cascade downstream of the IL-7R may be differentially regulated between CD4+ and CD8+ cells. IL-7Rα gene transcription is suppressed in response to IL-7 signaling, a process that involves different molecular mechanisms in CD4+ vs CD8+ T cells. CD8+ T cells use the transcriptional repressor GF11; the transcriptional repressor used by CD4+ T cells remains to be identified. If major differences exist in the repertoire of transcription activators and repressors used by different T cell subsets after IL-7R engagement, these factors could constitute important targets to control selectively T cell responses to various cytokines and therefore T cell homeostasis.

T cells injected into lymphopenic animals migrated into lymphoid follicles, and reconstituted their T cell areas. Restricted homing of transferred T cells into lymphoid organs suggests that lymphocyte migratory properties are crucial for the initiation of LIP. Pertussis toxin treatment of donor cells abrogates G protein-dependent migration of T cells and reduces LIP in recipient mice (43). IL-7 and additional resources, including chemokines, are present inside lymphoid follicles, and the concentration of these factors is presumably increased in lymphopenic conditions (23). This may be how T cells sense lymphopenia and how LIP is triggered. Experiments in vitro have shown that IL-7 decreases the activation threshold of T cells, thereby serving as a “cofactor” for activation (45). Our results confirm that a similar mechanism is presumably increased in lymphopenic conditions (23). This may be how T cells sense lymphopenia and how LIP is triggered. Experiments in vitro have shown that IL-7 decreases the activation threshold of T cells, thereby serving as a “cofactor” for activation (45). Our results confirm that a similar mechanism might be operational in vivo, thereby promoting LIP. The cells that deliver the signals promoting LIP remain to be defined. However, dendritic cells are good candidates because some of them localize inside lymphoid follicles, express MHC molecules, secrete cytokines, and sustain LIP in vitro (53).

Where does T cell proliferation during LIP occur? Most transferred cells localize to the PALS of lymphoid follicles (Fig. 3), the anatomical site where most proliferation occurs (43). However, it is unclear whether cells remain fixed in the spleen during LIP. Early after cell transfer, CFSE+CD4+ and CD8+ SP cells were found in the thymus of recipients, and their CFSE profiles mirrored those of peripheral T cells. Quantitative RT-PCR and analysis of thymic B cell phenotype together indicated that the thymus was enriched in IL-7 (Fig. 1C and N. Bosco, unpublished observation). That CFSE+ T cells were present in the thymus indicated that their survival could be maintained in anatomical sites rich in IL-7. Their presence in the thymus could also indicate that cells circulate during LIP. A homeostatic niche supporting T cell LIP could be simply defined as a location where the trophic factors are available. These locations for T cells are somehow flexible, a situation analogous to that described for B cells by Agenès and Freitas (54).

Transfer of B lymphocytes into IL-7r.Tg.CD3e−/− or IL-7r.Tg.Rag-2−/− recipients indicated that IL-7 was not involved in B cell LIP (not shown). This is consistent with our previous data using IL-7r.Tg.B6 mice, where the increase in mature B cell compartments is simply a consequence of increasing BM B lymphopoiesis and not due to a role for IL-7 in mature B cell survival (32). Thus, as previously reported, T and B cell homeostasis are independent of one another and depend on different resources (55).

In conclusion, we describe for the first time the pleiotropic effects of IL-7 during and after lymphocyte polyclonal LIP. We show that increasing IL-7 availability has effects on both CD4+ and CD8+ T cells but not on B cells. These results are relevant to the clinical settings in which IL-7 is being used as an adjunct for hemopoietic, in particular lymphocyte reconstitution, after BM transfer (10). Much less information is available on the role of IL-7 in human T cell LIP, but recent advances, including the generation of humanized RAG-1−/−, γc−/− mice reconstituted with human cord blood cells (56) could provide opportunities to explore the effect of human IL-7 on adaptively transferred human lymphocytes.

Acknowledgments

We thank Patrice Marche and Institut National de la Santé et de la Recherche Médicale for their support; Dr. Jörg Kirberg, Max Planck Institute for Immunobiology, Freiburg for P14.RAG-2−/− mice; Dr. Ton Rolink for invaluable help; and Drs. Simon Fillatreau and Didier Grunwald for confocal microscopy. We thank Eve Borel for mouse maintenance and Véronique Collignon for cell sorting. We thank Drs. Serge Candéas and Christophe Viret for their comments and constructive criticisms of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References


