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Elevated IL-7 Availability Does Not Account for T Cell Proliferation in Moderate Lymphopenia

Lisa Colleen Osborne,* Daniel Timothy Patton,* Jung Hee Seo,* and Ninan Abraham*†

Lymphopenia-induced proliferation (LIP) is a proliferative program initiated in response to T cell insufficiency caused by acute or chronic immunodepletion. Studies of lymphopenic mice have demonstrated that the cytokine IL-7 and TCR signaling are critical for LIP. We examined how these two factors impact T cell proliferation following transfer into moderately lymphopenic mice. In this study, we show that moderate lymphopenia (~25% of wild-type lymphocytes) of IL-7Rα knock-in mutant (IL-7Rα<sup>449F</sup>) mice supports T cell proliferation, although with decreased frequency and kinetics compared with cells transferred to severely lymphopenic (5% of wild-type lymphocytes) IL-7Rα<sup>−/−</sup> hosts. Although previous studies have demonstrated that elevated IL-7 levels play an important role in LIP, IL-7 availability was not elevated in IL-7Rα<sup>449F</sup> mice. However, moderate lymphopenia increased access of transferred T cells to self-peptide presented on APCs that can trigger TCR signaling and proliferation. Importantly, we did not detect significant changes in TCR Vβ usage of proliferated T cells recovered from either moderately or severely lymphopenic hosts. Our work demonstrates that polyclonal T cells retain a diverse TCR repertoire following proliferation mediated by either self-peptide–MHC interaction alone or in combination with IL-7, and that T cell reconstitution is most efficient in the presence of increased IL-7 availability. The Journal of Immunology, 2011, 186: 1981–1988.

Numerous situations may induce lymphopenia in the course of a lifetime. Primary immunodeficiencies, viral infection (such as in the case of HIV), radiation, chemotherapy, aging, and thymectomy can all result in profound lymphopenia and leave patients susceptible to infection. In these situations, efficient T cell reconstitution is imperative to restore cell-mediated immunity. In adult patients, T cell reconstitution is especially difficult owing to age-related thymic involution and decreased thymic output (1). In these cases, treatment with cytokines such as IL-7 is being examined for its clinical application to enhance T cell reconstitution (2–4). There are a number of factors that must be considered prior to clinical application of in vivo T cell restoration protocols. Potential drawbacks include spurring unregulated cell growth, leading to tumor development, clonal expansion, and activation of self-reactive T cells. Thus, information regarding safe, rapid T cell expansion that limits tumor and autoimmune potential while maintaining T cell function is necessary.

In lymphoreplete conditions, the naive T cell pool and TCR repertoire diversity are maintained by a combination of thymic output and low level peripheral T cell division, ensuring fitness for recognition of diverse antigenic stimuli (5, 6). Naive T cell survival requires signaling from IL-7 and the TCR. Due to competition for limiting amounts of IL-7 and interaction with MHC-presenting self-peptides, T cells receive low intensity signals and do not become overtly activated. In severely lymphopenic states, however, T cell competition for these factors is reduced and a program of T cell proliferation termed lymphopenia-induced proliferation (LIP) can be initiated to restore T cell numbers. The presence of IL-7 and the ability of T cells to interact with self-peptide presented on MHC by APCs are required for LIP (7–14). However, LIP results in conversion of naive T cells (CD4<sup>+</sup>/CD8<sup>+</sup>) into memory phenotype (CD4<sup>+</sup>/CD8<sup>+</sup>) T cells (15, 16). Memory phenotype (MP) T cells are more easily activated than naive T cells and no longer require costimulation for acquisition of effector function (8). Because LIP is mediated by interaction with self-peptides and results in accumulation of MP T cells with decreased activation requirements, it has been proposed that this could increase the risk of autoimmune disease development or narrowing of the TCR repertoire and decreased cell-mediated immunity (17, 18). It is essential to determine how to harness LIP for rapid T cell reconstitution while maintaining immune potential following lymphopenic episodes in patients (2).

Although the effects of severe lymphopenia are well studied, there are few reports analyzing the effect of moderate lymphopenia on peripheral T cell expansion. This is of interest in situations following long-term therapeutic toxicity or HIV infection, where immunodeficiency in situ is long-lasting but incomplete. To examine the effect of chronic moderate lymphopenia on T cell proliferation, we analyzed the proliferative capacity and characteristics of naive polyclonal wild-type (WT) T cells after transfer into IL-7Rα<sup>449F</sup> (moderate, T cell counts ~ 25% of WT) and IL-7Rα<sup>−/−</sup> (severe, T cell counts < 5% of WT) lymphopenic hosts (19, 20). Our data demonstrated that moderate lymphopenia supports proliferation of naive T cells, but at a much slower rate than is observed in IL-7Rα<sup>−/−</sup> hosts. Importantly, this was not dependent on elevated levels of available IL-7, but instead correlated with increased access to APCs. Furthermore, our data suggest that T cell repertoire diversity is maintained following proliferation. These data provide evidence that T cell reconstitution can occur...
in conditions of chronic moderate lymphopenia without limiting TCR diversity.

Materials and Methods

Mice

C57BL/6, B6.SJL-Pepc<sup>a</sup> Pepc<sup>b</sup> (CD45.1<sup>a</sup>), and IL-7Rα<sup>a</sup> mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME). IL-7Rα<sup>a</supeness mice were generated by homologous recombination to express the mutant receptor under control of the endogenous promoter and backcrossed to C57BL/6 at least 12 generations in our facility (19). Transgenic (Tg) IL-7 mice (IL-7 overexpressed in B and T cells by a modified IgH chain enhancer and promoter) (21) were backcrossed over 20 generations with C57BL/6 mice. Tg IL-7 and IL-7Rα<sup>a</sup> mice were cross-bred to generate IL-7Rα<sup>a</sup> mice that express the IL-7 transgenic and the mutant IL-7Rα. All mice were bred and maintained at the University of British Columbia, and experiments were performed in compliance with University Animal Care Committee and Canadian Council of Animal Care guidelines and approval.

Abs

Directly conjugated and biotinylated Abs were obtained from BD Pharmingen (Mississauga, Ontario, Canada) (anti-CD3ε, CD4, CD8α, CD11b, CD11c, CD44, CD45.1, TCR Vβ panel, BrdU) or eBioscience (San Diego, CA) (anti-CD45.2, IL-7Rα).

Flow cytometry

Surface staining for lymphocyte characterization was carried out according to standard procedures. Samples were collected on an LSR II cytometer (BD Biosciences, Mississauga, Ontario, Canada) and data were analyzed with FlowJo software (Tree Star, Ashland, OR).

Analysis of LIP

Naive T cells were isolated from the spleens and lymph nodes of 6- to 10-wk-old CD45.1<sup>a</sup> mice using the EasySep mouse T cell enrichment kit (StemCell Technologies, Vancouver, British Columbia, Canada) according to the manufacturer’s instructions. Anti-CD44 biotin was added to the depletion mixture when enriching for CD44<sup>a</sup> T cells. Cells were labeled with CFSE as previously described (19). Purified, labeled cells were transferred into age- and sex-matched unirradiated host mice (see figure legends for cell numbers transferred). After the indicated time points, total cell numbers and CFSE dilution profile were determined by flow cytometry.

IL-7 quantification

Analysis of in vivo CD127 regulation was performed as previously described (22). Briefly, 5 × 10<sup>6</sup> CD45.1<sup>a</sup> T cells were transferred i.v. into WT, IL-7Rα<sup>a</sup> mice, or IL-7Rα<sup>a</sup> hosts. Twenty hours later, spleens were isolated and CD127 surface expression on CD45.1<sup>a</sup> T cells was analyzed by flow cytometry. For splenic IL-7, spleens were harvested into cold PBS plus 4% FBS plus 2 mM EDTA. Organ weight was recorded and then placed in a 55°C heat block for 1–2 h. Samples were centrifuged at 16,000 <i>g</i> for 3 min. Spleen radioactivity was sampled in duplicate using a mouse IL-7 Quantikine kit (R&D Systems, Burlington, Ontario, Canada). After overnight incubation on the ELISA plate, the rest of the assay was carried out according to the manufacturer’s instructions.

Quantitative RT-PCR analysis of IL-7Ra message

Splenocytes harvested from WT or IL-7Rx<sup>449F</sup> mice were pooled, and total T cells were recovered using an EasySep mouse T cell enrichment kit (StemCell Technologies). Purified T cells (2 × 10<sup>6</sup>) were stimulated with 10 ng/ml IL-7 for either 6 or 24 h followed by immediate lysis in TRizol (Invitrogen) and RNA was extracted according to the manufacturer’s instructions. cDNA was created using a SuperScript III kit (Invitrogen), and the quantitative PCR was set up in duplicate using SsoFast Eva Green Master mix and a CFX96 PCR machine (Bio-Rad) using the following primer pairs: IL-7Ra sense, 5′-GGA TGG ACA GCT AGA TG-3′, antisense, 5′-GAG TTA GGC ATT TCA CTC GT-3′ (23); β-actin sense, 5′-AGT AAG GAC CTG ACT TCT C-3′; antisense, 5′-TGT GAT TTT CGC TTT C-3′ (24). An initial denaturation at 95°C for 30 s was followed by 40 cycles of 95°C for 5 s and 55°C for 5 s and a final melt curve from 65 to 95°C.

TCR Vβ analysis following LIP

After 4–5 wk of in vivo incubation, total splenic T cells were purified using an EasySep mouse T cell enrichment Kit (StemCell Technologies). Isolated cells were quantified and labeled with Abs to CD45.1, CD45.2, CD3, CD4, CD8, and a panel of TCR Vβ-chains.

Statistical analysis

All data except survival curves are presented as means ± SD and analyzed by a Student t test or one-way, repeated measures ANOVA followed by a Tukey post hoc test, as appropriate. Significance was set at <i>p</i> ≤ 0.05.

Results

Chronic moderate lymphopenia in IL-7Rx<sup>449F</sup> mice supports LIP

To determine whether the moderate lymphopenia observed in IL-7Rx<sup>449F</sup> mice affected LIP of transferred cells, we purified T cells from naive CD45.1<sup>a</sup> mice and transferred them i.v. to WT, IL-7Rα<sup>a</sup> mice, or IL-7Rα<sup>a</sup> hosts. Following 1 wk in vivo incubation, the total cell numbers and CFSE dilution profile of recovered CD45.1<sup>a</sup> cells were determined. As expected, the severe lymphopenia of IL-7Rα<sup>a</sup> host mice supported extensive cell division and accumulated significantly more transferred cells than did lymphoreplete WT hosts (Fig. 1A, 1B). At this time point, the moderate lymphopenia in IL-7Rα<sup>a</sup> hosts appeared insufficient to provide enough of the required factors, either IL-7 availability or self-peptide–MHC interactions, to support LIP since CFSE was not diluted and transferred CD45.1<sup>a</sup> cells did not accumulate (Fig. 1A, 1B). However, when in vivo incubation was increased to 4 wk, 38% of CD4 and 61% of CD8 T cells had undergone at least one division in IL-7Rx<sup>449F</sup> hosts whereas <10% of T cells from WT hosts had diluted CFSE (Fig. 1C, 1D). This level of proliferation was not as extensive as the division that occurred in IL-7Rα<sup>a</sup>-<sup>e</sup>-<sup>b</sup> hosts (all cells had undergone at least one division and >90% had completely diluted CFSE), but the moderate lymphopenia supported T cell proliferation and CD8 accumulation in a way that WT hosts did not. These results were confirmed by quantification of the recovered CD45.1<sup>a</sup> T cells (Fig. 1D). A previous report analyzing transient T lymphopenia demonstrated that in order for transferred T cells to proliferate, at least 90% of host T cells had to be depleted, and the slowly proliferating T cells maintained a CD44<sup>a</sup> naive phenotype (24). In contrast, in our model of chronic moderate lymphopenia, proliferating CD45.1<sup>a</sup> T cells readily acquired a CD44<sup>a</sup> memory-like phenotype, similar to what was observed on cells recovered from IL-7Rα<sup>a</sup>-<sup>e</sup>-<sup>b</sup> hosts (Fig. 1E). Thus, these data show that chronic moderate lymphopenia can support LIP with a similar phenotypic outcome to IL-7Rα<sup>a</sup>-<sup>e</sup>-<sup>b</sup> hosts, but with constrained kinetics.

IL-7 availability is sufficient to induce T cell proliferation, but it does not account for proliferation in IL-7Rx<sup>449F</sup> hosts

To address the hypothesis that increased IL-7 concentration is sufficient to drive T cell proliferation, we tested the ability of IL-7 overexpressing T cells from CD45.2<sup>a</sup> Tg IL-7 mice (21) to proliferate in a lymphoreplete (WT CD45.1<sup>a</sup>) host. Owing to the competitive environment, T cell proliferation was slow, but after 2 wk in vivo, Tg IL-7<sup>a</sup> T cells bearing the WT IL-7R (Tg IL-7R<sup>a</sup> mice) had significantly diluted CFSE (Fig. 2A). These findings are consistent with previous data demonstrating that elevated IL-7 or treatment with agonistic IL-7–α-IL-7 complexes are sufficient to drive T cell proliferation in the absence of lymphopenia (9, 25). In agreement with our previous report (19), T cell proliferation required IL-7Rα<sup>a</sup> Y449-mediated signals, as evidenced by failure of Tg IL-7<sup>a</sup> Tg IL-7Rα<sup>a</sup> T cells to dilute CFSE (Fig. 2A). Extending the in vivo incubation to 4 or 12 wk increased the CFSE dilution.
and CD8 T cell recovery of Tg IL-7;IL-7Rα449F T cells whereas very few Tg IL-7;IL-7Rα449F T cells were detected and those that were did not dilute CFSE (Fig. 2B and data not shown). In fact, at the 12 wk time point, Tg IL-7;IL-7Rα449F T cells could not be detected whereas Tg IL-7;IL-7Rα+/+ T cells had diluted CFSE to near extinction (data not shown), indicating that IL-7Rα Y449 is necessary not only for proliferation but also for T cell maintenance. Taken together, these data suggest that even in a lymphopenic host where competition for APC interaction is strong, IL-7 signaling is sufficient to drive T cell proliferation.

We then examined whether there was a measurable difference in the amount of bioavailable IL-7 in WT, IL-7Rα449F, and IL-7Rα−/− mice. When naive T cells are exposed to IL-7, IL-7Rα is down-regulated (26). As a nonquantitative measure of IL-7 availability, we transferred naive (CD44lo) CD45.1+ T cells into WT, IL-7Rα449F, and IL-7Rα−/− hosts i.v. after 4 wk, cells were analyzed for CFSE dilution and CD44 surface expression. Gated boxes show the percentage of cells that have undergone at least one division. A, C, and E. Flow cytometry plots are gated on live CD45.1+ CD4+ or CD8 T cells. Representative data from two to three independent experiments with three to five mice per genotype are shown. *p ≤ 0.05.

FIGURE 1. Chronic moderate lymphopenia supports slow T cell proliferation. Purified CFSE-labeled T cells (5 × 10⁶) from naive CD45.1+ mice were transferred i.v. into age- and sex-matched CD45.2+ WT, IL-7Rα449F, or IL-7Rα−/− hosts. After 1 wk (A, B) or 1 mo (C, D) in vivo incubation, CD45.1+ T cells were quantified and analyzed for CFSE dilution by flow cytometry. E. CD44lo T cells (5 × 10⁶) were purified from 6-wk-old CD45.1+ mice, CFSE labeled, and transferred i.v. into age- and sex-matched WT, IL-7Rα449F, or IL-7Rα−/− hosts. After 4 wk, cells were analyzed for CFSE dilution and CD44 surface expression. Gated boxes show the percentage of cells that have undergone at least one division. A, C, and E. Flow cytometry plots are gated on live CD45.1+ CD44+ CD3+ CD4 or CD8 T cells. Representative data from two to three independent experiments with three to five mice per genotype are shown. *p ≤ 0.05.
$7\text{R}^{449F}$ hosts showed minimal decreases in CD127 expression, and it was retained at significantly higher levels than on T cells recovered from IL-7R$^{a2a2}$ hosts (Fig. 3A). This result was confirmed by quantitative analysis of splenic IL-7 concentration. Stromal support cells in secondary lymphoid organs constitutively express IL-7 at very low levels, making protein detection difficult. To measure splenic IL-7, we dispersed entire spleens in collagenase IV to liberate IL-7 from cell surfaces or the extracellular matrix. To normalize for different spleen sizes caused by lymphopenia in IL-7R$^{a449F}$ and IL-7R$^{a2a2}$ mice, IL-7 concentration was expressed per milligram wet spleen weight. This analysis showed that IL-7 availability was elevated in IL-7R$^{a2a2}$ spleen tissue, but the amount of IL-7 detected was not statistically different between WT and IL-7R$^{a449F}$ spleens (Fig. 3B). Thus, in two separate assays, increased IL-7 availability could not be detected in the spleens of IL-7R$^{a449F}$ mice because IL-7R$^{a}$ expressing cells that bind IL-7 are acting as a cytokine sink. Failure to downregulate the receptor may lead to disrupted cytokine regulation. A previous report has demonstrated that IL-7R$^{a}$ expression on memory phenotype and regulatory T cells can limit peripheral T cell expansion (24). Both of these cell types are present in IL-7R$^{a449F}$ mice, and failure to down-regulate IL-7R$^{a}$ surface expression could contribute to further IL-7 sequestration and inhibit proliferation of transferred T cells.

T cell proliferation in IL-7R$^{a449F}$ mice correlates with increased access to APCs and self-peptide–MHC interactions

Because self-peptide–MHC interactions are a key requirement for LIP (12, 15), the TCR repertoire of peripheral T cell pools of WT and IL-7R$^{a449F}$ mice is an important factor. If IL-7R$^{a449F}$ hosts have a significant alteration in their TCR repertoire, this could lead to decreased competition for self-peptide recognition and an advantage for transferred WT CD45.1$^+$ T cells in IL-7R$^{a449F}$ hosts. Analysis of TCR repertoire by surface staining of TCR V$\beta$-chains showed that there was minimal difference in TCR V$\beta$ representation between WT and IL-7R$^{a449F}$ T cells (Fig. 4A). Thus, the IL-7R$^{a449F}$ mutation does not significantly affect TCR selection. In the absence of TCR repertoire deficiencies, it is unlikely that transferred CD45.1$^+$ T cells are proliferating in response to self-peptides that IL-7R$^{a449F}$ T cells are incapable of recognizing.

Because neither IL-7 availability nor TCR V$\beta$ usage appeared altered in IL-7R$^{a449F}$ mice, we evaluated whether APCs could be more accessible to transferred T cells. To address this question, we characterized and quantified splenic APCs based on surface
expression of CD11b and CD11c in WT and IL-7Rα449F mice. These analyses showed no difference in the absolute number of APCs between WT and IL-7Rα449F mice (Fig. 4B). However, there are far fewer host T cells in IL-7Rα449F mice, and this decreased competition for APC interaction may influence CD45.1+ T cell proliferation. Comparing the ratio of the absolute numbers of T cells to APCs in WT and IL-7Rα449F hosts showed that there are significantly fewer T cells per APC in IL-7Rα449F hosts (Fig. 4C).
4C). The increased availability of self-peptide–MHC interactions may be sufficient to drive the CD45.1+ T cell proliferation seen in IL-7Rα<sup>449F</sup> hosts.

To determine whether increased competition for APC access could inhibit T cell proliferation, we compared CFSE dilution of WT CD45.1+ T cells that were transferred into IL-7Rα<sup>449F</sup> hosts at either a low dose (5 × 10<sup>6</sup> T cells) or a high dose (20 × 10<sup>6</sup> T cells). In agreement with our hypothesis that increased T cell numbers would interfere with proliferation, CD8 T cells transferred at a high dose failed to dilute CFSE (31.8 ± 1.6% undivided) compared with CD8 T cells transferred at the low dose (19.4 ± 4.1% undivided, p < 0.01). Furthermore, of the CD8 T cells that started to proliferate, those transferred at the high dose did not dilute CFSE to the same extent as did those transferred at the low dose (Fig. 4D). As expected, T cells transferred into lymphopreplete WT mice did not proliferate (Fig. 4D). Thus, in addition to IL-7, availability of APCs appears to play a role in regulating T cell homeostatic proliferation.

Transferred polyclonal T cells retain TCR Vβ diversity

Limiting dilution analysis of transferred T cells has demonstrated that LIP can result in clonal expansion of transferred T cells that receive both self-peptide/MHC and IL-7 signaling, and that this self-peptide–mediated clonal expansion conferred susceptibility to autoimmune pathology in the host mice (27). However, detection of oligoclonal expansion required transfer of very low T cell numbers (<1000), much less than what is routinely transferred in these types of analyses. To test whether transfer of nonlimiting T cell numbers are subject to the same TCR repertoire limitation, we transferred 1–2 × 10<sup>5</sup> purified T cells from naive CD45.1+ mice and assayed proliferation and TCR Vβ usage of transferred T cells after 4 wk in vivo incubation in WT, IL-7Rα<sup>449F</sup>, and IL-7Rα<sup>−/−</sup> hosts. TCR Vβ usage of recovered cells was compared with the Vβ profile of cells prior to transfer and to T cells from CD45.1+ littermates that had not been transferred to control for differences in TCR repertoire mediated by aging. These comparisons demonstrated that the TCR repertoire diversity was maintained in transferred CD45.1+ T cells following LIP in both IL-7Rα<sup>449F</sup> and IL-7Rα<sup>−/−</sup> hosts (Fig. 5). Further analysis of repertoire diversity by TCR Vβ spectratyping may also provide a more complete picture of potential clonal dominance or restriction. Overall, this showed that TCR repertoire diversity is maintained following T cell transfer and proliferation induced by both moderate and severe lymphopenia, suggesting that it does not pose a significant risk for autoimmune development.

Discussion

We undertook this study to address the question of how T cells respond when they encounter a moderately lymphopenic environment and found that moderate lymphopenia limits the proliferative capacity of transferred polyclonal T cells compared with severe lymphopenia. Because LIP is regulated by IL-7 availability and TCR signaling mediated by interaction with APCs presenting self-peptide, we analyzed their ability to support T cell proliferation in IL-7Rα<sup>449F</sup> hosts. Interestingly, IL-7 availability was not elevated in IL-7Rα<sup>449F</sup> mice, but the reduced T cell numbers decreased competition with the transferred T cells for access to APCs and self-peptide/MHC-mediated TCR signals. This shows that the level of IL-7 available in WT mice is sufficient to support T cell proliferation, consistent with its role as an essential mediator of naive T cell homeostasis. However, the significant increase in both the frequency and kinetics of T cell proliferation in IL-7<sup>−/−</sup> hosts suggests that IL-7 availability must be increased above this threshold for efficient T cell reconstitution.

Retention of TCR diversity is a desirable characteristic in T cell reconstitution following lymphopenia, as oligoclonal or monoclonal expansion is more likely to lead to autoimmune pathology mediated by self-peptide–selected T cells. The data presented in this study suggest that the proliferation evoked by either moderate or severe lymphopenia is unlikely to favor excessive self-reactivity. This is consistent with clinical studies showing that IL-7 treatment actually increased naive T cell numbers with a diverse TCR repertoire (28, 29). Furthermore, these results agree with conclusions from a previous study of partial lymphopenia (24). To model situations of transient partial lymphopenia, WT mice were rendered partially lymphopenic (~10% of host T cells remaining) via Ab-mediated T cell depletion (24). In this model, very little proliferation of transferred cells was detected, and the cells retained a naive phenotype, leading to the conclusion that transient partial lymphopenia did not predispose to autoimmune pathology. In our system of chronic moderate lymphopenia (~25% of host T cells remaining), which may be more similar to

![FIGURE 5.](http://www.jimmunol.org/) Lymphopenia-induced proliferation does not cause preferential monoclonal expansion of transferred T cells. Purified T cells from young CD45.1+ mice were transferred to CD45.2+ WT, IL-7Rα<sup>449F</sup>, and IL-7Rα<sup>−/−</sup> hosts. Following 1 mo in vivo incubation, flow cytometric TCR Vβ analysis was performed on CD45.1+ T cells. Each dot represents an individual WT, IL-7Rα<sup>449F</sup>, or IL-7Rα<sup>−/−</sup> host or CD45.1+ mouse.
that LIP can act as a cofactor for autoimmune diseases due to clonal T cells. Interestingly, despite previous reports suggesting cognate Ag (27, 33, 34) or mouse models with compromised demonstration that acquisition of effector function can significantly activation of autoreactive T cells, and these studies have clearly situations of moderate lymphopenia, a program of IL-7 treatment may be required for more rapid T cell reconstitution, and this can likely be achieved without the drawback of autoreactive T cell expansion.

However, these results do not rule out that IL-7 or lymphopenia can contribute to proliferation or accumulation of autoreactive T cells. Numerous studies have shown that lymphopenia is associated with autoimmune pathology or increased cytotoxicity of tumor-reactive clones (18, 30–33). Thus, the lymphopenic environment does have the potential to support expansion and activation of autoreactive T cells, and these studies have clearly demonstrated that acquisition of effector function can significantly affect disease outcome. However, most of these studies analyzed Ag-specific monoclonal T cell populations and their responses to cognate Ag (27, 33, 34) or mouse models with compromised regulatory T cell function (18). To more closely mimic human T cell transplantation, we chose to analyze the behavior of polyclonal T cells. Interestingly, despite previous reports suggesting that LIP can act as a cofactor for autoimmune diseases due to acquisition of an effector memory phenotype and interaction with self-peptides, our work suggests that polyclonal T cells, although they do take on a CD44hi phenotype, do not undergo clonal expansion that could result in autoimmune pathology mediated by self-peptide reactivity. Further studies of TCR transgenic T cells in the presence or absence of the cognate Ag could permit further insight into the role of increased self-peptide–MHC interactions on T cell proliferation in the absence of elevated IL-7 levels.

Our data on IL-7Rα−/− mice compared with IL-7Rα+ mice and from transplanted Tg IL-7 T cells show that the amount of bioavailable IL-7 has a significant impact on T cell proliferation. In agreement with the hypothesis that IL-7 provides proliferative signals, we detected significant expansion of Tg IL-7 T cells in lymphopnelecongenic WT hosts where T cell competition for self-peptide–MHC interactions is high but IL-7 is not limiting. Furthermore, in the presence of high IL-7 levels in IL-7Rα−/− hosts, WT CD45.1+ T cells underwent rapid expansion, but this was limited in moderately lymphopenic IL-7Rα+ hosts where IL-7 availability is not elevated. However, IL-7Rα+ hosts do support significantly more proliferation of transferred T cells than do WT hosts. This may be more accurately referred to as “enhanced homeostatic proliferation” since it appears to result from less competition for T cells to interact with self-peptide–presenting APCs. In agreement with this idea, increasing the number of APCs, either by adoptive transfer or cytokine treatment, has previously been shown to increase T cell proliferation in the absence of IL-7 changes (22, 35).

Owing to its roles in thymocyte development and peripheral T cell homeostasis, IL-7 has emerged as a candidate immunotherapeutic for increasing T cell number and function in lymphopenic patients. Two independent clinical trials have been conducted and both report increased naive CD4 and CD8 T cell numbers in IL-7–treated patients (28, 29). Interestingly, analysis of naive T cell TCR repertoire following IL-7–mediated expansion showed that repertoire contraction did not occur, and, in fact, repertoire diversity was increased (28, 29). Our results lend further support to the idea that IL-7 may be a useful immunotherapeutic for T cell reconstitution following clinical T cell depletion in autoimmune patients, HIV patients, or in aging populations.

In the clinical trials, human naive T cells were preferentially expanded and retained their naive phenotype upon IL-7 treatment. In contrast, in LIP experiments, purified murine naive T cells take on a CD44hi memory-like phenotype. This is an interesting discrepancy that warrants further examination. In lymphopenic environments, competition for cytokine and self-peptide–MHC interactions is decreased, and together these are responsible for the conversion of naive to memory-like phenotype. However, in non-lymphopenic patients, competition for MHC interaction would still be intact while IL-7 treatment would increase cytokine availability. In vitro, high IL-7 concentrations are sufficient to drive T cell proliferation in the absence of TCR stimulation (36). This type of cytokine-driven proliferation paired with increased cell survival may explain the accumulation of naive T cells without memory T cell conversion seen in clinical trials. It will be interesting to determine whether IL-7 treatment of severely lymphopenic patients will result in naive or memory-like T cell accumulation. Promisingly, our analysis suggests that TCR repertoire diversity will still be maintained, but care must be taken to monitor patients for signs of autoimmune pathology due to the association of IL-7Rα deregulation in such diseases.

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Disclosures

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