This information is current as of April 19, 2017.

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*J Immunol* 2002; 169:3752-3759; doi: 10.4049/jimmunol.169.7.3752

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TCR and IL-7 Receptor Signals Can Operate Independently or Synergize to Promote Lymphopenia-Induced Expansion of Naive T Cells

Benedict Seddon and Rose Zamoyska

TCR and cytokine signals induce naive T cells to undergo spontaneous divisions as part of a homeostatic response to conditions of T cell deficiency. The conditions under which these signals evoke the homeostatic response and their interaction with each other are poorly understood, and yet are very important clinically in considering strategies for immune reconstitution. Here, we show that p56
\(^{lck}\)-mediated TCR signals and IL-7R signals are each able to stimulate T cell proliferation in lymphopenic hosts independently of one another, but can also synergize to facilitate proliferation. Furthermore, the relative contribution to the homeostatic response by TCR and cytokine signals is not fixed and critically depends on both the degree of lymphopenia and specific characteristics of individual T cell clones. Finally, we show that only lck and not fyn can mediate the TCR-driven proliferation, while neither lck nor fyn is required for IL-7R-induced proliferation. The Journal of Immunology, 2002, 169: 3752–3759.

Cell populations in peripheral lymphoid organs are maintained at constant numbers as a consequence of tight homeostatic control (1). Thymic production and expansion of Ag-specific T cells in the periphery are balanced by the death of cells competing for limiting survival factors. Homeostasis of memory T cells is regulated by specific cytokines (2) that act to allow expansion of the memory pool and limit its size, but appears not to require interaction of the TCR with self-MHC Ags (3, 4). In contrast, maintenance of the naive T cell pool is dependent on both TCR and cytokine signals. It is now well established that interaction of the TCR with self-MHC ligands is required for prolonged survival of naive T cells (5–10). Additionally, cytokines whose receptors use the common \(\gamma\)-chain have been shown to be important in providing survival signals for naive T cells (11). Specifically, IL-7 has been implicated as an essential survival factor in vivo (12, 13).

Naive T cells also have the capacity to proliferate in response to conditions of lymphopenia, while largely maintaining a naive phenotype, as part of a homeostatic response to T cell deficiency. This occurs in mice following thymectomy (14), but is also seen following adoptive transfer of T cells into hosts rendered lymphopenic by sublethal irradiation or into hosts lacking T cells through genetic defects (8, 15–18). The signals that drive this homeostatic proliferation are dependent on both TCR and cytokine signals. It is now well established that interaction of the TCR with self-MHC ligands is required for prolonged survival of naive T cells (5–10). Additionally, cytokines whose receptors use the common \(\gamma\)-chain have been shown to be important in providing survival signals for naive T cells (11). Specifically, IL-7 has been implicated as an essential survival factor in vivo (12, 13).

Naive T cell survival, induction of homeostatic proliferation requires concurrent activation of both IL-7R and TCR signaling pathways.

Previous studies in this laboratory using mice bearing an inducible p56
\(^{lck}\) (lck) transgene (20) have shown that, in contrast to survival signals, the TCR signals that induce homeostatic proliferation have an absolute requirement for the expression of lck (21). In the present study we show that even when TCR signaling is abrogated by the absence of both src family kinases, lck and fyn, IL-7R-mediated signals are able to drive homeostatic proliferation under conditions of acute lymphopenia. Therefore, stimulation of naive T cells through either the TCR or IL-7R signaling pathway can independently result in homeostatic proliferation with distinct kinetics and duration. While the overall response is a synergy of these combined signals, the relative contribution of these pathways depends on both the degree of lymphopenia and the relative responsiveness of individual T cell clones to these two stimuli.

**Materials and Methods**

**Mice**

Generation of inducible lck transgenic mice has been described previously (20). Mice were fed doxycycline (dox) in food (1 mg/g) to maintain lck transgene expression. Lck
\(^{1}\mu m\)fyn
\(^{m}\) mice were derived by intercrossing rTA-C
\(^{\text{Cre}}\)/lck
\(^{m}\)/fyn
\(^{m}\) and Lck
\(^{1}\mu m\)/lck
\(^{m}\)/fyn
\(^{m}\) strains. F5
\(^{\text{Cre}}\)/recombinase-activating gene (RAG)
\(^{m}\), F5
\(^{\text{Cre}}\)/RAG
\(^{m}\)/fyn
\(^{m}\), lck
\(^{m}\)/fyn
\(^{m}\), lck
\(^{m}\)/fyn
\(^{m}\), RAG
\(^{m}\), class II
\(^{m}\)/RAG
\(^{m}\)/fyn
\(^{m}\), and C57BL/10 mice were bred in a conventional colony free of pathogens at the National Institute for Medical Research (London, U.K.).

**Purification, labeling, and adoptive transfer of T cells**

Lymphocytes were teased from lymph nodes of donor mice, and single-cell suspensions were prepared. For experiments requiring purified T cell preparations, lymphocytes were labeled with 100 \(\mu l/10^6\) cells of a mixture of anti-B220-biotin (BD PharMingen) at 1 μg/ml for 30 min at 4°C. After washing, cells were incubated with M280-streptavidin Dynal beads (Dynal, Chantilly, VA) for 30 min, after which cells were separated by application of a magnet. Contaminating class II MHC expressing or B220+ cells were consistently <1%. Cells were labeled with 1 μM CFSE (Molecular Probes, Eugene, OR).
development and, in contrast to lck biotin-IL-7R recipients lacking the expression of both lck and another src family kinase, p59

Flow cytometry
Flow cytometry was conducted using 10⁶ thymocytes or lymph node cells. Cells were incubated with saturating concentrations of Abs in 100 µl PBS/BSA (0.1% azide (1 mM) for 1 h at 4°C, followed by three washes in PBS/BSA/azide. Biotin-conjugated Ab labeling was developed with streptavidin-PE-Cy7 (Caltag, Burlingame, CA). For recipients of CFSE-labeled T cells, cell suspensions were prepared from superficial cervical, brachial, axillary, inguinal, and mesenteric lymph nodes of individual mice. Up to 3 × 10⁷ lymph node cells were labeled with the indicated Abs at saturating concentrations in 20 µl/10⁶ cells. The MAbs used in this study were as follows: PE-CD4 (GK1.5; BD PharMingen), PerCP-CD8α (53-6.7; BD Pharmingen), FITC-TCR (H57-597; BD Pharmingen), allophycocyanin-CD8 α (53-6.7; BD Pharmingen), biotin-IL-7R α (A7R34), allophycocyanin-B220 (RA3-6B2; BD Pharmingen), and PE-IGM (BD Pharmingen). Negative control samples for biotin-IL-7R α staining were performed by incubation of cells with unlabeled IL-7R α mAb before labeling with biotin-IL-7R α and streptavidin-PE-Cy7.

Four-color cytometry staining was analyzed on a FACS caliber instrument (BD Biosciences, Mountain View, CA), and data analysis was performed with CellQuest software (BD Biosciences).

Results
T cells transferred to T cell-deficient hosts proliferate independently of TCR signals
To assess the role of TCR signals in driving homeostatic proliferation of naive T cells, we examined the behavior of T cells from mice with an inducible lck transgene (20). These mice have an lck transgene under a tetracycline-sensitive promoter (Lck1) and a second tet-on trans-activator domain (rtTA-C) transgene under control of human CD2 regulatory elements, targeting expression to T cells. Since Lck1/rtTA-C/lck

OR in Dulbecco’s PBS (Life Technologies, Grand Island, NY) for 10 min at 37°C and washed twice. Cells were transferred into various recipient mice via tail vein injections. Treatment of recipient mice with purified αL-7R mAb (A7R34) in PBS was performed on a 7-day cycle of i.p. injections of 300 µg/mouse of mAb on days 1, 3, and 5 for the duration of the experiment. Controls received injections of PBS alone. Successful treatment with αL-7R Ab was determined by showing that B cell development was completely blocked in the bone marrow of recipient lck

After which dye levels are below the range of detection. Typically in these cases, 2 wk following transfer into “empty” hosts, both CD4⁺ and CD8⁺ WT T cells underwent a number of cell divisions (Fig. 1A, left column), and a similar pattern of cell divisions was observed in T cells from Lck1inds mice transferred to dox-fed recipients (Fig. 1A, middle column). Surprisingly, in the absence of continued lck expression (−dox, Fig. 1A, right columns) both CD4⁺ and CD8⁺ T cells from Lck1inds mice underwent significant proliferation, although this was generally less than that of WT T cells. Proliferation was not mediated by residual lck in these cells, as the low levels of lck expressed by Lck1inds peripheral T cells are completely lost within 48 h after dox removal (data not shown), while most proliferation in this situation is observed between days 7 and 14. Furthermore, cells from mice that had been off dox for 4 wk before transfer showed an equivalent number of divisions (Fig. 1A). While lck

We have shown previously that lck is required to transduce the TCR signals that induce homeostatic proliferation (21). Therefore, we considered that this lck-independent proliferation might not be TCR mediated. This possibility was assessed in two ways. Firstly, homeostatic proliferation of lck-deficient T cells in hosts lacking specific MHC ligands was assessed. Lymph node T cells from either WT or Lck1inds mice were rigorously depleted of class II expressing cells, CSFE labeled, transferred into dox-free class II MHCneg RAGneg recipients, which lack host T cells, class II MHC ligands, and NK cells, and were analyzed after 2 wk. Both lck-deficient and WT CD4⁺ T cells proliferated in an identical way in the absence of class II MHC ligands, while remaining CD44low over this time period (Fig. 1B). Secondly, the behavior of T cells from Lck1inds mice that also lack p59 (fyn) was assessed. In the absence of both transgenic lck and endogenous fyn expression, T cells fail to survive long term (29) due to an inability to transduce TCR-mediated survival signals. However, naive cells survive long enough to assess their proliferative response upon transfer into T cell-deficient recipients. T cells from either Lck1inds or Lck1inds fynneg mice were CSFE labeled and transferred into empty lckneg fynneg recipients in the absence of dox. After 2 wk, analysis of lymph nodes of recipient mice showed that CD4⁺ T cells from Lck1inds fynneg mice proliferated to an equivalent extent as T cells from Lck1inds mice despite lacking the expression of both lck and fyn (Fig. 1C). Together, these data clearly illustrate that homeostatic proliferation in T cell-deficient hosts can occur in the complete absence of TCR-mediated signals.

TCR and IL-7R signals drive homeostatic proliferation in T cell-deficient hosts
Recent studies have highlighted a role for IL-7 in the homeostatic proliferation of naive T cells (13, 19). Therefore, we asked whether lck-independent proliferation by Lck1inds T cells in T-deficient
hosts might be mediated by IL-7. T cells from WT and Lck\textsuperscript{1ind} mice were CFSE labeled and transferred into empty \textit{lck\textsuperscript{més/fyn\textsuperscript{neg}}} hosts. The effect of IL-7 on donor T cells was assessed by treating groups of recipient mice with repeated injections of an Ab that binds IL-7R (αIL-7R), thereby inhibiting the biological activity of IL-7 in vivo. We could confirm that the αIL-7R Ab was at saturating levels by showing that B cell development was completely blocked in the bone marrow of recipient \textit{lck\textsuperscript{més/fyn\textsuperscript{neg}}} mice. Immature B220\textsuperscript{high}IgM\textsuperscript{−} (R1) and B220\textsuperscript{low}IgM\textsuperscript{+} (R2) subsets were ablated compared with PBS-treated controls (Fig. 2A, right column), leaving only a population of mature B220\textsuperscript{high}IgM\textsuperscript{+} cells. Analysis of lymph node T cells from host mice 10 days after transfer showed that blockade of IL-7R resulted in a considerable reduction in the proliferation of WT cells, reflected by the increased size of the undivided peak, particularly in the CD4\textsuperscript{+} subset (Fig. 2A). Strikingly, proliferation of T cells from Lck\textsuperscript{1ind} mice in dox-free \textit{lck\textsuperscript{més/fyn\textsuperscript{neg}}} hosts was essentially abrogated by treatment with the αIL-7R mAb (Fig. 2B). Significantly, these data show that homeostatic proliferation of naive T cells in T cell-deficient hosts can be completely accounted for by a combination of \textit{lck} and IL-7R-mediated signals.

**Titation of T cell space in different combinations of \textit{lck} and \textit{fyn} deficiencies.**

The data described to date show that homeostatic proliferation can be induced by activation of the IL-7R signaling pathway independently of signals through the TCR. However, we showed previously that the presence of as little as 14% of the normal T cell cohort (i.e., in \textit{lck\textsuperscript{més}} hosts) prevented this expansion in the absence of \textit{lck}-mediated TCR signals (21). Therefore, we examined the relative contributions of TCR- and IL-7R-mediated signals under a range of lymphopenic conditions of increasing severity. CFSE-labeled T cells from WT and Lck\textsuperscript{1ind} mice were transferred into mice that were 1) WT for \textit{lck} and \textit{fyn} (47% T cells in peripheral LN), 2) \textit{lck\textsuperscript{més/fyn\textsuperscript{1+}}} (14% of WT T cell numbers), 3) \textit{lck\textsuperscript{més/fyn\textsuperscript{1−}}} (3% of WT T cell numbers), and 4) \textit{lck\textsuperscript{més/fyn\textsuperscript{neg}} (<1% of WT T cell numbers). Mice receiving Lck\textsuperscript{1ind} T cells were taken off dox to switch off \textit{lck} expression, and all recipients were analyzed on
day 33. As expected, WT T cells transferred into full \(\text{fyn}^{-/-}\ lck^{+/+}\) hosts did not undergo homeostatic proliferation (Fig. 3A), whereas the same cells transferred to the various \(\text{lck}^{\text{neg}}\) lymphopenic hosts proliferated in an increasing manner that reflected the degree of T cell deficiency in the recipient (Fig. 3, B–D). A useful indicator of the extent of proliferation was obtained from the relative size of the undivided peak in each of the hosts, which became smaller as host T cell numbers were reduced. Lck\(^{\text{ind}}\) T cells in which \(\text{lck}\) expression was lost. Groups of recipient mice were treated with repeated injections of an \(\text{oIL-7R}\) mAb, while controls received PBS. Ten days after cell transfer, lymph node cells of recipient mice were stained with CD4 and CD8, and CFSE staining was analyzed by FACS. Percentages are given for the fraction of CFSE-positive cells that remained undivided. Recoveries of CFSE-labeled cells were: WT CD4\(^+\) cells, 1.1%; WT CD8\(^+\) cells, 3.7%; \(\text{oIL-7R}\)-treated WT CD4\(^+\) cells, 0.5%; \(\text{oIL-7R}\)-treated WT CD8\(^+\) cells, 3%; \(\text{lck}^{\text{ind}}\) CD4\(^+\) cells, 2.5%; Lck\(^{\text{ind}}\) CD8\(^+\) cells, 2%; \(\text{oIL-7R}\)-treated Lck\(^{\text{ind}}\) CD4\(^+\) cells, 1.6%; \(\text{oIL-7R}\)-treated Lck\(^{\text{ind}}\) CD8\(^+\) cells, 2.4%. Bone marrow cells from femurs of recipient mice were extracted, and a sample was stained for the expression of B220 and IgM to confirm the efficacy of \(\text{oIL-7R}\) mAb treatment. Data are representative of two independent experiments.

By turning off \(\text{lck}\) expression in transferred Lck\(^{\text{ind}}\) cells, we could eliminate the TCR signal and assess the specific contribution of IL-7R-driven proliferation in the different hosts. In common with WT T cells, proliferation was greatest in the most lymphopenic \(\text{lck}^{\text{neg}}\) hosts (Fig. 3D). However, proliferation of T cells lacking \(\text{lck}\) was far more sensitive than WT cells to the presence of host T cells, such that as few as 3% of normal T cell numbers present in \(\text{lck}^{\text{neg}}\) hosts was sufficient to dramatically reduce IL-7-mediated proliferation (Fig. 3C). The presence of 14% of normal T cell numbers (\(\text{lck}^{\text{neg}}\) hosts) was sufficient to completely inhibit IL-7-driven proliferation (Fig. 3B).

### Competition for IL-7 limits the extent of IL-7R-driven proliferation

Since IL-7R-driven proliferation is sensitive to so few host T cells, we asked whether this sensitivity would ultimately limit the extent to which T cells could proliferate. We compared the kinetics of IL-7R- vs TCR-induced proliferation by transfer of CFSE-labeled T cells from WT and Lck\(^{\text{ind}}\) mice to dox-free \(\text{lck}^{\text{neg}}\) hosts and assessed proliferation on days 16 and 33. In the absence of \(\text{lck}\) T cells from Lck\(^{\text{ind}}\) mice underwent an initial burst of proliferation within the first 16 days, but underwent very little further division between days 16 and 33 (Fig. 4A). In contrast, WT CD4\(^+\) and CD8\(^+\) T cells continued to proliferate between days 16 and 33 (Fig. 4A). This suggests that the IL-7R-induced component is relatively short-lived, and expansion after ~day 14 relies on a competent TCR signaling component. A possible explanation for the short-lived nature of IL-7R-driven proliferation is that the expansion of donor T cells results in either competition for or consumption of available IL-7, such that signaling through IL-7R alone becomes insufficient to drive further proliferation. Alternatively, the transferred T cells may become refractory to stimulation through IL-7R in the absence of additional TCR signals.

To distinguish between these possibilities, unlabeled T cells were allowed to expand in empty \(\text{lck}^{\text{neg}}\) hosts, and their influence on the behavior of a second cohort of CFSE-labeled cells was determined. Lck\(^{\text{neg}}\) mice were preconditioned by reconstituting them with unlabeled T cells from Lck\(^{\text{ind}}\) mice. Fourteen days later, CFSE-labeled Lck\(^{\text{ind}}\) T cells were injected. At 28 days recipients were taken, and proliferation of CD4\(^+\) CD44\(^{\text{low}}\) cells present in lymph nodes was assessed (Fig. 4Bii). As a control, untreated \(\text{lck}^{\text{neg}}\) hosts were injected with CFSE-labeled T cells from WT and Lck\(^{\text{ind}}\) mice labeled with CFSE and transferred to WT \(\text{lck}^{\text{neg}}\) hosts (A), \(\text{lck}^{\text{neg}}\) hosts (B), \(\text{lck}^{\text{neg}}\) hosts (C), or \(\text{lck}^{\text{neg}}\) hosts (D). Percentages indicate the fraction of CFSE-positive cells that remained undivided. Data are representative of at least two independent experiments.
cells from Lck\(^{\text{ind}}\) mice, and CD\(^4^\)CD\(^4^\)low lymph node cells of recipients were examined 14 and 28 days after cell transfer (Fig. 5A). Consistent with the previous experiment (Fig. 4Bi), donor T cells in unconditioned recipients underwent several divisions within the first 14 days, but were little changed thereafter (Fig. 4Bi). Significantly, preconditioning with unlabeled T cells, seen as the CFSE-negative peak in Fig. 4Bi, was sufficient to prevent division of CFSE-labeled lck\(^{\text{neg}}\)-negative T cells. The failure of freshly transferred T cells to proliferate in these hosts suggests that IL-7 was not available at a level sufficient to induce proliferation as a result of the prior transfer of unlabeled Lck\(^{\text{ind}}\) T cells. Therefore, the data support the view IL-7R-driven proliferation is limited by the absence of fyn (Fig. 5B) was at least equivalent to and even slightly increased compared with that in WT F5 cells (Fig. 5A).

Surprisingly however, F5 RAG\(^{\text{neg}}\)lck\(^{\text{ind}}\) T cells demonstrated a total dependence on lck for any proliferative response even in these acutely lymphopenic hosts (Fig. 5D). Thus, the behavior of lck\(^{\text{neg}}\)F5 T cells differs from that of the majority of polyclonal CD8\(^+\) cells (Fig. 1), in that proliferation does not occur to IL-7R signals alone and instead is entirely dependent on the expression of lck.

An explanation for this was revealed by examination of IL-7R expression by F5 T cells, which was greatly reduced compared to levels seen in WT F5 cells (Fig. 4Ai). This reduction was not obvious in the absence of inducible lck expression (data not shown). The specific signaling requirements for homeostatic proliferation of F5 T cells were examined by transferring CFSE-labeled lymph node cells from F5 RAG\(^{\text{neg}}\)lck\(^{\text{ind}}\) into lck\(^{\text{neg}}\)fyn\(^{\text{neg}}\) recipients with or without dox. Additionally, we compared whether there was a requirement for the expression of fyn by similarly transferring CFSE-labeled T cells from F5 RAG\(^{\text{neg}}\)lck\(^{\text{ind}}\) into lck\(^{\text{neg}}\)fyn\(^{\text{neg}}\) donors. Control mice received CFSE-labeled T cells from F5 RAG\(^{\text{neg}}\) mice.

Analysis of lymph nodes at 14 day (Fig. 5) showed that, as seen with polyclonal cells, both F5 RAG\(^{\text{neg}}\) (Fig. 5A) and dox-fed F5 RAG\(^{\text{neg}}\)lck\(^{\text{ind}}\) (Fig. 5C) donor T cells underwent homeostatic proliferation. In contrast to the requirement for lck, proliferation in the absence of fyn (Fig. 5B) was at least equivalent to and even slightly increased compared with that in WT F5 cells (Fig. 5A).
with that by polyclonal B10 controls even though expression appeared normal in double-negative and double-positive F5 thymocytes (Fig. 6). However, this low level IL-7R expression in peripheral F5 T cells was evidently sufficient to provide survival signals, as similar numbers of F5 and WT T cells were recovered 14 days after their transfer to T cell-deficient hosts (data not shown).

Nonproliferative IL-7R signals enhance TCR-driven homeostatic proliferation

Since the expression of IL-7R in F5 T cells is by itself too low to induce homeostatic proliferation, we were able to address the question of whether we could observe synergy between TCR and IL-7R signals for the overall proliferative response in WT F5 T cells. T cells from F5 RAG<sup>−/−</sup> mice were CFSE labeled and transferred into lck<sup>−/−</sup>fyn<sup>−/−</sup> recipients treated with either PBS or repeated injection of αIL-7R mAb to block IL-7R activity in vivo. At 7 and 14 days after transfer, lymph node cells were taken and analyzed. T cells transferred into PBS-treated recipients underwent at least five divisions during the 14-day period. Significantly, treatment of mice with αIL-7R mAb greatly reduced the number of divisions undergone by F5 T cells (Fig. 7). These data strongly suggest that IL-7R-mediated signals were indeed providing a positive signal to facilitate TCR-mediated homeostatic expansion by F5 T cells, demonstrating a synergy between IL-7R and TCR signals. Finally, these data confirm that TCR-mediated signals are sufficient to induce homeostatic expansion in the absence of contributions from the IL-7R.

Discussion

Recent studies have shown that the ability of naive T cells to proliferate in response to conditions of lymphopenia depend on both TCR and IL-7R-derived signals and have also suggested that proliferation depends on the activation of both these pathways. Naive T cells are acutely sensitive to their host environment and can make homeostatic responses to a broad range of insults to the T cell pool, ranging from the mild effects of thymectomy (14) to being placed in irradiated hosts or hosts completely lacking endogenous T cells (24–27). Previous studies have not addressed how the different signals that induce homeostatic proliferation contribute to a proportionate response under these range of conditions or indeed if there is any cooperative interactions between the different signals. Our findings here address these important questions in two ways. Firstly, we show that IL-7R and TCR signals are able to stimulate independent pathways, each resulting in homeostatic proliferation. Secondly, we show that although both pathways

![FIGURE 6](http://www.jimmunol.org/)

**FIGURE 6.** F5 T cells express lower levels of IL-7R than WT T cells. Thymus and lymph nodes were taken from WT (A) and F5 RAG<sup>−/−</sup> (B) mice. Thymocytes were labeled with mAbs for CD4, CD8, and IL-7R. Dot plots are of CD4 vs CD8 expression for total thymus. Squares indicate gates used to analyze double-negative (DN), double-positive (DP), and CD8 single-positive thymic subsets. Lymph node cells were stained with mAbs for CD8, TCR, and IL-7R. Dotted lines indicate background staining. ■, IL-7R expression.

![FIGURE 7](http://www.jimmunol.org/)

**FIGURE 7.** Nonproliferative IL-7R signals synergize with TCR signals to drive homeostatic proliferation. T cells from F5 RAG<sup>−/−</sup> mice were labeled with CFSE, transferred to groups of lck<sup>−/−</sup>fyn<sup>−/−</sup> recipients (3 × 10<sup>6</sup>/mouse), and either treated with αIL-7R mAb or given PBS as a control. On days 7 and 14 after transfer, lymph node cells from recipient mice were labeled with mAbs for CD8 and TCR, and CFSE staining of gated CD8<sup>+</sup>TCR<sup>+</sup> cells is shown. Percentages indicate the frequency of donor cells that remain undivided. Host B cell development in bone marrow was blocked (data not shown), confirming that αIL-7R mAb treatment was successful. Data are representative of three independent experiments. Identical results were obtained when RAG<sup>−/−</sup> mice were used as recipients.
would normally be active, the relative contributions to the homeostatic response by TCR and IL-7R signals are not fixed and depend not only on the degree of lymphopenia, but also on the sensitivity of individual T cell clones to these two signals.

Earlier reports have suggested that induction of homeostatic proliferation requires coincident activation of both TCR and IL-7R pathways (13), and consistent with this, we observed greatest proliferation when both TCR and IL-7R pathways were active (Fig. 1). However, we also found that proliferation could, in fact, be induced by independent activation of either TCR or IL-7R signaling pathways. Neither an absence of host class II MHC ligands nor inactivation of TCR signaling by loss of lck and fyn expression prevented IL-7R-induced proliferation (Fig. 1), and conversely, complete IL-7R blockade did not prevent TCR-mediated proliferation (Figs. 2 and 7). Assessing the contributions of these two triggers of homeostatic proliferation in response to varying degrees of lymphopenia revealed different roles for these signals. TCR signals induced sustained proliferation over the range of conditions tested. In contrast, IL-7R signals induced proliferation only in the most lymphopenic hosts. It appears that induction of TCR-independent proliferation requires a critical threshold of IL-7R signaling that cannot be achieved in hosts with low numbers of T cells (Figs. 3 and 4), in which there is presumably competition for IL-7, and this may explain why this proliferation is ultimately self-limiting (Fig. 4A). It is significant to note that IL-7 has been shown to play a major role in driving proliferation in sublethally irradiated hosts (13, 19) that still contain residual host T cells, suggesting that irradiation may stimulate an increase in IL-7 production. Indeed, we also found that Lck\(^{\text{Tm}}\) T cells proliferate vigorously in sublethally irradiated hosts, confirming that in this environment proliferation can occur independently of TCR-induced signals (our unpublished observations).

Although we demonstrate that IL-7 and TCR signals can operate independently, we were able also to show synergy and not simply an additive effect of these signals. Under conditions where IL-7 was unable to directly induce TCR-independent proliferation in F5 RAG\(^{\text{wt}}\) T cells, it was apparent that IL-7R signals synergized with TCR signals to drive stronger proliferation than that mediated by TCR signals alone (Fig. 7). The point at which these signals interact is not known. The proliferation in response to IL-7R triggering clearly occurs in the absence of either lck or fyn (Fig. 1), whereas the TCR component has an absolute requirement for lck, but, interestingly, not fyn, suggesting distal activation of a common program of cell division, rather than an influence on the sensitivity of the TCR to self-Ag. Consistent with this view, the expression of CD5, used as an indicator of TCR survival signals, is not influenced by blocking IL-7R (our unpublished observations).

The status of the naive T cell is also an important consideration, as demonstrated by the experiments involving Lck\(^{\text{wt}}\) F5 T cells, which express only low levels of IL-7R (Figs. 5 and 6) and fail to respond to IL-7 signals in the absence of contributory signaling through the TCR. It is also worth noting that not all polyclonal T cells respond to IL-7R signals, as we found that in the absence of lck expression a significant proportion of CD4\(^{+}\) and CD8\(^{+}\) cells failed to proliferate. By the same token, not all T cell specificities may be able to respond to self-MHC peptide complexes or even the synergistic combination of both of these signals. Furthermore, whether this proliferation results in up-regulation of markers such as CD44 varies and presumably reflects the avidities of individual T cells. It is likely that individual TCR transgenic mice represent a subset of the polyclonal repertoire, exhibiting either a responsive (e.g., OT-1 (19)), partially responsive (e.g., F5 (18)), or non-responsive (e.g., H-Y (5)) phenotype, rather than being representative of the entire T cell population. However, these TCR transgenic examples are valuable for illustrating the characteristics of individual T cells that contribute to their ability to divide in response to lymphopenia and need to be considered together with the status of the host environment.

The preceding discussion may also explain apparent contradictions between our observation that IL-7R signals and TCR signals can drive homeostatic proliferation independently of one another and those of other groups who showed that proliferation of polyclonal and OT-1 TCR transgenic T cells was abrogated in IL-7-deficient hosts (13, 19). Additionally, these studies monitored proliferation over 7 days, while we followed the behavior of T cells over a longer time period. Significantly, proliferation of F5 T cells in aIL-7R-treated hosts at d7 (Fig. 7) appears very similar to that described for OT-1 T cells in IL-7-deficient hosts, as the TCR-mediated drive is quite slow, in contrast to that stimulated through IL-7R, and it is not until the second week that significant proliferation is observed. In this regard it is significant to note the low expression of IL-7R by F5 compared with the high levels expressed by OT1 T cells (19) may also contribute to the observed differences in their responsiveness to IL-7R signals.

The signaling requirements for homeostatic proliferation, therefore, contrast with those of T cell survival, even though the same combination of TCR and IL-7R signals is involved. Fig. 8 suggests a model of how these signals may be integrated for these two processes. Both TCR and IL-7R signals are absolutely required for long term survival (29), whereas either of these signals alone cannot initiate homeostatic proliferation. Furthermore, we found no evidence that fyn contributed to either IL-7R- or TCR-induced homeostatic proliferation, whereas fyn plays a significant role in transducing TCR survival signals (29). Finally, these data demonstrate that the signaling pathway downstream of the IL-7R does not involve the src family kinases lck and fyn.
In conclusion, our data show that T cell homeostasis is regulated by complex interaction of TCR and IL-7R signaling pathways. As suggested by others (28), IL-7 may provide an additional feedback for regulating the size of the T cell pool. Whereas TCR signals induce a homeostatic response over a broad range of lymphopenias, IL-7 appears to regulate naive T cell homeostasis at several levels. Under conditions of extreme lymphopenia, both IL-7R and TCR signals can directly induce proliferation, and it is this combination that results in the overall homeostatic response. Under milder conditions, IL-7R signals do not directly induce proliferation, but, rather, synergize with existing TCR signals to induce a response. In a full host, IL-7 and TCR signals act as survival factors, limiting repertoire expansion. Therefore, interactions of TCR and IL-7R signals at multiple levels allow fine-tuning of the repertoire to a broad range of conditions.

Acknowledgments

We thank Trisha Norton, Keith Williams, and Rachel Simmons for assistance with mouse breeding and typing, and Peter Tomlinson for technical help. Our thanks also go to Prof. J. di Santo for the use of γc−/− mice, Prof. S. Nishikawa for the anti-IL-7Rα mAb A7R34, and to Brigitta Stockinger, George Kassiotis, and Dimitris Koussis for critical reading of the manuscript and discussion.

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