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TCR Signals Mediated by Src Family Kinases Are Essential for the Survival of Naive T Cells

Benedict Seddon and Rose Zamoyska

The role of TCR signals triggered by recognition of self MHCs in maintaining the survival of naive peripheral T cells remains controversial. Here we examine the role of the Src family kinases, p56\(^{Lck}\) and p59\(^{fyn}\) (Fyn), in the survival of naive T cells. We show that long term survival requires a combination of signals transduced by Src family kinases and signals through the IL-7R. In the absence of either one, naive T cells die slowly, but if both signals are removed, cell loss is greatly accelerated. The TCR signal can be mediated by either Fyn or Lck at wild-type levels of expression, but not by Lck alone if expressed suboptimally. The disappearance of T cells in the absence of Fyn and Lck was associated with a complete loss of TCR\(\gamma\) chain phosphorylation and down-regulation of CD5, both of which are also MHC contact dependent, indicating that the Src family kinases are critical for transducing a TCR-MHC survival signal. The Journal of Immunology, 2002, 169: 2997–3005.

T cell receptor signals regulate the differentiation and function of T cells throughout their lifespan. TCR signals that are evoked following recognition of peptide-MHC complexes determine thymocyte selection and play an important role in peripheral naive T cell homeostasis. Under conditions of lymphopenia, T cells have the capacity to undergo spontaneous cell division, independently of foreign Ag, driven by TCR interactions with self-MHC peptide complexes similar to those that mediate thymic selection processes (1–4). However, less clear is the role these signals play in maintaining the peripheral naive T cell repertoire in a full steady state condition.

The question of whether MHC-TCR interactions are required for prolonged T cell survival is one that has been addressed by a number of studies in recent years. While many of these evoke a role for MHC-TCR interactions in T cell maintenance (1, 5–9), a significant number of others have suggested that survival is not linked to self-MHC recognition (10–12). It has been argued that since MHC ligands also induce T cell expansion under conditions of lymphopenia, in the absence of MHC it is difficult to differentiate between a defect in expansion vs one in survival. Conversely, studies that failed to find a requirement for MHC ligands in promoting T cell survival have involved T cell transfers to MHC-deficient environments under conditions where it is difficult to absolutely exclude the possibility of cotransferring MHC-expressing APCs or their precursors. The situation is further confused in that cytokines also play an essential role in promoting the survival of naive T cells (13–15), and their impact on TCR-mediated survival signals has, in general, not been addressed in these studies.

Previous studies in our laboratory have attempted to characterize the signals involved in peripheral T cell homeostasis by directly manipulating the intracellular signaling molecules important in transducing TCR signals. Using mice bearing a tetracycline-inducible transgene for the Src family kinase, p56\(^{Lck}\) (Lck\(^{1\text{mos}}\)), we showed that expression of Lck was essential for transducing the TCR signals that mediate homeostatic proliferation, but surprisingly Lck-mediated signals were not required for long term survival of naive T cells (16). Given that Lck plays such a central role in initiating and propagating TCR signals (17, 18), the dissociation of T cell survival from Lck expression might argue against the existence of a TCR-mediated survival signal. However, p59\(^{fyn}\) is another Src family kinase expressed in T cells that has been shown to be able to substitute for some of the functions of Lck (19). In the present study we generated mice with the inducible lck transgene that lacked both endogenous Lck and Fyn and show that Src family kinases are crucial for the prolonged survival of naive T cells and, therefore, that survival is indeed TCR signal dependent.

Materials and Methods

Mice

Generation of inducible Lck-transgenic Lck\(^{1\text{mos}}\) mice was described previously (20). Briefly, mice expressing the rtTA trans-activator domain under control of the human CD2 promoter on an endogenous Lck\(^{\text{wt}}\) background (rtTA-C/Lck\(^{\text{wt}}\)) were intercrossed with mice bearing the mouse lck transgene under control of a tetO/CMVmin promoter, also on an endogenous Lck\(^{\text{wt}}\) background (Lck\(^{1\text{mos}}\)/Lck\(^{\text{wt}}\)). Mice were fed doxycycline (dox) in food (1 mg/g) throughout pregnancy and before weaning. Lck\(^{1\text{mos}}\)/rtTA-C/Lck\(^{\text{wt}}\) (Lck\(^{1\text{mos}}\)) offspring were fed dox-containing food to maintain lck transgene expression. Lck\(^{1\text{mos}}\)/Fyn\(^{1\text{mos}}\) mice were similarly derived by intercrossing rtTA-C Lck\(^{1\text{mos}}\)/Fyn\(^{1\text{mos}}\) and Lck\(^{1\text{mos}}\)/Fyn\(^{1\text{mos}}\) strains. C57BL/10 wild-type, Lck\(^{\text{wt}}\), Fyn\(^{\text{wt}}\), and Lck\(^{1\text{mos}}\)/Fyn\(^{1\text{mos}}\) mice were bred in a conventional colony free of pathogens at National Institute for Medical Research (London, U.K.). All lines used were the H-2\(^{b}\) haplotype.

Flow cytometry and cellularity determinations

Flow cytometry was conducted using 2–5 \(\times\) 10^6 thymocytes, lymph node cells, or spleen cells. Lymph node cell suspensions were prepared from superficial cervical, brachial, axillary, inguinal, and mesenteric lymph nodes of individual mice. Cell concentrations were determined using a Coulter Counter (Scharf Instruments, Reutlingen, Germany). Cells were incubated with saturating concentrations of Abs in 100 µl PBS/BSA (0.1% v/v, 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 (Catlg, San Francisco, CA). The mAb used in this study were as follows: PE-CD4 (GK1.5; BD Pharmingen, San Diego, CA), PerCP-CD8a (53-6.7; BD, San Diego, CA).
BD Pharmingen), allophtococyanin-CD8a (53-6.7; BD Pharmingen), FITC-TCR (H57-597; BD Pharmingen), allophtococyanin-CD44 (Leinco Technologies), and IL-7R (A7R34) and FITC-CD5 (BD Pharmingen). Four-color cytometry staining was analyzed on a FACScan (BD Biosciences, Mountain View, CA), and data analysis was performed with CellQuest software (BD Biosciences). Statistical significance of reductions in cell recoveries compared with controls was tested using one-tailed Student’s t test (unpaired).

Western blot analysis

Cell suspensions from thymus, lymph node, and spleen were recovered by centrifugation and resuspended in ice-cold lysis buffer (50 mM Tris-HCl (pH 8.0); 150 mM NaCl; 1% Triton X-100; 5 μg/ml each of chymostatin, leupeptin, pepstatin A, and antipain (Sigma-Aldrich; St. Louis, MO); 1 mg/ml iodoacetamide (Sigma-Aldrich); and 0.2 mg/ml Pefabloc (Roche, Indianapolis, IN)) for 30 min, and postnuclear supernatants were obtained by centrifugation. Immunoprecipitation, SDS-PAGE, and Western blotting were conducted as reported previously (21). Total cell lysates for Lck immunoblotting were loaded at 2 × 10^6 T cell equivalents/lane, while anti-CD3ζ immunoprecipitates were from the equivalent of 5 × 10^7 T cells using H146-968 mAb coupled to protein A-Sepharose beads (Sigma-Aldrich). Lysates and immunoprecipitates were separated on 12.5% SDS-PAGE under reducing conditions, blotted onto Immobilon-P membranes (Millipore, Bedford, MA), and probed using anti-phosphotyrosine RC20-HRP (Transduction Laboratories, Lexington, KY), rabbit anti-Lck sera, rabbit anti-Fyn (Santa Cruz Biotechnology, Santa Cruz, CA), anti-CD3ζ (H146-968), and rabbit anti-actin sera (Sigma-Aldrich). Unconjugated IgG were detected using protein A-Sepharose beads (Sigma-Aldrich). Lysates were washed using PBS/Tween (0.5%) between sequential probing steps. Rabbit anti-Lck serum and the H146-968 anti-CD3ζ-secreting hybridoma were gifts from A. Magee and R. Kubo, respectively.

Treatment of mice with anti-IL-7R mAb

A7R34, a gift from Prof. S. Nishikawa (Department of Molecular Genetics, Kyoto University, Kyoto, Japan), was purified from hybridoma tissue culture supernatants with Mono-S Sepharose (Pharmacia, Piscataway, NJ) and dialyzed into PBS. Treatment of mice with 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.

Results

Normal thymic development in Fyn-deficient mice bearing an inducible lck transgene

To assess the requirement of both Fyn and Lck for naive T cell survival, mice with an inducible lck transgene (20) were backcrossed to a Fyn^neg background. These mice have an lck transgene under a tetracycline-sensitive promoter (Lck1) and a second tet-on trans-activator domain (rtTA-C) transgene under the control of human CD2 regulatory elements, targeting expression to T cells. Lck-inducible (Lck1^ind) mice lack endogenous Lck and express the lck transgene in response to administration of the tetracycline-derivative dox.

Thymic development appears normal in Fyn^neg mice (22, 23). To confirm this was also true of Lck1^indFyn^neg mice, thymic phenotypes were analyzed from mice fed since gestation with dox. As described previously, we were able to overcome the block in thymic development found in Lck^neg mice to near-normal levels by expression of an inducible lck transgene (20) (Fig. 1A). Lck^neg/Fyn^neg mice exhibit a complete block in thymocyte development and have virtually no double-positive or mature single positive thymocytes (19, 24) (Fig. 1, A and B). Introduction of the lck1^ind transgene was able to restore thymic differentiation in Lck^neg/Fyn^neg mice to levels comparable with those observed on Lck^neg/Fyn^- background. Consistent with earlier studies, we found that the loss of Fyn had little impact on either the frequency or number of thymocytes, providing Lck is present in the wild-type (WT) or inducible form (Fig. 1, A and B). Furthermore, expression of TCR by single-positive and double-positive thymocytes from WT or Lck1^ind/Fyn^- mice was comparable to that expressed by the corresponding Fyn^- strain mice (Fig. 1C).

Lck1^ind/Fyn^- mice have reduced numbers of peripheral naive T cells

Analysis of thymi from Lck1^ind/Fyn^- mice suggested that thymic development and production of mature single-positive T cells occurs comparably to those in Lck1^ind/Fyn^- controls, so we examined
their peripheral naive T cell numbers. In the presence of endoge-
nous Lck, the frequencies of TCR$^{\text{high}}$ cells and CD4$^{+}$ and CD8$^{+}$
subsets found in lymph node from Fyn$^{\text{neg}}$ mice were similar to
those in WT mice (Fig. 2A). Interestingly, this was not found to be
the case when lymph node cells from Lck$^{1\text{ind}}$Fyn$^{\text{neg}}$ mice were
compared with those from Lck$^{1\text{ind}}$Fyn$^{+}$ controls. The overall fre-
quency of TCR$^{\text{high}}$ lymph node cells was consistently lower in
Lck$^{1\text{ind}}$Fyn$^{\text{neg}}$ mice than in Lck$^{1\text{ind}}$Fyn$^{+}$ controls (Fig. 2A, histo-
grams), although the ratios of CD4$^{+}$ to CD8$^{+}$ cells were similar
(Fig. 2A, dot plots). Analysis of the absolute numbers of naive
phenotype CD4$^{+}$CD44$^{\text{low}}$ and CD8$^{+}$CD44$^{\text{low}}$ cells in defined
lymph nodes and spleens confirmed this observation. The numbers
of naive T cells in lymph nodes and spleen from Fyn$^{\text{neg}}$ mice were
similar to those in WT mice (Fig. 2B). However, there was a re-
producible and statistically significant reduction (~2.5-fold) in the
numbers of naive CD4$^{+}$CD44$^{\text{low}}$ and CD8$^{+}$CD44$^{\text{low}}$ cells found in
lymph node and spleen of Lck$^{1\text{ind}}$Fyn$^{\text{neg}}$ mice compared with
Lck$^{1\text{ind}}$Fyn$^{+}$ controls (Fig. 2B). Examination of the frequency of
CD44$^{\text{high}}$ T cells in lymph node and spleen revealed no significant
difference between Fyn$^{\text{WT}}$ strain mice and their Fyn$^{\text{neg}}$ counter-
parts (Fig. 2C).

**Peripheral T cell pool size correlates with availability of Src
kinases**

Naive peripheral T cells in Fyn$^{\text{neg}}$ mice are found in comparable
numbers to those in WT mice, so it was surprising to find such
a significant reduction in the numbers of naive T cells in the periph-
ery of Lck$^{1\text{ind}}$Fyn$^{\text{neg}}$ mice compared with Lck$^{1\text{ind}}$Fyn$^{+}$ controls.

**FIGURE 2.** Peripheral naive T cells are reduced in Lck$^{1\text{ind}}$Fyn$^{\text{neg}}$ mice. Single-cell suspensions were prepared from specific
lymph nodes (see Materials and Methods) and spleen taken from WT ($n = 10$), Fyn$^{\text{neg}}$ ($n = 10$), Lck$^{1\text{ind}}$ ($n = 7$), and Lck$^{1\text{ind}}$Fyn$^{\text{neg}}$ ($n = 15$) mice. Cells were counted, and samples
were stained for the expression of TCR, CD4, CD8, and CD44 and were analyzed by FACS. Histograms are of TCR expression by lymph node cells (A, upper row), and percentages indicate the frequencies of TCR$^{\text{high}}$ cells. Dot plots are of CD4 vs CD8 expression by TCR$^{\text{high}}$ lymph node cells (A, lower row). B. Bar charts show total numbers and numbers of CD4$^{+}$CD44$^{\text{low}}$ and CD8$^{+}$CD44$^{\text{low}}$ cells detected in lymph node and spleen of Lck$^{\text{WT}}$ and Lck$^{1\text{ind}}$
strains (\[\text{■}, \text{numbers from the corresponding Fyn}^{\text{WT}} \text{strain}; \[\text{□}, \text{numbers from its Fyn}^{\text{neg}} \text{counterpart})]. Numbers indicate the fold difference between Fyn$^{\text{WT}}$
and Fyn$^{\text{neg}}$ cell numbers and were statistically significant as indicated (*, $p < 0.0001$). C, Bar charts show the percentage of T cells expressing high levels
of CD44 in lymph node and spleen of Lck$^{\text{WT}}$ and Lck$^{1\text{ind}}$ strains (\[\text{■}, \text{corresponding Fyn}^{\text{WT}} \text{strain}; \[\text{□}, \text{its Fyn}^{\text{neg}} \text{counterpart}).
We have previously reported that there are differences in expression of the induced lck1 transgene between thymocytes and peripheral cells (16). Therefore, we compared expression of lck and fyn in the thymus and periphery of Lck1ind and WT mice on Fyn- and Fynneg backgrounds; lck expression in the thymus of Lck1ind/Fyn+ mice was comparable to that seen in WT and Fynneg mice (Fig. 3). However, while LckWT strains express equivalent levels of Lck protein in thymus and periphery, expression of the lck1 transgene in the periphery of both Lck1ind/Fyn+ and Lck1ind/Fynneg mice was considerably lower. No change was observed in the level of Fyn expressed in the periphery of WT and Lck1ind/Fyn- T cells (Fig. 3), and consistent with other studies, Fyn expression was much lower in thymus than in the periphery (25).

These data suggest a correlation between the size of the naive T cell pool and the levels of Src family kinases expressed by peripheral T cells. Although T cells from Lck1ind/Fyn+ mice have only low levels of lck1 transgene expression, fyn expression is normal. Conversely, Fynneg mice lack WT fyn expression but have normal levels of lck. In contrast, Lck1ind/Fyn+ mice lack WT fyn and only express ~20% of WT levels of lck in the periphery; this, it seems, is insufficient to maintain survival of peripheral T cells.

*Naive T cells do not survive the absence of both lck and fyn*

To test whether there is a requirement for Src family kinases for the prolonged survival of peripheral T cells, lck1 transgene expression was switched off. Dox was removed from the diets of cohorts of Lck1ind/Fyn+ mice (Lck1 OFF Fyn+), Lck1ind/Fynneg mice (Lck1 OFF Fynneg), and WT mice (Lck1 ON Fyn-). Dox was removed from the diets of Lck1 OFF Fynneg and Lck1 OFF Fyn+ mice. Lysates (2 × 10⁶ T cells/lane) were separated on a duplicate SDS-PAGE gel and Western blotted with either anti-Lck or anti-Fyn serum. Blots of thymus lysates were probed for actin as a loading control, while splenocyte lysates were probed for CD3ζ. Data are representative of three independent experiments.

![Figure 3](http://www.jimmunol.org/)

**Figure 3.** Reduced expression of Lck in the periphery of Lck1ind/Fynneg mice. Total cell lysates were prepared from thymus and spleen of WT, Lck1ind, Fynneg, and Lck1ind/Fynneg mice. Lysates (2 × 10⁶ T cells/lane) were separated on a duplicate SDS-PAGE gel and Western blotted with either anti-Lck or anti-Fyn serum. Blots of thymus lysates were probed for actin as a loading control, while splenocyte lysates were probed for CD3ζ. Data are representative of three independent experiments.

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Fyn, there was total loss of ζ phosphorylation within 1 wk of cessation of dox feeding (Fig. 5B).

**Fyn and Lck regulate CD5 expression in peripheral naive T cells**

Recent studies have shown that levels of CD5 expressed by peripheral T cells are modulated by TCR interactions with MHC in a manner that reverts the avidity of a specific TCR for self MHC-peptide complexes, and that, certainly for CD4+ cells, this is in part dependent on Lck expression (31). To determine whether this is also true of CD8+ cells and whether Fyn can influence the adaptation of CD5, we examined T cells from Lck1ind/Fyn+ and Lck1ind/Fyn− mice for their expression of CD5. Fig. 6 shows representative histograms of TCR and CD5 expression by CD44low (Fig. 6A) and CD84high (Fig. 6B) cells from WT, Lck1OFF/Fyn+, and Lck1OFF/Fyn− mice. Levels of TCR expression were constant between T cells from the different mouse strains (Fig. 6, A and B, left histogram). However, both Lck and Fyn were found to influence the levels of CD5 expressed in CD4+ and CD8+ cell subsets, since CD5 levels were reduced in Lck-deficient cells compared with those in WT cells and were lowest in T cells lacking both Lck and Fyn. Fig. 6C summarizes CD5 expression, expressed as a percentage of WT levels, by CD4+CD44low and CD8+CD44low cells from Fyn+, Lck1OFF/Fyn+, Lck1OFF/Fyn−, and Lck1OFF/Fyn− mice. Interestingly, while Lck and Fyn mediated the adaptation of CD5 levels in both CD4+ and CD8+ cells, the influence of these two kinases was not the same for both subsets. CD5 expression on CD4+ cells was more sensitive to the presence of Lck, since its reduction in Lck1ind/Fyn+ON mice, which express low levels of Lck, and in Lck1ind/Fyn−OFF mice which have no Lck, was much more marked in the CD4+ vs the CD8+ subset (Fig. 6C). In contrast, CD5 expression on CD8+ cells was more sensitive to the presence of Fyn, shown firstly by the reduced expression of CD5 on CD8+ cells in comparison with CD8+ cells in Fyn− mice (LckWT) (Fig. 6C). Secondly, in the absence of Lck (or in the presence of reduced levels of Lck), in CD8+ cells compared with only a 20% drop in CD4+ cells (Fig. 6C). These data demonstrate that Fyn contributes to the sensory adaptation of CD5 on peripheral T cells and reinforce the view that naive T cells are continually making contact with MHC-peptide complexes, as deprivation of this contact has been demonstrated to reduce CD5 levels (31, 32).

**TCR and IL-7R signals synergize to maintain prolonged naive T cell survival**

As the data in Fig. 4 show, failure of naive T cells to express both Lck and Fyn resulted in their disappearance. However, although the TCR-mediated survival signal, as visualized by CD3ζ-chain phosphorylation and CD5 expression, was lost within 1 wk of switching off Lck expression (Fig. 5), disappearance of T cells was considerably slower. Studies of T cell survival in the absence of either MHC or TCR expression report a similarly slow rate of T cell decay (9, 32, 33). In contrast, T cells in vitro die rapidly without stimulation, suggesting that factors other than TCR signals contribute to T cell survival. The importance of cytokines whose receptors use the common γ-chain in the survival of naive T cells has been well documented (13), in particular IL-7 (14, 15, 34). To determine whether the slow decline in naive T cell numbers in the absence of continued TCR signals was because cells were still...
receiving survival signals from IL-7, the fate of T cells were followed after treatment of mice with an IL-7R/H9251-specific mAb (H9251 IL-7R), that blocks the biological activity of IL-7.

At time zero the numbers of naive T cells in the lymph nodes of groups of 6- to 8-wk-old Lck1 ind Fyn /H11001 and Lck1 ind Fyn neg mice were determined. The remaining littermates were taken off dox and treated with saturating levels of H9251 IL-7R Ab or PBS as a control. As shown previously, the loss of Lck (Lck1 OFF Fyn /H11001 mice) had little effect on the survival of CD44 low naive cells (Fig. 7). The removal of either the IL-7R-mediated survival signals by treatment of Lck1 OFF Fyn /H11001 mice with H9251 IL-7R Ab or TCR-mediated survival signals by the absence of both Lck and Fyn (Lck1 OFF Fyn neg ) resulted in similar reductions of naive T cell survival over the 28 days. However, the combined loss of both these survival signals (Lck1 OFF Fyn /H11001 mice treated with H9251 IL-7R) precipitated a severe decline in naive T cell numbers. Interestingly, CD8+ cells seemed to be even more sensitive to the loss of these survival signals than CD4+ cells. Loss of naive T cells in H9251 IL-7R-treated mice was not a cytolytic effect of the coating mAb, since the numbers of memory CD8+ cells, whose survival is not dependent on IL-7 (34), were unaffected in treated hosts compared with controls even though they express higher levels of IL-7R than naive T cells (data not shown). Data are pooled from at least four independent experiments.

Discussion

The role of MHC-derived TCR signals in maintaining long term survival of naive T cells remains controversial, with recent studies both supporting (9, 32, 33) and refuting (10–12) roles for TCR and MHC. A further complication in assessing the requirement for TCR signals is that cytokines also play an essential role in maintaining naive T cells (13–15). In the present study we were able to...
address these important issues in two ways; firstly, by generating mice bearing an inducible \textit{lck} transgene on an endogenous \textit{Lck}^{neg}Fyn^{neg} background, we were able to show that long term naive T cell survival was strictly dependent on TCR signals mediated by Src family kinases, and secondly, that short term survival of T cells in the absence of these kinases was IL-7R signal dependent, since in the absence of both TCR and IL-7R signals, T cells disappeared rapidly.

The essential role of Src family kinases in the prolonged survival of naive T cells was apparent by examining Lck1^{neg}Fyn^{neg} mice even when expression of the \textit{lck} transgene was maintained. Lck1^{neg}Fyn^{neg} mice have reduced numbers of both naive CD4^{+} and CD8^{+} cells compared with Lck1^{ind} controls (Fig. 2), even though thymic development and mature single-positive cell production are normal in both strains (Fig. 1). The defect in peripheral T cell survival was not seen in Lck1^{neg}Fyn^{neg} mice expressing WT Fyn, which was more abundant in peripheral T cells than Lck (25). Similarly, no defect in survival was seen in Fyn^{neg} mice expressing WT Lck, probably because WT levels of Lck are \sim 5 times greater than those found in resting peripheral T cells from mice expressing the induced \textit{lck} transgene. However, in Lck1^{neg}Fyn^{neg} mice, T cells can rely only on the low level of \textit{lck} transgene expression for their continued survival, and this appears to be insufficient.

The implication that Src family kinases are required for naive T cell survival was confirmed when Lck1 expression was switched off in Lck1^{neg}Fyn^{neg} mice (Fig. 4). T cell numbers declined with kinetics similar to those described previously following ablation of either MHC expression (9) or expression of the TCR itself (33). However, both Lck and Fyn have been implicated in the signaling of other cell surface molecules as diverse as CD28 (35), CD44 (36), L-selectin (37), and cytokine receptors (38, 39). In principle, therefore, the requirement of Lck and Fyn for T cell survival may not be specifically for transducing TCR signals. While involvement of other surface receptors is a possibility that cannot be excluded, we could show that phosphorylation of CD3\_zeta in ex vivo unstimulated T cells was entirely Src kinase dependent (Fig. 5), and its absence correlated with a failure in prolonged T cell survival. Furthermore, there is good evidence that phosphorylation of TCR\_zeta is dependent on interaction of TCR with MHC molecules both in vitro (31) and in vivo (9, 11).

Quantitative analysis revealed that the extent of TCR\_zeta-chain phosphorylation broadly correlates with expression levels of Lck and Fyn. TCR\_zeta-chain phosphorylations in WT and Fyn^{neg} mice are similar, suggesting that Lck at WT levels is sufficient to maintain normal phosphorylation. When the levels of Lck are greatly reduced (Lck1^{neg}Fyn^{neg} mice) or when Lck is absent (Lck1^{neg}Fyn^{neg} mice), the extent of TCR\_zeta phosphorylation is \sim 50\% that in WT mice. This phosphorylation must be mediated by Fyn, as all Phos-\zeta disappears when Fyn is also absent. It will be interesting to determine whether Fyn and Lck preferentially phosphorylate different immunoreceptor tyrosine-based activation motifs on \zeta, because while those phosphorylated by Fyn are entirely sufficient to promote T cell survival, they are not sufficient to transduce the TCR signals that drive homeostatic proliferation of naive T cells under conditions of lymphopenia (16). Furthermore, in another study we could find no evidence that Fyn played any role in mediating either TCR or IL-7R signals that induce homeostatic proliferation by T cells under conditions of lymphopenia (42) strongly suggesting that promotion of cell survival and induction of homeostatic proliferation involve discrete signaling pathways.

Further evidence suggesting that Src kinase-dependent survival signals were TCR dependent came from analysis of CD5 expression. Previous studies have shown that the maintenance of CD5 expression was MHC dependent, was correlated with the degree of TCR\_zeta-chain phosphorylation, and was influenced by the expression of Lck, all of which evoke an MHC-dependent TCR signaling mechanism (31). Here we show that adaptation of CD5 expression is regulated by Fyn as well as Lck (Fig. 6). Interestingly, we found evidence that these two Src family kinases have different influences for CD4^{+} and CD8^{+} subsets. This may reflect more general preferences for CD4^{+} and CD8^{+} subsets for the use of either Lck or Fyn for TCR signaling, and it is interesting to note that in Lck^{neg} mice, the presence of Fyn seems to favor the selection of CD8^{+} cells over CD4^{+} cells (40). In addition, CD5 expression was found to correlate well with cell survival. T cells from Lck1^{neg}Fyn^{neg} and Lck1^{neg}Fyn^{neg} mice that exhibited defects in naive T cell survival also had the lowest levels of CD5.

The role of IL-7 as a survival factor for naive T cells is well documented (13–15, 34). However, the interaction between IL-7 and TCR survival signals has not previously been addressed. In the

**FIGURE 7.** TCR and IL-7R signals synergize to promote the survival of naive peripheral T cells. Lck1^{neg}Fyn^{neg} and Lck1^{neg}Fyn^{neg} mice, aged 6–8 wk, were taken off dox and treated with either anti-IL-7R mAb (oIL-7R) or PBS as a control. Just before treatment, numbers of CD4^{+}, CD44^{low}, CD8^{+}, and CD44^{low} cells in lymph node were determined in groups of mice by counting total numbers of lymph node cells and analyzing the expression of CD4, CD8, TCR, and CD44 by FACS. After 2 and 4 wk of treatment, cell suspensions were made from lymph nodes, and numbers of naive T cells were determined. [44x200]Lck neg Fyn neg background, we were able to show that long term

**FIGURE 7.** TCR and IL-7R signals cooperate to promote the survival of naive peripheral T cells. Lck1^{neg}Fyn^{neg} and Lck1^{neg}Fyn^{neg} mice, aged 6–8 wk, were taken off dox and treated with either anti-IL-7R mAb (oIL-7R) or PBS as a control. Just before treatment, numbers of CD4^{+}, CD44^{low}, and CD8^{+}CD44^{low} cells in lymph nodes were determined in groups of mice by counting total numbers of lymph node cells and analyzing the expression of CD4, CD8, TCR, and CD44 by FACS. After 2 and 4 wk of treatment, cell suspensions were made from lymph nodes, and numbers of naive T cells were determined. [44x200]Lck neg Fyn neg background, we were able to show that long term

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present study we found that blocking one or another of these signals compromised T cell survival to a broadly similar extent. Significantly, when both IL-7R and TCR signals were blocked in αIL-7R-treated Lck<sup>502</sup>Fyn<sup>low</sup> mice, nearly all naive T cells were lost within 2 wk (Fig. 7). Therefore, it appears that TCR and IL-7R signals have a limited capacity to sustain naive T cells independently of one another, and it is not until both signals are blocked that a rapid attrition of T cells is observed. While in a “full” host, these signals are individually insufficient to sustain survival for any longer than a few weeks, combined they can maintain naive T cells in the periphery for many months. A similar synergy between TCR and IL-7R signals occurs when homeostatic proliferation is induced in T cells under conditions of lymphopenia (42). In this way the size of the T cell pool is regulated by both stromal cell IL-7 production and access to MHC expressing APCs, and is ultimately limited by whichever of the two is lesser.

In the light of these findings, therefore, the differences in T cell half-lives previously reported (9, 32, 33) in the absence of TCR or its ligand are more likely a reflection of the host cytokine milieu than a function of TCR signaling itself. It is clear that even in a full host, IL-7 can prolong T cell survival in the absence of TCR signaling for a limited period. IL-7 can induce homeostatic proliferation of naive T cells independently of TCR signals, but depends on the extent of lymphopenia within a host (42). Therefore, under lymphopenic conditions, greater availability of IL-7 may increase its capacity to maintain T cell survival in the absence of TCR signals than is observed in full hosts, and this may explain reports of naive T cell survival upon adoptive transfer to MHC-deficient hosts (10, 11). Finally, we found that CD8<sup>+</sup> cells were more reliant on both TCR and IL-7R signals than were CD4<sup>+</sup> cells, exhibiting a more rapid loss in the absence of either or both signals. This greater reliance may reflect a greater sensitivity to such signals, and it is interesting to note that CD8<sup>+</sup> cells survive better than CD4<sup>+</sup> cells in an intact host (41).

In conclusion, it is clear from the present study that Src kinase expression is essential for prolonged survival of naive T cells. We cannot exclude the possibility that Lck and Fyn mediate survival signals via surface receptors other than the TCR; however, evidence in favor of the latter is very strong. CD3ζ chain phosphorylation, which has been shown to be MHC dependent (9, 11), is absolutely Src kinase dependent; sensory adaptation of CD5 expression, also dependent on TCR-MHC interactions (31), correlates with both Src kinase expression and T cell survival. Therefore, the most direct interpretation of these data is that a TCR survival signal for naive T cells is delivered by MHC and is mediated by Src family kinases.

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References
42. Seddon, B., and R. Zamoyska. T cell receptor and IL-7 receptor signals can operate independently or synergize to promote lymphopenia-induced expansion of naive T cells. J. Immunol. In press.