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IL-7Rα Gene Expression Is Inversely Correlated with Cell Cycle Progression in IL-7-Stimulated T Lymphocytes[1]

Louise Swainson,[2]*†‡§ Els Verhoeyen,[2]¶‖# François-Loïc Cosset,¶‖# and Naomi Taylor[3]*†‡§

IL-7 plays a major role in T lymphocyte homeostasis and has been proposed as an immune adjuvant for lymphopenic patients. This prospect is based, at least in part, on the short-term expansion of peripheral T cells in rIL7-treated mice and primates. Nevertheless, in vivo, following initial increases in T cell proliferation and numbers, lymphocytes return to a quiescent state. As the bases for this cell cycle exit have not yet been elucidated, it is important to assess the long-term biological effects of IL-7 on quiescent human T lymphocyte subsets. In this study, we find that IL-7-stimulated CD4$^+$ naive lymphocytes enter into cell cycle with significantly delayed kinetics as compared with the memory population. Importantly though, these lymphocytes exit from the cell cycle despite the continuous replenishment of rIL-7. This response is distinct in memory and naive CD4$^+$ lymphocytes with memory cells starting to exit from cycle by day 10 vs day 18 for naive cells. Return to quiescence is associated with a cessation in IL-7R signaling as demonstrated by an abrogation of STAT-5 phosphorylation, despite an up-regulation of surface IL-7Rα. Indeed, an initial 10-fold decrease in IL-7Rα mRNA levels is followed by increased IL-7Rα expression in naive as well as memory T cells, with kinetics paralleling cell cycle exit. Altogether, our data demonstrate that IL-7 promotes the extended survival of both naive and memory CD4$^+$ T cells, whereas cycling of these two subsets is distinct and transient. Thus, IL-7 therapy should be designed to allow optimal responsiveness of naive and memory T cell subsets. ***The Journal of Immunology,*** **2006, 176: 6702–6708.**

The IL-7 cytokine plays a major role in the in vivo maintenance of polyclonal naive and memory T cells, positively regulating the survival, differentiation and proliferation of thymocyte and peripheral T lymphocyte populations. The maintenance of a polyclonal T cell pool is referred to as homeostasis, and is required for the persistence of immunologic memory as well as the maintenance of naive T cells that can respond to novel Ags. Under conditions of lymphopenia, IL-7 supports homeostatic T cell proliferation, promoting the expansion of T cells with a diverse TCR repertoire (1–4). This appears to be of physiological consequence as there are increased serum IL-7 levels in patients with lymphopenia, secondary to HIV infection or chemotherapy (5–8). Administration of exogenous IL-7 can enhance thymic activity and/or promote the short-term expansion of mature peripheral T cells (9, 10). Notably, IL-7 treatment appears to result in the preferential expansion of de novo-generated recent thymic emigrants (11, 12). These properties have lead to the proposal that rIL-7 be used as an adjuvant immune therapy. Indeed, preclinical murine and primate studies have demonstrated that exogenous IL-7 enhances T cell survival and proliferation (1, 4, 13–16).

In vivo, the 25-kD IL-7 glycoprotein is produced mainly by stromal cells, monocytes, and epithelial cells (17). Under physiological conditions, the concentration of endogenous IL-7 relative to the number of peripheral T cells is likely to be extremely low and as such, may limit the homeostatic expansion of peripheral cells. This is crucial because the size of the T cell pool remains relatively constant during the lifetime of an individual. Indeed, under normal circumstances, the vast majority of peripheral T cells are quiescent, in the G_0 phase of the cell cycle. In support of the hypothesis that low levels of IL-7 contribute to this quiescent state, a significant proportion of peripheral T cells enter into cycle upon administration of exogenous IL-7 (3, 9, 10, 18–21). Nevertheless, the cycling status of these cells appears to be transient and lymphocytes return to quiescence, even following continued administration of rIL-7. Importantly, the biological mechanisms underlying this return to a quiescent state have not been elucidated.

We and others have previously shown that naive as well as memory human T lymphocytes respond to IL-7 stimulation ex vivo, as monitored by the activation of proximal signaling molecules, antiapoptotic effectors, and cell cycle entry (22–28). Nevertheless, all these studies were performed during a short IL-7 stimulation period (6–8 days). The potential role for exogenous IL-7 as an adjuvant therapy in lymphopenic patients necessitates an in-depth understanding of the effects of this cytokine on the long-term fate of both naive and memory T lymphocytes. Moreover, it is important to determine whether the IL-7-stimulated responsiveness of T lymphocyte subsets is differentially regulated following extended exposure to the cytokine. In this study, we demonstrate that naive and memory CD4$^+$ lymphocytes display distinct kinetics of cell cycle entry in response to continuous long-term exposure to IL-7, with naive lymphocytes entering into cycle with significantly delayed kinetics. Intriguingly, the responsiveness of both naive and memory CD4$^+$ lymphocytes to IL-7 is transient, with memory T cells exiting cell cycle at earlier time

*Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5535, Montpellier, France; †Institut de Génétique Moléculaire de Montpellier, Montpellier, France; ‡Université Montpellier, Montpellier, France; §Institut Fédératif de Recherche 122, Montpellier, France; ¶Institut National de la Santé et de la Recherche Médicale, Unité 758, Lyon, France; ‖Ecole Normale Supérieure de Lyon, Lyon, France; and #Institut Fédératif de Recherche 128, BioSciences Lyon-Gerland, Lyon, France

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[2] L.S. and E.V. contributed equally to this study and are listed alphabetically.

[3] Address correspondence and reprint requests to Dr. Naomi Taylor, Institut de Génétique Moléculaire de Montpellier, 1919 Route de Mende, 34293 Montpellier, Cedex 5, France. E-mail address: taylor@igmm.cnrs.fr

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points. This exit reflects a relative refractoriness to IL-7-mediated signaling, as monitored by activation of the proximal signaling intermediate STAT-5. Importantly, transcription of the IL-7Rα subunit of the IL-7R is down-regulated following short term IL-7 exposure but is then differentially up-regulated on naive and memory lymphocytes, with kinetics paralleling cell cycle exit. The ensemble of these data indicates important differences in the IL-7 reactivity of naive and memory $CD4^+$ T lymphocytes. Finally, our data point to intrinsic feedback mechanisms that limit the responsiveness of naive and memory T cell subsets following long-term continuous exposure to the IL-7 cytokine.

Materials and Methods

Cell isolation and culture conditions of primary T cells

Adult peripheral blood, obtained from healthy adult donors after informed consent, was collected in heparinized tubes. $CD4^+$ T cells were purified by negative selection using the Rosette tetrameric complex system (StemCell Technologies), wherein Abs recognize surface Ag on B cells, monocytes, NK cells, or $CD8^+$ cells and another recognizes glycophorin A on the surface of RBC. Non-$CD4^+$ T cells were then pelleted upon Ficoll-Hypaque (Sigma-Aldrich) separation, and nonbound $CD4^+$ T cells were recovered. To isolate naive and memory T cell subsets by negative selection, $CD4^+$ lymphocytes were incubated with anti-CD45RO (DakoCytomation) or anti-CD45RA (Immunotech) mAbs, respectively, in combination with anti-HLA-DR and anti-CD69 mAbs to eliminate preactivated T cells. The nonbound naive ($CD45RO^-$) and memory ($CD45RA^-$) cells were recovered following addition of anti-mouse IgG-conjugated magnetic beads (Dynal Biotech). The purity of each cell isolation was monitored on a FACSCalibur (BD Pharmingen) after staining with appropriate fluorochrome-conjugated mAbs and was consistently >90%.

Lymphocytes were cultured in RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% (vol/vol) FCS, penicillin and streptomycin, and human rIL-7 (10 ng/ml; Cytheris). IL-7 was replenished every 2–3 days during the entire culture period at a concentration of 10 ng/ml. Where indicated, lymphocytes were stimulated with anti-CD3/CD28 beads at a bead to cell ratio of 1:1 (Xcyte Therapies).

Flow cytometry for surface markers and cell cycle analysis

To detect expression of the α-subunit of the IL-7R (CD127), cells were incubated for 20 min on ice with the PE-conjugated mAb (Immunotech) at the indicated time points. Background fluorescence was measured using an Ig isotype control Ab. Cells were washed with PBS (Bio Media) and then analyzed on a FACSCalibur (BD Pharmingen). To assess survival, lymphocytes were incubated with propidium iodide (10 μg/ml). Data analyses were performed using CellQuest software (BD Pharmingen) or FlowJo (Tree Star).

Cell cycle analysis was performed by staining DNA and RNA with 7-aminoactinomycin D (7-AAD)[4] and pyronin Y, respectively. A total of 5×10^5 cells were labeled with 7-AAD (Sigma-Aldrich) at a final concentration of 20 μM for 30 min at room temperature followed by 5 μM pyronin Y (Sigma-Aldrich) for 10 min on ice. Cells were immediately analyzed on a FACSCalibur flow cytometer.

STAT-5 phosphorylation analysis

At the indicated time points, cells were prepared for intracellular staining by fixation (Cytofix; BD Pharmingen), and permeabilization (PhosFlow Perm III; BD Pharmingen). The phosphorylation state of STAT-5 was assessed using an anti-phospho-STAT-5 (Y694) Ab coupled to Alexa Fluor 647, according to the manufacturer's instructions (BD Pharmingen). Control fluorescence was analyzed using Alexa Fluor 647-coupled control IgG Abs (BD Pharmingen).

Quantitative analysis of IL-7Rα mRNA levels

At the indicated time points, 1×10^6 cells were lysed and total RNA was extracted by GenElute mammalian total RNA miniprep kit (Sigma-Aldrich). cDNAs were prepared by reverse transcription and quantitative PCR was performed using the Quantitect SYBR green PCR Master mix (Qiagen) with 2 μl of cDNA in a final volume of 20 μl and the following primers at a final concentration of 500 nM. Primers for IL-7Rα were 5′-TACCGTGAGCGACAAAGATG-3′ (forward) and 5′-GCTGAATCATTGGGTCACCT-3′ (reverse). Primers for GAPDH were 5′-ACACCCACTCCTCCACCTTT-3′ (forward) and 5′-TCCACCACCCTGTTGCTGTA-3′ (reverse). Amplification of IL-7Rα and GAPDH cDNAs were performed using the LightCycler 2000 instrument (Roche). The cycling conditions comprised a denaturation step for 15 min at 95°C, followed by 40 cycles of denaturation (95°C for 15 s), annealing (59°C for IL-7Rα or 62°C for GAPDH for 20 s), and extension (72°C for 15 s). After amplification, melting curve analysis was performed with denaturation at 95°C for 5 s, then continuous fluorescence measurement from 70°C to 95°C at 0.1°C/s. Each sample was amplified in duplicate.

[4] Abbreviations used in this paper: 7-AAD, 7-aminoactinomycin D; MFI, mean fluorescence intensity.

Results

Distinct cell cycle entry and exit kinetics of naive and memory $CD4^+$ T cells

We and others have previously reported increased cell cycle entry of memory T cells as compared with naive T cells following IL-7 stimulation (23–26, 29, 30). Nevertheless, these published experiments were all performed during a short IL-7 stimulation period (<6 days). Intriguingly, we now find that following extended culture in the presence of exogenous IL-7, the percentage of cycling naive T cells increases, with levels of cell cycle entry surpassing those observed in equivalently treated memory T cells (Fig. 1). Thus, by day 8 of culture in the continued presence of rIL-7, a higher number of naive T cells are in cycle as compared with memory cells, as monitored by visualizing the RNA and DNA contents of the cells. These data indicate that naive T cells actively respond to IL-7 stimulation, albeit with slower kinetics than their memory counterpart.

Due to the late entry of IL-7-stimulated naive T cells into cell cycle, it was of interest to study the long-term cell cycle kinetics of these lymphocytes. To this end, fresh media supplemented with rIL-7 (10 ng/ml) was added to the cultures every 2–3 days, maintaining an IL-7 concentration of at least 10 ng/ml. This dose has been found to be optimal for inducing IL-7-mediated responses (26). It was therefore somewhat surprising to find that $CD4^+$ memory T cells began to exit from the cell cycle within 10 days post-IL-7 stimulation, with <10% of cells in cycle following 14 days of culture in the continuous presence of this cytokine (Fig. 1). In marked contrast, the percentages of naive T cells in cycle increased during the first 14–15 days of continuous IL-7 stimulation. Moreover, the actual proportion of naive cells progressing to $S/G_2/M$ phases of the cell cycle was consistently higher than that observed in memory cells, with percentages of 13 vs 4% in the representative experiment shown in Fig. 1. Nevertheless, like their memory counterpart, naive cells too exited from the cell cycle, albeit with much slower kinetics. Only after >30 days of continuous culture did cell cycle progression of naive cells decrease to levels lower than 10% (Fig. 1 and data not shown). These data were confirmed by assessing cell size, another measure of T cell activation (data not shown). It is important to note that although there were small differences in cell cycle entry and exit when these parameters were assessed in naive and memory $CD4^+$ T cells isolated from different donors, the same trend was observed in all 10 individual donors studied (data not shown). Taken together, these data indicate that naive and memory T cells respond only transiently to IL-7, at least as assessed by cell cycle entry. The kinetics of both cell cycle entry and exit differ for these two subsets with significantly extended IL-7-mediated cycling of naive T cells.

IL-7 induces transient activation of the STAT pathway in $CD4^+$ T lymphocytes

Because IL-7 responsiveness can be monitored as a function of STAT-5 activation, the kinetics and magnitude of STAT-5 phosphorylation in naive and memory T cells were studied. STAT-5 was phosphorylated in the vast majority of naive and memory

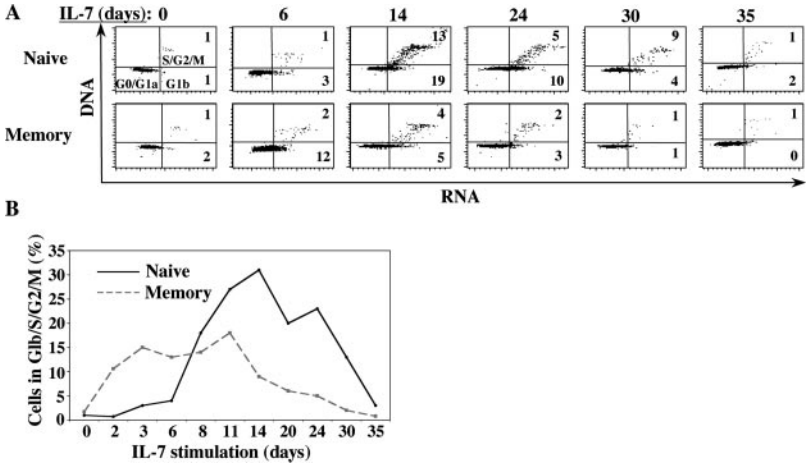

FIGURE 1. Distinct cell cycle entry and exit kinetics of IL-7-stimulated naive and memory T cells. *A*, Cell cycle progression of freshly isolated naive and memory $CD4^+$ T cell populations was analyzed following 0–35 days of IL-7 stimulation (10 ng/ml). Cells were stained for DNA and RNA levels using 7-AAD and pyronin Y, respectively. In each dot plot, cells (*lower left quadrant*) have not yet entered the G_{1b} phase of the cell cycle. The percentages of cells in the G_{1b} (*lower right quadrant*) and $S/G_2/M$ phases (*upper right quadrant*) are indicated. Note, cultures were replenished with rIL-7 (10 ng/ml) every 2–3 days during the entire period. *B*, Quantification of a representative experiment showing cell cycle entry, as defined by cells in $G_{1b}/S/G_2/M$, following 0–35 days of IL-7 stimulation. Results are representative of data obtained in eight independent experiments.

$CD4^+$ lymphocytes within 30 min following IL-7 stimulation, as assessed by intracellular staining with an Ab recognizing the Y694-phosphorylated form of STAT-5 (Fig. 2). The magnitude of STAT-5 phosphorylation, determined by the mean fluorescence intensity (MFI) of staining, was maximal at day 2 of stimulation and then decreased slowly thereafter. Nevertheless, it is important to note that in the context of continual rIL-7 stimulation, STAT-5 was phosphorylated in the majority of naive lymphocytes during the first 20 days of culture. In marked contrast, there was an absence of detectable STAT-5 phosphorylation in >50% of equivalently treated memory T cells by day 20 (Fig. 2). Upon further culture, there was an abrogation of proximal IL-7 signaling, as demonstrated by a lack of phospho-STAT-5 in both naive and memory T lymphocytes. Thus, these data are in accord with the cell cycle progression experiments discussed, demonstrating that IL-7-induced signaling is transient in both naive and memory T cells, with extended IL-7 responsiveness in naive T cells.

IL-7R expression as a function of cell cycle progression and signaling

Receptor signaling is generally accompanied by an internalization of the ligand-receptor complex. Therefore in the case of IL-7, wherein activation of the proximal STAT-5 effector was defective following extended IL-7 stimulation, it was of interest to determine the status of IL-7R expression at the cell surface. The IL-7R is composed of the IL-7R α-chain as well as the γ_c chain, a subunit also found in the IL-2, IL-4, IL-9, IL-15, and IL-21 receptors. Preliminary experiments indicated that surface levels of the γ_c chain were not significantly modulated by IL-7 stimulation, whereas the IL-7R α-chain was rapidly down-regulated, within 30 min of addition of the cytokine (Fig. 3 and data not shown). Surface expression descended to a nadir within 12 h. IL-7-stimulated naive and memory lymphocytes differed with respect to this internalization, in that by day 6 of stimulation, there was consistently a small percentage (3–5%) of memory T cells expressing IL-7Rα as compared with naive T cells where this receptor subunit was not detected (Fig. 4).

Surface expression of IL-7Rα increased in both naive and memory T cells following extended IL-7 stimulation. Importantly, this increase was inversely correlated with IL-7 signaling, as monitored by the STAT-5 phosphorylation and cell cycle progression experiments described. Indeed, the percentages of cells expressing IL-7Rα and the level of IL-7Rα expression (MFI) increased at significantly earlier time points in memory T cells as compared with naive cells. Specifically, there was a lag of at least 8–10 days; IL-7Rα was detected on memory cells by day 10–12 and on naive cells, only after 20 days of culture. Thus, surface expression of IL-7Rα is inversely proportional to the level of IL-7 signaling following extended exposure to rIL-7. Furthermore, receptor levels on the naive and memory T cell subsets are up-regulated with distinct kinetics.

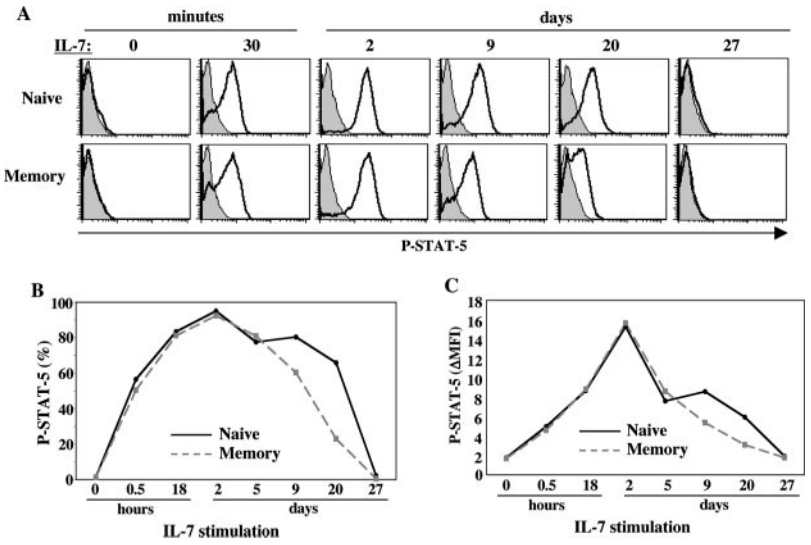

FIGURE 2. STAT-5 is rapidly phosphorylated in IL-7-stimulated T cells but is not maintained during extended culture. Naive and memory $CD4^+$ lymphocytes were cultured in the continuous presence of rIL-7 (10 ng/ml) and at the indicated time points, STAT-5 phosphorylation was monitored. Cells were permeabilized and stained with the Alexa Fluor 647-conjugated polyclonal Ab, recognizing the Tyr^{694}-phosphorylated form of STAT-5 (P-STAT-5). *A*, Representative histogram plots depicting staining (open histograms) relative to control IgG fluorescence (shaded histograms) are shown. *B*, Quantification of the percentages of naive and memory T cells harboring phosphorylated STAT-5 at each time point. *C*, Quantification of the level of phosphorylated STAT-5 as monitored by ΔMFI relative to control staining at each time point.

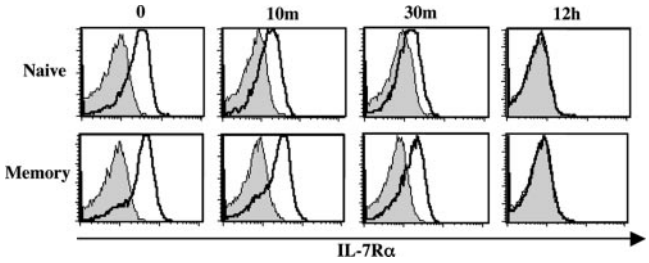

FIGURE 3. Rapid internalization of IL-7Rα following IL-7 stimulation. Surface expression of IL-7Rα on naive and memory CD4$^+$ T cell subsets was analyzed at early time points (0–12 h) following IL-7 stimulation. Cells were stained with a PE-conjugated anti-IL-7Rα mAb (open histograms), and at each time point fluorescence relative to control IgG (shaded histograms) is shown.

IL-7R gene expression in IL-7-stimulated CD4$^+$ T cells

The finding that surface IL-7Rα expression was up-regulated following extended exposure to rIL-7 was surprising. These data suggested that either 1) the treated lymphocytes had ceased to internalize IL-7Rα following interaction with the cytokine, and/or 2) there was an increased production and/or transport of the IL-7Rα subunit to the surface following extended IL-7 stimulation. As such, we proceeded to assess the level of IL-7Rα transcripts in naive and memory T cells following IL-7 stimulation. Using real-time PCR, it was intriguing to note that the baseline level of IL-7Rα transcripts in naive T cells was ~3-fold higher than that detected in memory T cells (Fig. 5*A*). Following IL-7 stimulation, the level of IL-7Rα transcripts in both naive and memory cells decreased slowly until day 8. Interestingly though, IL-7Rα gene expression then diverged in IL-7-stimulated naive and memory T cells. IL-7Rα mRNA levels increased in memory cells, correlating with increased surface expression (Fig. 4), whereas mRNA levels in naive cells remained suppressed for a further 10 days of culture. Furthermore, in naive T cells, the decrease in IL-7Rα mRNA was much more pronounced with mRNA levels decreasing by 30-fold as compared with a decrease of only 6-fold in memory cells (Fig. 5*A*). Significant increases in IL-7Rα mRNA levels were detected only after 20–24 days of culture in the presence of IL-7. Thus, it appears that the re-emergence of IL-7Rα at the cell surface of both naive and memory T cells is regulated at the level of transcription. Moreover, the transcriptional control of IL-7Rα following IL-7 stimulation is distinct in naive and memory T cells.

The dramatic down-regulation of IL-7Rα mRNA levels by day 8 post-IL-7 stimulation was somewhat surprising in light of our observations that IL-7-mediated signaling, as monitored by STAT-5 phosphorylation, could be detected for at least 20 days post-IL-7 stimulation (Fig. 2). Two possibilities are as follows: 1) signaling is maintained in the absence of continuous interactions between IL-7 and its receptor; or 2) signaling is maintained despite the low levels of IL-7Rα transcripts. The first hypothesis is unlikely because we determined that STAT-5 phosphorylation decreases to undetectable levels within 48 h following removal of rIL-7 from the culture (data not shown). It was therefore of interest to compare IL-7Rα mRNA levels in IL-7-stimulated cells with its level in other cells wherein expression is low or absent. To this end, it has recently been shown that IL-7Rα expression is minimal in TCR-activated T cells (2, 31–33). Indeed, following TCR engagement, IL-7Rα mRNA levels quickly diminished, with significantly faster kinetics than in IL-7-stimulated T cells (Fig. 5*B*). Moreover, although IL-7Rα mRNA levels decreased in both culture conditions, the level of IL-7Rα mRNA was ~10-fold lower in the TCR-stimulated cells (Fig. 5*B*). Thus, the low level of IL-7Rα transcripts detected in IL-7-stimulated T cells is likely to be sufficient to allow for continued low level signaling during the first 20–25 days of IL-7 stimulation, at least in naive T cells. Nevertheless, IL-7Rα mRNA levels are not the limiting factor in IL-7 responsiveness as gene expression is increased at late time points when the cells are refractory to IL-7-mediated signals.

IL-7 promotes long-term survival of naive and memory CD4$^+$ T cells

The augmented IL-7Rα mRNA levels in naive and memory CD4$^+$ T cells coincided with a relative incapacity of the cell to transduce IL-7-mediated signals, as assessed by activation of the STAT-5 transcription factor and cell cycle progression (Figs. 1 and 2). Nevertheless, when cultured in the presence of IL-7, CD4$^+$ lymphocytes demonstrated extended viability. To specifically address this point, we quantified survival of IL-7-stimulated naive and memory CD4$^+$ T lymphocytes following 0–35 days of IL-7 stimulation. During the entire 35 day culture period, the viability of both naive and memory T cells was greater than 80% (Fig. 6*A*), whereas all cells were dead in the absence of cytokine stimulation (data not shown). Thus, although the responsiveness of these CD4$^+$ lymphocytes to IL-7 is significantly muted following extended exposure to the cytokine, these data indicate that survival is promoted

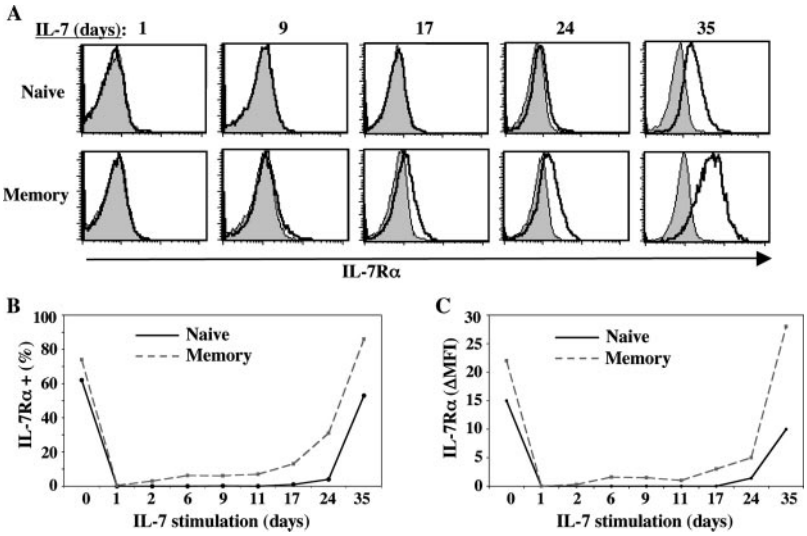

FIGURE 4. Surface IL-7Rα expression is up-regulated at late time points following IL-7 stimulation. *A*, Surface expression of IL-7Rα was assessed on naive and memory T cells following 0–35 days of culture in the continuous presence of rIL-7 (10 ng/ml). Cells were stained with a PE-conjugated anti-IL-7Rα mAb, and histograms at selected time points depicting fluorescence relative to control IgG are shown. *B*, Quantification of the percentages of naive and memory T cells expressing IL-7Rα at the cell surface. *C*, Quantification of the relative expression of surface IL-7Rα as monitored by the ΔMFI relative to control staining. Results are representative of data obtained with seven different donors.

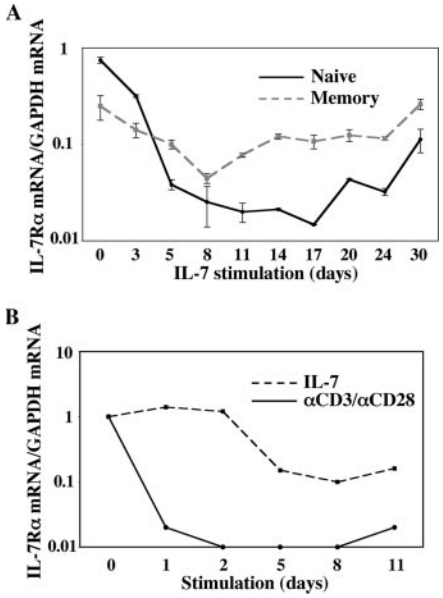

FIGURE 5. IL-7Rα expression in IL-7-stimulated T cells is regulated at the mRNA level. *A*, Naive and memory $CD4^+$ T lymphocytes were stimulated with rIL-7 (10 ng/ml). At the indicated time points, total RNAs were extracted and reverse transcribed. cDNAs were amplified with primers specific for IL-7Rα and GAPDH. The relative level of mRNA transcripts was assessed by real-time PCR in duplicate samples, and the mean number of molecules of IL-7Rα (±SD) relative to GAPDH at each time point is shown. *B*, The levels of IL-7Rα transcripts in TCR-stimulated $CD4^+$ T cells, via anti-CD3/CD28 mAbs, were compared with those detected in IL-7-stimulated cells at the indicated time points. The levels of IL-7Rα relative to GAPDH are shown at each time point, with the value at day 0 arbitrarily designated as 1.

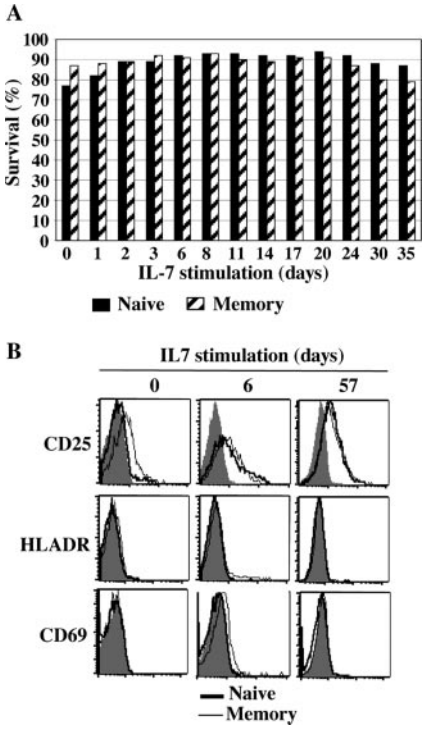

FIGURE 6. Survival and phenotype of IL-7-stimulated naive and memory T cells. Naive and memory $CD4^+$ T lymphocytes were isolated from peripheral blood by negative selection. Cells were cultured in the presence of IL-7 (10 ng/ml) for 1–57 days, and cytokine was replenished every 2–3 days. *A*, Cell death was analyzed by propidium iodide staining, and the percentage of live cells is indicated. Data from one of six representative experiments are shown. *B*, Expression of the CD25, CD69, and HLA-DR activation markers was assessed on IL-7-stimulated naive and memory T cells at days 0, 6, and 57 of culture.

over a long time period. This long-term IL-7-induced response likely reflects the transduction of earlier IL-7-mediated signals as survival is maintained even following withdrawal of this cytokine (data not shown). Altogether these data indicate that IL-7-induced survival of both naive and memory T cells largely surpasses the duration of a direct IL-7-mediated signal and cell cycle effects. Moreover, the phenotype of these naive and memory cells is largely maintained for over 50 days in culture (Fig. 6*B*). As previously reported, neither IL-7-stimulated naive nor memory $CD4^+$ T cells demonstrated an up-regulation of CD69 or HLA-DR activation markers, although CD25 was up-regulated as previously reported (34). Notably, this up-regulation remained relatively constant over 50 days of stimulation (Fig. 6*B*). Finally, the naive phenotype of the cells, as monitored by CD45RA expression, was maintained on ~90% of cells during long-term IL-7 stimulation and intriguingly, CD45RA was up-regulated on >40% of long-term cultured memory cells (data not shown). Thus, long-term IL-7 stimulation does not appear to induce extensive expression of memory markers and CD45RA cells maintain their naive phenotype.

Discussion

The present study shows that extended IL-7 stimulation preferentially promotes cell cycle progression of naive $CD4^+$ T cells as compared with the memory counterpart. Intriguingly, proximal IL-7 signaling responses are equivalently induced in the two subsets at early time points, but are extended for over 3 wk in naive cells. Surface levels of IL-7R on freshly isolated naive and memory cells are equivalent (24, 25), but we demonstrate that the basal level of IL-7Rα mRNAs is 3-fold higher in naive cells than memory cells. This result may reflect the greater dependence of naive cells on IL-7-induced survival signals. Importantly though, our data reveal that IL-7-mediated homeostatic proliferation cannot be maintained for perpetuity and is regulated by complex mechanisms.

We show that transcription of the IL-7Rα subunit of the receptor is suppressed in both naive and memory T cells following IL-7 stimulation. Transcriptional suppression of IL-7Rα occurred with relatively slow kinetics, with only a 2-fold decrease in IL-7Rα mRNA levels observed after 3 days of continuous IL-7 stimulation. The suppression of IL-7Rα transcription appears to occur more rapidly in murine T cells, with a 60–70% decrease following overnight exposure to rIL-7 (35). This may reflect intrinsic differences between murine and human T cells, or alternatively, the in vivo environment from which these cells were isolated; the source of the human T cells used in this study was peripheral blood, whereas the source of murine T cells was the lymphoid milieu of lymph nodes. Nonetheless, in both murine and human T cells, this regulatory feedback mechanism may serve to inhibit further signaling in cells that have already received an IL-7 survival signal (35). Indeed, we find that the time period during which an IL-7-mediated signal promotes survival largely exceeds the period during which a proximal signaling response is observed. Moreover, the "magnitude" of the IL-7 signaling response is likely to condition the fate of the lymphocyte; IL-7 can induce metabolic and proliferative responses in addition to a survival response (28, 36–39), and these responses can be distinguished by the timing and administered dose of exogenous IL-7 (L. Swainson, manuscript in preparation). Further work will be necessary to determine whether

the signaling intermediates induced in naive and memory subsets are equivalent, at both a qualitative and quantitative level. Our preliminary data indicate that IL-7-mediated cell cycle progression is associated with higher levels of induced glucose transporter expression (L. Swainson, unpublished observations).

The initial down-regulation of surface IL-7Rα levels on IL-7-stimulated lymphocytes is the result of a rapid receptor internalization, with very low Ab binding within 30 min poststimulation. This internalization consistently occurred with more rapid kinetics in naive lymphocytes as compared with memory cells, but the physiological consequence is not clear because activation of STAT-5 was observed in ~50% of both T cell subsets by 30 min. Our finding that STAT-5 phosphorylation was detected in human lymphocytes cultured in the continuous presence of IL-7, even under conditions wherein IL-7Rα mRNA levels were decreased by 6- to 30-fold, strongly suggests that an IL-7-mediated signal was being transmitted. This hypothesis is supported by two observations: 1) STAT-5 phosphorylation is abrogated within 24–48 h following removal of IL-7 (L. Swainson, unpublished observations), and 2) the relative number of IL-7Rα transcripts in TCR-stimulated T cells drops to ~10-fold lower levels than in IL-7-stimulated T cells (Fig. 5*B*). Thus, in contrast to TCR-engaged lymphocytes, wherein an IL-7 signal cannot be relayed (2, 31–33), we propose that IL-7 signaling occurs in long-term stimulated naive and memory $CD4^+$ T lymphocytes. Nevertheless, signaling is not optimal and as such, cells eventually exit from the cell cycle. Thus, these data point to a complex regulatory mechanism by which initial stimulation with IL-7 induces cell cycle entry of a diverse pool of lymphocytes, with a bias toward the cycling of naive T cells. Following this initial stimulation, subsequent signaling is dampened, due in large part to a suppression of IL-7Rα gene expression, and lymphocytes return to the quiescent state. It will be important to determine the manner in which IL-7 stimulation modulates the ability of a given T cell to then respond to Ag-TCR stimulation.

Several recent studies have found that IL-7Rα levels are down-regulated in vivo, in response to viral infections and aging. Specifically, the level of IL-7Rα transcription is inversely correlated with disease progression in HIV-infected patients (40, 41), and IL-7Rα is expressed only at very low frequencies on EBV- and CMV-specific T cells (42). Thus, persistent Ag exposure can suppress IL-7Rα expression, resulting in the exhaustion of a stable T cell population (43). This result likely accounts for the higher numbers of IL-7Rα-negative T cells detected in the peripheral blood of the elderly and significantly, these cells show decreased survival in response to IL-7 (44). Our results demonstrate that the decreased levels of IL-7Rα can also occur as a result of continuous IL-7 exposure, but in the absence of Ag stimulation, receptor levels are subsequently up-regulated at the mRNA level as well as at the cell surface. The mechanisms resulting in enhanced IL-7Rα transcription in T lymphocytes are only beginning to be elucidated, but at least in precursors, the transcriptional activator PU.1 promotes transcription (45). In murine T cells where PU.1 is not expressed, it appears that the GA binding protein promotes IL-7Rα transcription (46) while suppression of this gene is mediated via the transcriptional repressor factor GFI1 (35, 47). Nevertheless, it is not clear whether IL-7Rα repression in human T cells proceeds via the same mechanism (44). Irrespective of the precise mechanisms implicated in this regulation, it appears that the reappearance of IL-7Rα on activated T cells is associated with a survival advantage conferring protective immunity (48).

rIL-7 has recently entered phase I clinical studies in patients with advanced cancers (National Cancer Institute Study CYT 99 007), with the goal of enhancing the immune response. Indeed, an elegant murine study has found that rIL-7 is a potent vaccine adjuvant and improves T cell survival (49). The data presented in this study, revealing distinctions in the reactivity as well as kinetics of naive and memory T cells to IL-7, will help optimize the administration of IL-7 in future clinical trials. Finally, our elucidation of inhibitory feedback mechanisms limiting IL-7 responsiveness need to be taken into account in clinical studies assessing an immunostimulatory role for rIL-7 in lymphopenic patients.

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Disclosures

The authors have no financial conflict of interest.

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