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Differential Regulation of Human IL-7 Receptor α Expression by IL-7 and TCR Signaling[1]

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IL-7Rα is essential for the development and homeostatic maintenance of mature T cells. Studies in humans and mice have shown that IL-7Rα expression is reduced by its cognate cytokine, IL-7, and Ag, suggesting that active regulation of IL-7 responsiveness is necessary to balance T cell numbers. We show that IL-7- or TCR/CD28-mediated signaling induced a rapid down-regulation of IL-7Rα expression on naive T cells on the mRNA and protein level, with a mild (10-fold) or strong (50-fold) gene suppression, respectively. In both situations, the down-regulation of IL-7Rα was blocked by cyclohexamide and actinomycin D, indicating the involvement of an active mechanism dependent on new transcription and protein synthesis. Upon IL-7 withdrawal, IL-7Rα mRNA and surface protein reappeared in a transcription-dependent manner within 7 h. Yet, IL-7Rα was hardly re-expressed during the same period after TCR/CD28-activation. Likewise, T cells that were activated through CMV in vivo did not re-express IL-7Rα after in vitro culture. Functionally, IL-7-induced down-regulation of IL-7Rα did not hinder the responsiveness of naive T cells to IL-7. Conversely, down-regulation of IL-7Rα on TCR/CD28-activated cells limited IL-7 responsiveness. Strikingly, ectopic expression of IL-7Rα cells on TCR/CD28-activated cells conferred a selective advantage in the response to IL-7. In conclusion, our data show that IL-7- and TCR/CD28-mediated signaling differentially regulate IL-7Rα expression on human T cells with a transient and chronic effect, respectively. The stringent and active regulation of IL-7Rα may constitute a homeostatic mechanism to curtail unwarranted T cell expansion. ***The Journal of Immunology,*** **2008, 180: 5201–5210.**

The role of IL-7Rα in T cell ontogeny and maintenance is well established. The variable expression found at different stages of T cell development, activation, and differentiation infers that the IL-7Rα locus is under active control. IL-7 regulates the homeostasis of naive T cells in the periphery together with signals from low affinity interactions between TCR and self-peptide-major histocompatibility complexes (1–3). IL-7 belongs to the common cytokine-receptor γ-chain family. It binds a dimeric receptor constituted by the high affinity IL-7Rα-chain (CD127) and the γ-chain (CD132) (4). IL-7 may be a limiting survival factor because its levels in the circulation correlate inversely with T cell numbers (5–7). Both human and mouse naive T cells expanded in IL-7 maintain their phenotype, except for the conspicuous down-regulation of IL-7Rα (8, 9). For murine T cells, it has been established that this is an active process regulated at the transcriptional level (9, 10). These findings have led to the assumption that the down-regulation of IL-7Rα constitutes a homeostatic mechanism to regulate IL-7 consumption by naive T cells (9). Although a similar mechanism may be expected, the certainty of this principle remains to be demonstrated in humans.

Studies with human and mouse T cells have demonstrated that antigenic stimulation also causes a down-regulation of cell surface IL-7Rα protein in T cells (10–14). Current models explain the biological significance of this dynamic regulation as a homeostatic mechanism that controls IL-7 responsiveness. We performed a comprehensive characterization on the impact of IL-7- or combined TCR/CD28-activation on the regulation of IL-7Rα in human naive T cells. Our data demonstrated that the expression of IL-7Rα in T cells was actively and differentially controlled by these two distinct signals. Markedly, whereas IL-7 induced a transient down-regulation of IL-7Rα, TCR/CD28-mediated signaling caused a chronic down-regulation of this receptor. These dissimilarities are reflected in the subsequent responsiveness to IL-7.

Materials and Methods

Reagents and mAbs

The mAbs PE-conjugated CD4, PerCP-conjugated CD3, allophycocyanin-conjugated CD3, PerCP-conjugated CD8, and Alexa Fluor 647-conjugated pSTAT5 were purchased from Becton Dickinson. Isotype controls consisted of mouse IgG1. Allophycocyanin-conjugated CD8, PE-conjugated and allophycocyanin-conjugated CD45RA were obtained from Coulter. CD27-FITC (clone 3A12) and allophycocyanin-conjugated HLA-A2 tetramer loaded with the CMV pp65-derived NLVPTMVATV peptide and HLA-B7 tetramer loaded with the CMV pp65-derived TPRVTGGGAM peptide were obtained from Sanquin. PE-conjugated anti-IL-7Rα (CD127) was purchased from Immunotech. Microbeads coated with anti-human CD4 or CD8 (CD4 or CD8 microbeads) were purchased from Miltenyi Biotec, MACS. Cells were stained with indicated mAbs and were analyzed on a FACSCalibur (BD Biosciences). Cyclohexamide (CHX)[5] and actinomycin D (Act D) were obtained from Sigma-Aldrich.

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[5] Abbreviations used in this paper: CHX, cyclohexamide; Act D, actinomycin D; IRES, internal ribosomal entry site; O/N, overnight; MFI, mean fluorescence intensity; TSLP, thymic stromal lymphopoietin.

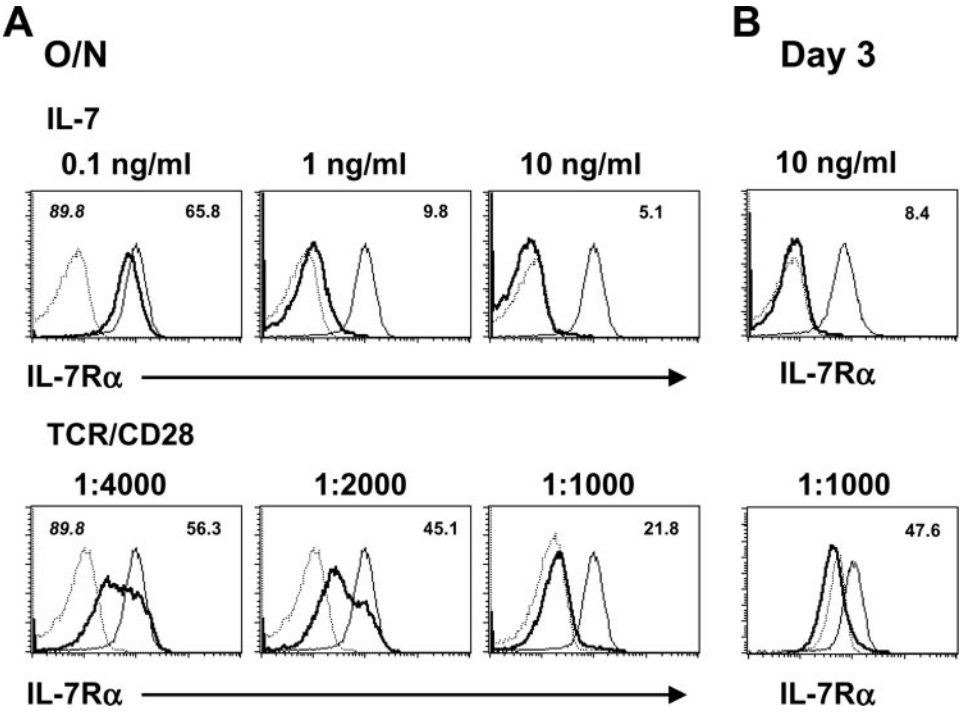

FIGURE 1. IL-7Rα expression is down-regulated by both IL-7 and TCR/CD28-triggering in a dose-dependent manner. Cord blood-derived naive $CD4^+$ T cells were stimulated O/N with IL-7 (*top row*) or combined anti-CD3/CD28 mAbs (*bottom row*). The expression IL-7Rα was measured by FACS analysis. *A*, Overnight (O/N) cultures with a graded concentration of IL-7 or anti-CD3 mAb, the latter combined with a fixed amount of anti-CD28 mAb (5 μg/ml). *B*, Three-day cultures with the highest concentration of IL-7 or combined anti-CD3/CD28 mAb. Ex vivo naive cells (thin line); stimulated cells (bold line); and isotype control (dashed line). Numbers in the histograms represent the MFI of treated (right) or untreated naive (italic) cells. Data are from one representative experiment of three performed.

Cell preparation

Human PBMCs from healthy donors, and umbilical cord blood mononuclear cells were isolated by Ficoll-Isopaque density gradient centrifugation (Nycomed, Pharma). Naive $CD4^+$ or $CD8^+$ T cells were purified from total umbilical cord blood mononuclear cells by positive selection using the MACS system, as described previously (15). The sample purity was assessed by FACS, with PE-conjugated CD4 and PerCP-conjugated CD3 mAb or PerCP-conjugated CD3 and allophycocyanin-conjugated CD8. The purity ranged 90–95%.

The J16 clone was derived from the human T acute lymphoblastic leukemia cell line Jurkat by limiting dilution and was selected for CD95 sensitivity (16).

CFSE labeling

Purified naive $CD4^+$ T cells were pelleted and resuspended in PBS at a final concentration of $5–10 \times 10^6$ cells/ml. Next, cells were labeled in 0.5 μM (final concentration) of CFSE (Molecular Probes Europe BV) in PBS for 10 min at 37°C. Cells were washed and subsequently resuspended in IMDM supplemented with L-Glutamine, 25 mM HEPES (Biowhittaker), containing 10% human pool serum (Biowhittaker), streptamycin (100 ng/ml) (Invitrogen Life Technologies), penicillin (10 U/ml) (Yamanouchi, Pharma), and 3.57×10^{-4} % (v/v) β-mercapto ethanol (Merck) (culture medium).

Cell culture and viability

Cells were cultured in culture medium for the indicated time points at 37°C in 5% CO_2 atmosphere. For cytokine activation, cells were cultured in the presence or absence of a range of concentrations (0.1–10 ng/ml) of IL-7 (Strathmann) or IL-15 (R&D Systems). For combined TCR/CD28 activation, cells were cultured with plate-bound anti-CD3 mAb (0.25–1.0 μg/ml) (clone 16A9; Sanquin) in the presence or absence of soluble anti-CD28 mAb (5 μg/ml) (clone 15E8; Saquin). Cell viability was assessed on the FACS by propidium iodide (5 μg/ml) exclusion or live gating based on forward scatter/side scatter.

Real-time PCR

Total RNA was extracted from freshly isolated cells (resting) and cytokine- or TCR/CD28-stimulated T cells using GenElute Mamalian total RNA Miniprep kit (Sigma-Aldrich). Oligodeoxythymidine-primed cDNA was synthesized using avian myeloblastosis virus reverse transcriptase (Roche Molecular Biochemicals). From these cDNA pools, specific targets where amplified by PCR performed with Lightcycler FastStart DNA Master SYBR Green I from Roche, using the sense and antisense IL-7Rα primers 5′-TCGCAGCACTCACTGACC-3′ and 5′-CGGGAAGGAGCCAATGAC-3′, the wGFI1 primers 5′-TGACTTGGGGAAGGAATTTA-3′ and 5′-CCAGTGATGAGGTTTTCACA-3′, the GAPBα primers 5′-AGCATCAGTGCAATCTGCTA-3′ and 5′-TTCCCAGGTGAGCTTCTATC-3′ and the 18S primers 5′-GGACAACAAGCTCCGTGAAGA-3′ and 5′-CAGAAGTGACGCAGCCCTCTA-3′, respectively. The results were normalized to 18S rRNA.

IL-7Rα cloning, retroviral constructs, and transduction

A cDNA encoding human IL-7Rα was obtained via RT-PCR on RNA isolated from human naive T cells (Tap Polymerase). Forward primer: 5′-ATGAATTCCACCATGACAATTCTAGGTACAACTT-3′; reverse primer: 5′-CTCGAGTCACTGGTTTTGGTAGAAGC-3′. The forward primer has an *Eco*RI site followed by a Kozak sequence and a start codon. The reverse primer has a stop codon followed by a *Xho*I site. The PCR product was cloned into a pGEM T-vector (Promega) and after sequence verification (BigDye sequencing kit, ABI), recloned following *Eco*RI and *Xho*I digestion into the *Eco*RI and *Xho*I site of Lazarus-linker-internal ribosomal entry site (IRES)-GFP vector that has previously been published (17). Correct orientation was verified by restriction analysis. The retroviral plasmids (IL-7Rα-IRES-GFP (IL-7Rα) or control-IRES-GFP vector (Mock)) were transfected into the helper virus-free amphotropic producer cell line Phoenix with Fugene-6 (Roche), according to the manufacturer's protocol. The medium was refreshed the next morning, and 24 h later retroviral supernatants were collected, centrifuged and frozen in cell-free aliquots at −70°C.

Transduction of total T cells or Jurkat (J16) cells was performed according to standard protocols, as described previously (16). In brief, transduction of cells was performed by 1 cycle of overnight exposure to viral supernatant on retronectin-coated (Takara Shuzo) 24-well plates. Following overnight culture, cells were washed and transferred to 24-well tissue culture-treated plates (Costar) with new medium supplemented with IL-2 (20 U/ml) and IL-15 (1 ng/ml) to maintain viability. The efficiency of transduction was estimated by determining the percentage of GFP-positive cells by flow cytometry 3 days after transduction. To remove the dead cells, transduced cells were subjected to Ficoll-Isopaque density gradient centrifugation (Nycomed, Pharma) before use.

Statistical analysis

The two-tailed Mann-Whitney *U* test was used for analysis of differences between groups. A p value <0.05 was considered significant.

Results

IL-7Rα expression is down-regulated on T cells activated by IL-7 or TCR/CD28 engagement

We started by determining the effect of IL-7 or combined TCR/CD28 activation on the expression of IL-7Rα at protein level in

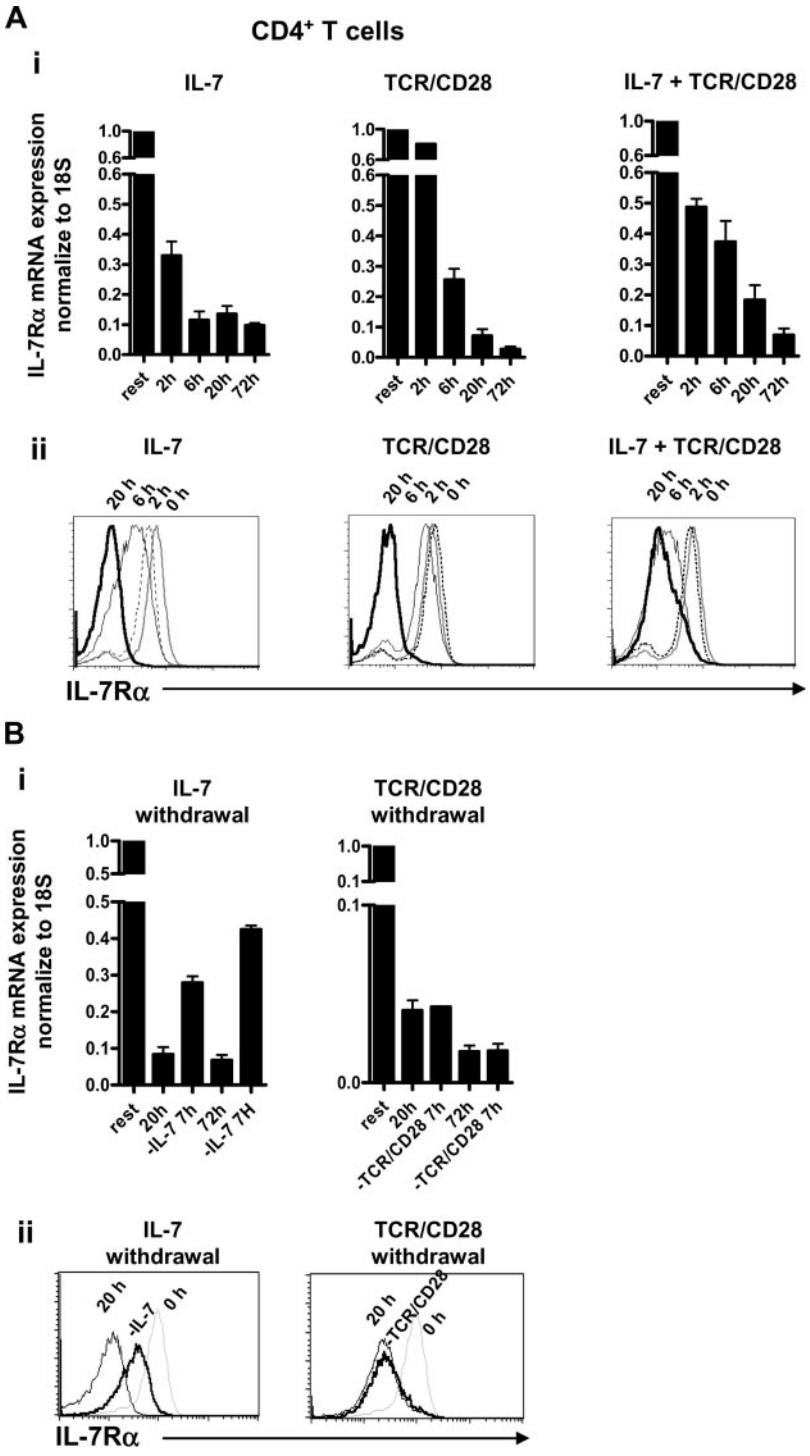

FIGURE 2. IL-7Rα expression is transiently or chronically reduced by IL-7 or TCR/CD28 activation, respectively. *A*, Kinetic analyses on the impact of IL-7, TCR/CD28 activation in the expression of IL-7Rα. Cord blood-derived naive $CD4^+$ T cells were stimulated with IL-7 (10 ng/ml) (*left*), combined anti-CD3/CD28 mAbs (1 and 5 μg/ml, respectively) (*middle*), or with both stimuli (*right*). *B*, Recovery of IL-7Rα expression following IL-7 or TCR/CD28 activation. Activated cells were washed and cultured in medium for 7 h. At the indicated time points: *i*, RNA was isolated and the expression of IL-7Rα was quantified by real-time PCR. Results were normalized to the values obtained from ex vivo naive cells (rest). Graphs show the mean expression and SD of three experiments. *ii*, IL-7Rα expression was analyzed by FACS analyses. In *B ii*, 20-h activated cells (thin line) were washed and cultured in medium for 7 h (bold line); ex vivo naive cells (dashed line).

human naive T cells. Cells were cultured overnight (O/N) with a graded concentration of either IL-7 or anti-CD3 mAb combined with fixed doses of anti-CD28 mAb. As depicted in Fig. 1*A*, both stimuli induced a rapid decrease of IL-7Rα cell surface expression of protein in a dose-dependent manner. Using the concentrations of both stimuli that yielded the most pronounced effect, we next evaluated the effect of prolonged activation. Naive T cells cultured in IL-7 remained IL-7Rα low (Fig. 1*B*, *upper row*). In TCR/CD28-activated cells, the expression of IL-7Rα was diminished compared with resting naive cells, although an increased mean fluorescence intensity (MFI) was observed in cells activated for 3 days as compared with O/N stimulation (Fig. 1*B*, *lower row*). However, as the MFI from isotype control was also increased, the stickiness of 3-day TCR/CD28-activated blasts may explain the high background staining. The same results were obtained using naive $CD8^+$ T cells (data not shown). Thus, human T cells rapidly downregulated IL-7Rα expression upon stimulation via its cognate cytokine, IL-7, or via TCR/CD28 engagement.

IL-7Rα expression is differentially regulated by IL-7 and TCR/CD28-mediated signaling

It has recently been shown that IL-7 suppresses transcription of the IL-7Rα gene in murine and human T cells (9, 10). Further, separate studies in humans and mice have demonstrated that IL-7Rα

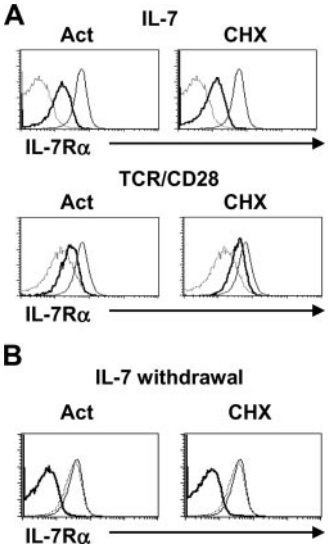

FIGURE 3. Regulation of IL-7Rα expression by IL-7 and TCR/CD28 activation requires both new transcription and protein synthesis. *A*, Cord blood-derived naive CD4$^+$ T cells were stimulated O/N with IL-7 (10 ng/ml) (*top row*) or combined anti-CD3/CD28 mAbs (1 and 5 μg/ml, respectively) (*bottom row*) in the presence (bold line) or absence (dashed line) of Act D (*left*) or CHX (*right*). *B*, Recovery of IL-7Rα expression following O/N activation with IL-7. Activated cells were washed and cultured O/N in medium in presence (bold line) or absence (dashed line) of Act D (*left*) or CHX (*right*). Resting naive cells (thin line). The expression of IL-7Rα was measured by FACS analysis. Data are from one representative experiment of two performed.

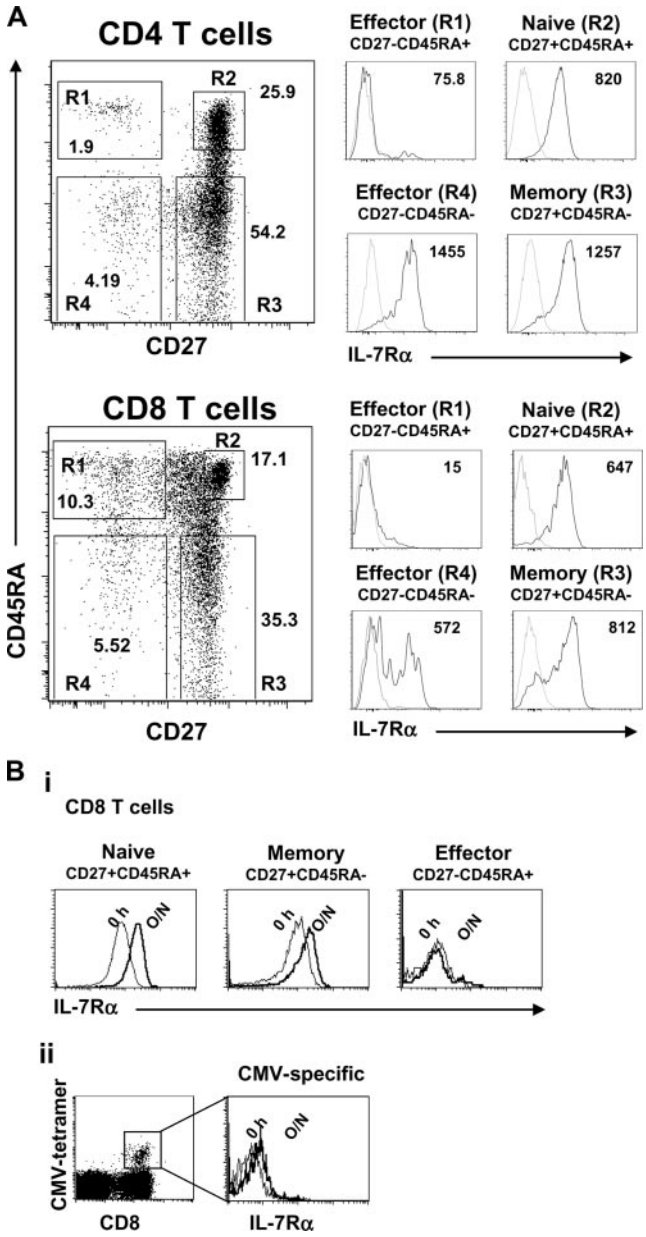

FIGURE 4. Fully differentiated T cells do not re-express IL-7Rα. *A*, Heterogeneous expression of IL-7Rα in human T cell subsets. Expression of IL-7Rα was assessed by FACS analyses on both CD4$^+$ (*top*) and CD8$^+$ (*bottom*) peripheral blood human T cell subsets. Data are from one representative experiment of six performed. Numbers in the histograms represent the MFI for IL-7Rα. Thin line (IL-7Rα staining) and bold line (isotype control). *B*, Impaired IL-7Rα re-expression in fully differentiated CD8$^+$ T cells. Expression of IL-7Rα on CD8$^+$ T cells subsets (*i*) or CMV-specific cells cultured in medium O/N (bold line) (*ii*) as compared with ex vivo analyzes (thin line). Data are one representative experiment of two performed.

expression is lost in T cells after antigenic stimulation (10–12, 18), although it is incompletely resolved how this is regulated at the molecular level. A comparative analysis was performed regarding the effect of IL-7 and TCR/CD28 activation on the expression of IL-7Rα at both the mRNA and protein level. Naive T cells cultured in IL-7 rapidly down-regulated the IL-7Rα expression (t1/2 for IL-7Rα mRNA <2 h). The suppression of IL-7Rα at mRNA level was sustained by prolonged culture in IL-7 (10-fold reduction) (Fig. 2*A*, *left*). IL-7Rα expression was also down-regulated by TCR/CD28 activation, although with a slower initial kinetic compared with IL-7 stimulation (t1/2 for IL-7Rα mRNA ≈ 4 h). Interestingly, and in contrast to IL-7 signaling, prolonged activation via TCR/CD28-engagement further decreased IL-7Rα mRNA levels (ultimately to a 50-fold reduction at 72 h) (Fig. 2*A*, compare *left* and *middle panel*). Similar results were obtained upon TCR-engagement in the absence of CD28 and using naive CD8$^+$ T (data not shown). At the protein level, the disappearance of IL-7Rα from the cell surface followed a similar kinetic, with a more rapid down-regulation in presence of IL-7. At the 20 h time point, the lost of IL-7Rα was equivalent on both populations (Fig. 2*A ii*). The combined activation by both stimuli induced a profound down-regulation of IL-7Rα both at mRNA (with t1/2 for IL-7Rα mRNA around 2 h) and protein level (Fig. 2*A*). To investigate the impact of IL-7 and TCR/CD28 activation on the recovery of IL-7Rα expression, cells activated for either 20 or 72 h were washed thoroughly and cultured in medium for an additional 7 h. Naive T cells rapidly up-regulated IL-7Rα mRNA and protein following IL-7-withdrawal either in short or prolonged cultures. After removal of the stimulus, TCR/CD28-activated cells remained, however, IL-7Rα low both at mRNA and protein level during the same period (Fig. 2*B*, compare *right* and *left panels*). Our data demonstrated that, in contrast to the transient effect of IL-7, TCR/CD28-mediated signaling had a chronic impact on the expression of IL-7Rα.

Modulation of IL-7Rα expression by IL-7- and TCR/CD28-mediated signaling entails new protein synthesis

The effect of IL-7 and TCR/CD28 activation on the transcription of IL-7Rα mRNA suggested the existence of a mechanism that suppressed the IL-7Rα gene. To evaluate whether this process was dependent on new transcription and protein synthesis, naive T cells were activated with either stimuli in the presence of ActD (inhibiting transcription) or CHX (inhibiting translation). Both treatments partially blocked the TCR/CD28- or IL-7-induced down-regulation of IL-7Rα (Fig. 3*A*). Likewise, the recovery of IL-7Rα

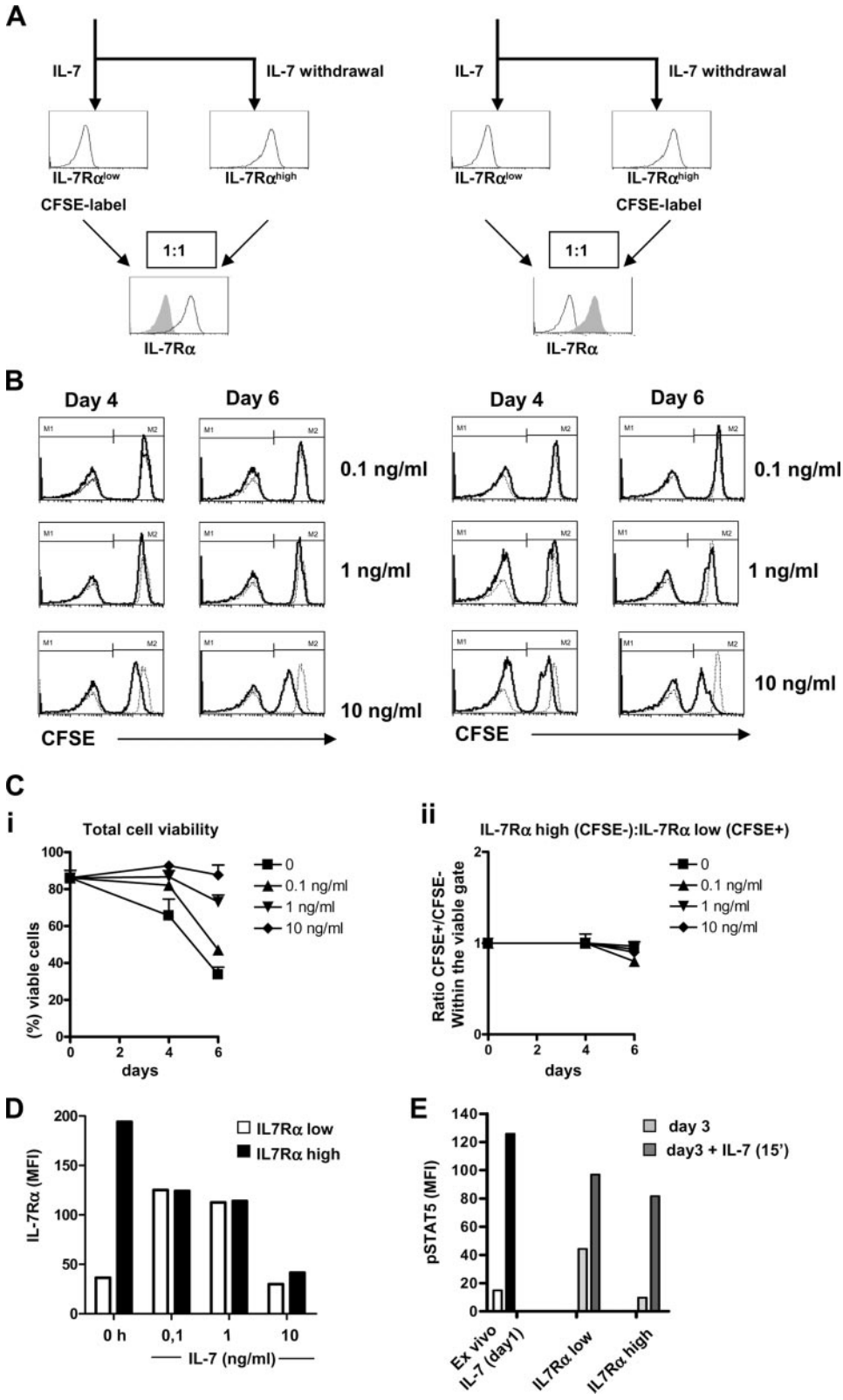

FIGURE 5. IL-7-induced down-regulation of IL-7Rα does not limit the subsequent response of naive T cells to IL-7. *A*, Cord blood-derived naive $CD4^+$ T cells were cultured for 3 days in presence of IL-7 (10 ng/ml). At the day 2, a fraction of cells were removed from IL-7 cultures, washed, and cultured O/N in plain medium allowing recovery of IL-7Rα expression. The other fraction remained always in presence of IL-7. At day 3, one of the populations was labeled with CFSE and the other left unlabelled (on the left: IL-7Ra ^{low} cells were labeled with CFSE; on the right: IL-7Ra ^{high} cells were labeled with CFSE). Cells were subsequently mixed in a 1:1 ratio and cultured in a graded concentration of IL-7 or left unstimulated for extra 6 days. *B*, At the indicated time points, proliferation was assessed by FACS analysis. *C i*, Total cell viability measured by FACS analysis (viable gating and PI exclusion). *C ii*, The ratios between IL-7Rα^{low} ($CFSE^+$) and IL-7Rα^{high} ($CFSE^-$) cells were calculated in the total live gating. Graphs show the mean expression and SD of three experiments. *D*, IL-7Rα expression was analyzed on IL-7Rα^{low} naive cells (white bars) and IL-7Rα^{high} naive cells (black bars) 2 days following culture in different doses of IL-7. Graphs represent the MFI for IL-7Rα staining measured by FACS analysis. *E*, STAT-5 is phosphorylated at same extent in IL-7Rα^{low} naive cells and IL-7Rα^{high} naive cells upon restimulation with IL-7. At day 3 (light gray bars), both populations were pulsed with IL-7 (10 ng/ml) for 15 min (dark gray bars), and the level of STAT-5 phosphorylation (pSTAT5) was monitored by FACS analysis using an Ab that recognizes the Tyr694-phosphorylated form of STAT5. Ex vivo isolated naive cells and cells cultured in IL-7 (10 ng/ml) for 1 day are shown as control. Graphs represent the MFI for pSTAT5 staining.

was blocked following IL-7 withdrawal (Fig. 3*B*). A further reduction in IL-7Rα expression was noted in previously TCR/CD28-activated cells and similar results were obtained using naive $CD8^+$ T cells (data not shown). Recent observations have shown that the expression of murine IL-7Rα is regulated by the transcriptional repressor GFI-1 and the transcription factor GABPα (9, 19). We analyzed whether these genes were differentially regulated at the transcription level by the two distinct stimuli in human naive $CD4^+$ and $CD8^+$ T cells. Yet, neither IL-7 nor TCR/CD28 activation significantly altered the expression of GABPα and GFI-1 in either subset (data not shown). Thus, although suppression of IL-7Rα expression by both IL-7- and TCR/CD28-mediated signaling is an active process dependent on both new transcription and protein synthesis, the molecular factors regulating this process in human T cells remain to be identified.

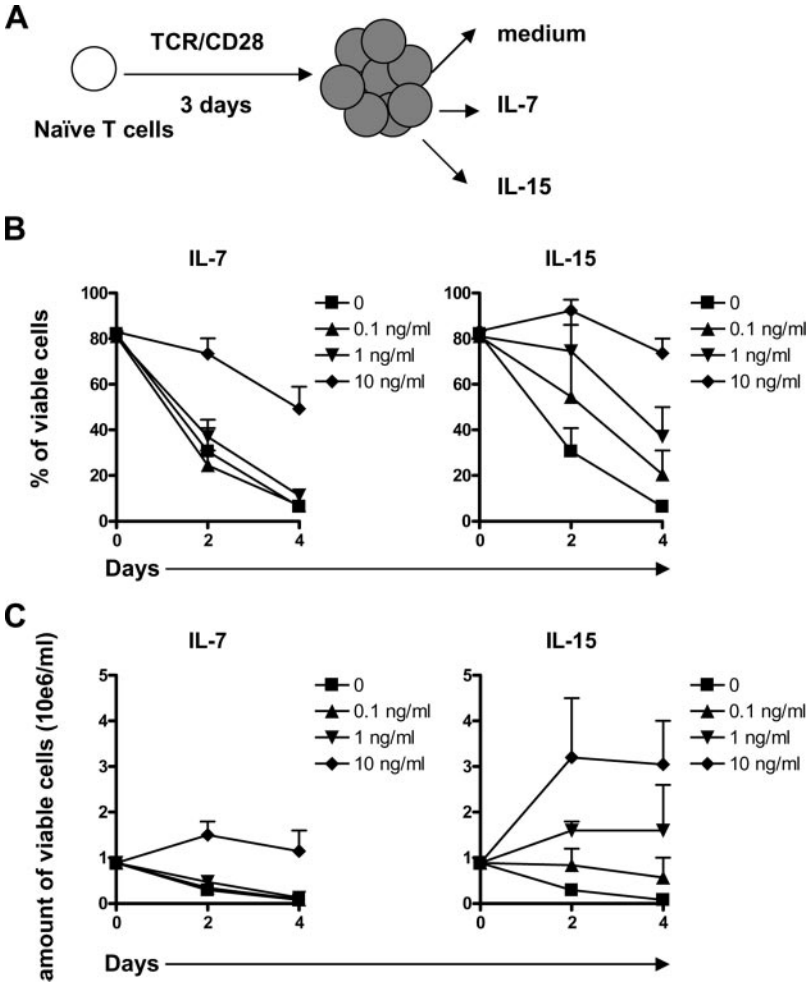

FIGURE 6. Low responses of TCR/CD28-induced IL-7Rα^{low} cells to IL-7. *A*, Cord blood-derived naive CD4$^+$ T cells were activated with combined anti-CD3/CD28 mAbs (1 and 5 μg/ml, respectively) for 3 days to ensure a decrease of IL-7Rα expression (see Fig. 2). On day 3, cells were washed and plated at a cell density of 1×10^6/ml in a graded concentration of IL-7 and IL-15. *B*, At the indicated time points viability (PI exclusion) was assessed by FACS. *C*, Viable cell numbers in the cultures are represented. Graphs show the mean expression and SD of three experiments.

Fully differentiated T cells lose the ability to re-express IL-7Rα

The majority of memory CD8$^+$ T cells specific for persistent viruses, e.g., CMV, lack expression of IL-7Rα. It has been implied that this reflects a constant contact with Ag during latency that drive T cells into a more differentiated stage (11, 18). Based on phenotypic distinctions (20, 21), we tested whether IL-7Rα expression would be altered in different T cell subsets, including CMV-specific cells, once removed from their in vivo environment. Overall, IL-7Rα was expressed in the majority of the subsets, but effector CD27$^-$CD45RA$^+$ CD8$^+$ T cells. Memory T cells exhibited consistently higher levels of IL-7Rα as compared with naive T cells. IL-7Rα^{low} cells were predominantly found in effector CD27$^-$CD45RA$^+$ CD8$^+$ T cells and in small fraction of both CD4 and CD8 T cells included within the CD27$^-$CD45RA$^+$ and CD27$^-$CD45RA$^-$ gate, respectively (Fig. 4*A*). Culture in medium enhanced IL-7Rα expression in CD4$^+$ T cell subsets, but not in CD27$^-$CD45RA$^+$ CD4$^+$ T cells (data not shown). Within the CD8$^+$ T cell subset, both naive and memory cells upregulated IL7Rα expression, whereas CD45RA$^+$CD27$^-$ T cells did not (Fig. 4*B i*). Up-regulation was blocked by treatment with Act D and CHX, suggesting that IL-7Rα expression was actively restrained in vivo (data not shown). Yet, effector-type CD8$^+$ T cells remained IL-7Rα^{low} after in vitro culture. Similarly, T cells that were activated through CMV infection in vivo, and that showed for the majority the same effector phenotype, failed to re-express IL-7Rα (Fig. 4*B ii*). Thus, in peripheral blood, IL-7Rα^{low} T cells were only found in fully differentiated cells and those cells apparently lost the ability to re-express this receptor. Thus, chronic activation in vivo, as for CMV, has profound consequences on IL-7Rα expression.

IL-7-induced down-regulation of IL-7Rα does not hinder responsiveness of naive T cells to IL-7

Having established a distinct effect of IL-7 or TCR/CD28 activation on the expression of IL-7Rα, we next investigated whether these differences were functionally relevant. Recently, it has been suggested that suppression of IL-7Rα by IL-7 constitutes a mechanism to regulate IL-7 consumption by naive T cells (9). Accordingly, down-regulation of IL-7Rα in IL-7-signaled cells would facilitate the use of IL-7 by IL-7Rα^{high} naive T cells. To test this for human T cells, a competitive assay was performed assessing the responsiveness of IL-7Rα^{low} and IL-7Rα^{high} naive T cells to IL-7, using CFSE as a tag (Fig. 5*A*). In mixed cultures, IL-7Rα^{low} naive T cells proliferated only in presence of a high concentration of IL-7 (Fig. 5*B*, *lower panel*). At intermediate and lower doses of IL-7, viability was sustained in a dose-dependent manner (Fig. 5*C*, *upper panel*). Yet, analyses of the ratios between IL-7Rα^{low} and IL-7Rα^{high} naive T cells, included in the total viable gate, demonstrated that both populations had identical survival or proliferative capacity at all concentrations of IL-7 (Fig. 5*C*, *bottom panel*). To exclude side effects of CFSE, IL-7Rα^{high} naive T cells were labeled with CFSE with identical results (Fig. 5, *A* and *B* and data not shown). The expression of IL-7Rα was transient as both populations displayed similar levels of the receptor once cultured in different doses of IL-7 (Fig. 5*D*). To measure the responsiveness of

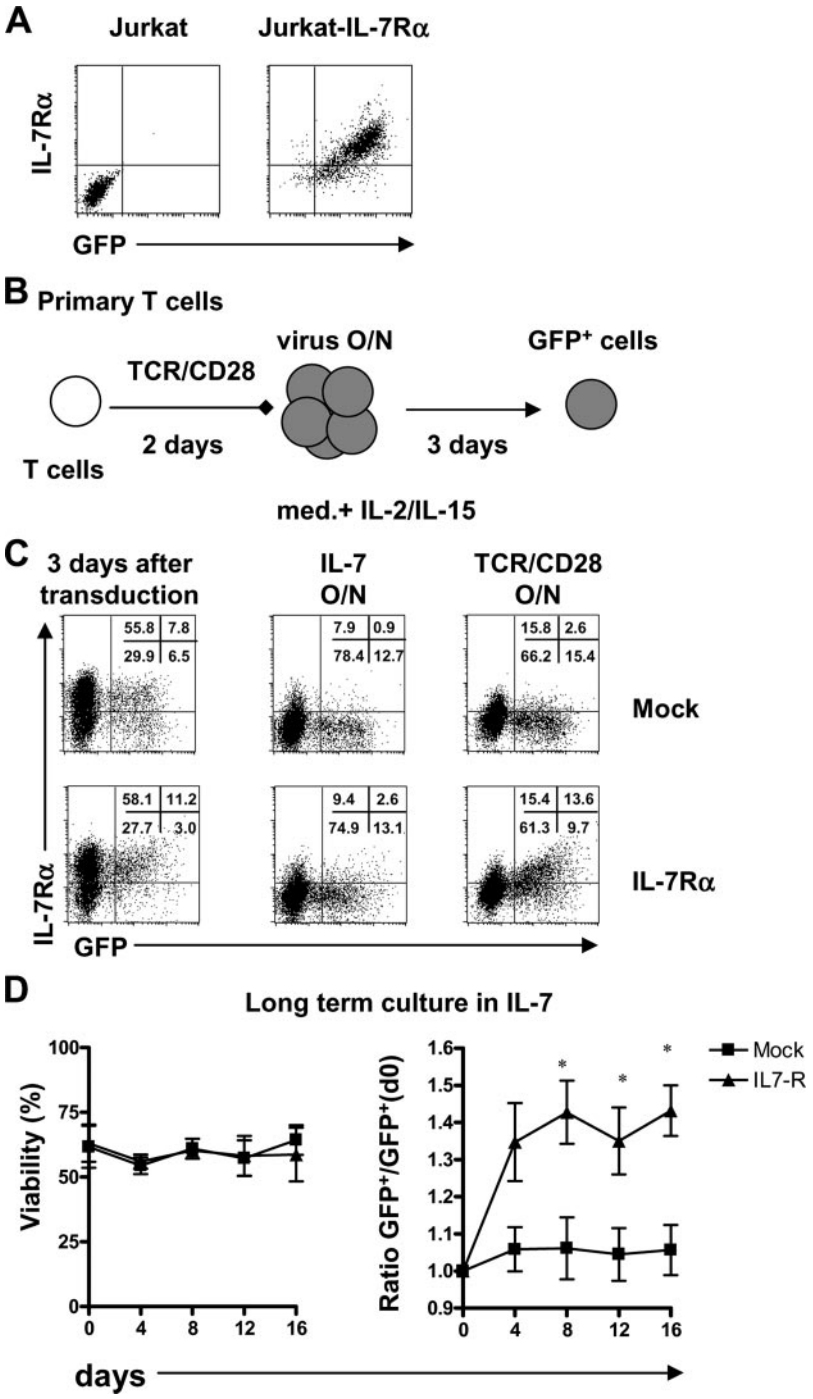

FIGURE 7. Retrovirally induced expression of IL-7Rα on TCR/CD28 activated cells confers a selective advantage to IL-7. *A*, Jurkat cells were retrovirally transduced with human IL-7Rα-IRES-GFP (IL-7Rα), sorted based on GFP expression and stained for IL-7Rα (*right*). Untransduced cells are shown as comparisons (*left*). *B*, Schematic representation of transduction procedure in primary T cells. T cells were initially activated via combined TCR/CD28 mAbs. At day 2, cells were exposed overnight (O/N) to viral supernatants. Subsequently, cells were washed and cultured in fresh medium supplemented with IL-2 (20 U/ml, ~1 ng/ml) and IL-15 (1 ng/ml) to maintain viability. Three days after transduction, GFP^+ cells were obtained. *C*, IL-7Rα expression in cells expressing control-IRES-GFP (Mock) (*top row*) or human IL-7Rα-IRES-GFP (IL-7Rα) (*bottom row*) immediately after transduction (*left*) and following O/N IL-7 (*middle*) or TCR/CD28 (*right*) activation. *D*, Prolonged culture of transduced cells in presence of IL-7. Cells were cultured in fresh medium supplemented with IL-7 (10 ng/ml) every 4 days. *Left*, Total viability in the cultures. *Right*, At each time point the percentage of GFP^+ cells within the viable gate was measured by FACS analyses for each population. Results were normalized to the percentage of GFP^+ cells on day 0. Statistical analysis for differences within groups: day 4, $p = 0.0571$; day 8, 12 and 16, $p = 0.0286$. Graphs show the mean expression and SD of four experiments.

$IL\text{-}7R\alpha^{low}$ and $IL\text{-}7R\alpha^{high}$ naive T cells to IL-7 at an earlier time point, we monitored the ability to both subsets to activate STAT5, a bona-fide down-stream target of IL-7 signaling (4). Upon short culture in IL-7, STAT5 phosphorylation was equivalent in both subsets, although the initial basal level of phosphorylated STAT5 was higher in $IL\text{-}7R\alpha^{low}$ cells compared with $IL\text{-}7R\alpha^{high}$ cells (Fig. 5*E*). Overall, our data suggest that the proliferative and survival response of $IL\text{-}7R\alpha^{low}$ and $IL\text{-}7R\alpha^{high}$ naive T cells to IL-7 was indistinguishable.

TCR/CD28-induced IL-7Rα^{low} cells display a limited responsiveness to IL-7

In parallel, we tested whether the persisting down-regulation of IL-7Rα on T cells by TCR/CD28 signaling would influence IL-7 responsiveness (Fig. 6*A*). The survival and proliferative capacity of TCR/CD28-activated cells were assessed following culture in the presence of a graded concentration of IL-7 and IL-15. In the absence of cytokines, cell viability and numbers dropped dramatically within 2 days (Fig. 6). Addition of IL-7 only affected T cell viability at a high concentration. Under this condition a subtle increase in viable cell numbers was observed, suggesting that cells slightly divided in response to higher amounts of IL-7 (Fig. 6, *B* and *C*, *left panel*). Conversely, the responsiveness to IL-15 was higher, with dose-dependent effects on both viability and cell numbers (Fig. 6, *B* and *C*, *right panels*). The apoptosis of activated T cells was also inhibited at 1 ng/ml IL-2 (data not shown). These data suggest that, in concordance with the strong down-regulation of IL-7Rα induced by

TCR/CD28, activated T cells displayed a limited ability to respond to IL-7.

Ectopic IL-7Rα expression on TCR/CD28-activated cells confers a selective advantage to IL-7

The observations that IL-7Rα is prominently down-regulated by TCR/CD28-mediated signaling and that TCR-activated cells have a poor response to IL-7 prompted us to test whether these two phenomena are strictly related. We addressed the functional consequences of retrovirally driven expression of IL-7Rα on the survival and expansion of TCR/CD28-activated T cells to IL-7. To validate the viral construct, IL-7Rα-negative Jurkat cells were transduced with retrovirus-expressing human IL-7Rα-IRES-GFP (IL-7Rα) (Fig. 7*A*). Next, primary T cells were transduced with retrovirus-expressing human IL-7Rα-IRES-GFP (IL-7Rα) or retrovirus containing empty expression vector (Mock) (Fig. 7*B*). Transduction efficiencies, expressed as percentage of GFP^+ cells, ranged between 10 and 25%. The expression of IL-7Rα was heterogeneous in control cells, both Mock GFP^- and GFP^+ cells, and IL-7Rα-GFP^- cells. In IL-7Rα-GFP^+ cells was detected a substantial increase in the expression of IL-7Rα. IL-7 activation down-regulated the cell surface levels of the endogenous as the exogenous IL-7Rα-chain. Thus, the IL-7Rα-chain was undetectable at cell surface even when its expression was driven by a constitutive active promoter, indicating protein cellular redistribution. Yet, and contrasting with control cells, the expression of IL-7Rα was sustained in IL-7Rα-GFP^+ cells following TCR/CD28-activation (Fig. 7*C*). Thus, our data suggest that the cell surface expression of exogenous IL-7Rα was regulated by IL-7 as the endogenous chain, but not by TCR/CD28-activation. Subsequently, we determined the competitive fitness of transduced cells in prolonged cultures supplemented with a high concentration of IL-7 (10 ng/ml) every 4 days. During the course of the experiment, cells did not expand considerably (data not shown, comparable to Fig. 6*C*) and cell survival was maintained (Fig. 7*D*, *left*). Strikingly, IL-7Rα-GFP^+ cells exhibited a selective initial advantage to IL-7 compared with control cells (Fig. 7*D*, *right*). Thus, constitutive expression of IL-7Rα increased the response of TCR/CD28-activated T cells to IL-7 and suggests that TCR-induced down-modulation of IL-7Rα is a major factor in limiting the response of Ag-activated T cells to IL-7.

Discussion

T cell maintenance involves the coordinate regulation of distinct variables, among others, T cell numbers, homeostatic cytokines, and their receptors (4, 22, 23). In particular, the IL7Rα-chain, which is part of the dimeric IL-7 receptor, is pivotal for T cell homeostasis. Its tight regulation has been suggested to be an essential mechanism to limit IL-7 consumption (5, 9, 22). We compared the impact of IL-7 and TCR/CD28 on the expression of this receptor in human naive T cells and demonstrated that IL-7 and TCR/CD28 activation differentially regulates IL-7Rα expression in a transient or chronic manner, respectively. These differences are reflected on the ability of cells to respond to IL-7. In contrast to IL-7 activation, the down-regulation of IL-7Rα by TCR/CD28 stimulation limits IL-7 responsiveness. Strikingly, ectopic expression of IL-7Rα on TCR/CD28-activated cells augments the responsiveness to IL-7, indicating that TCR signaling may impact the response of T cells to IL-7 by restraining the expression of its cognate receptor.

It has been demonstrated that suppression of murine IL-7Rα expression by IL-7 entails newly synthesized proteins (9). Our data demonstrate an equivalent dynamic mechanism in the human system, regulating the suppression of IL-7Rα by IL-7 or TCR/CD28 activation (Fig. 3). Recent data have shown that GFI-1 is a key transcriptional repressor involved in IL-7-induced suppression of the mouse IL-7Rα gene, specifically in naive $CD8^+$ T cells. Yet, the mechanism in $CD4^+$ T cells remains to be deciphered (9). Furthermore, the transcriptional factor GABPα has been shown to regulate IL-7Rα expression in mouse T cells (19). In line with a previous study by Kim et al. (24), we found no clear association between expression of IL-7Rα and GFI-1 or GABPα in human lymphocytes, except a subtle increased in GFI-1 expression in naive $CD8^+$ T cells culture with IL-7 (data not shown). Thus, in human lymphocytes, the expression of IL-7Rα appears to be regulated in a GFI-1- or GABPα-independent manner.

In the absence of IL-7 and IL-7Rα, the homeostasis of naive T cells is severely compromised in both humans and mice (1, 2, 25–27). Hence, the transient effect of IL-7 in the expression of IL-7Rα in naive T cells (Fig. 2 and 3) may constitute a homeostatic mechanism to maintain the responsiveness of these cells to IL-7. As IL-7 is suggested to be limiting in vivo (5–7), the active regulation of IL-7 responsiveness is necessary for T cell homeostasis (9, 22). Recently, it has been proposed that suppression of IL-7Rα constitutes a mechanism to maximize the use of IL-7 (9). In this viewpoint, down-regulation of IL-7Rα on naive T following contact with IL-7 remove signaled-cells from the cellular competitive pool allowing other unsignalled naive cells to bind IL-7. Although our in vitro experimental setting may differ from an in vivo context in terms of the bioavailability of IL-7 signals, we found no evidence of this altruistic model in a competitive assay using limiting amounts of IL-7. In fact, the survival and proliferative response to IL-7 of naive T cells expressing high or low levels of IL-7Rα is indistinguishable even in presence of limiting amounts of IL-7 (Fig. 5). The cell surface detection of IL-7Rα is prevented as long as IL-7 is present. In this scenario, a continuous and sufficient newly formed IL-7Rα protein may be shuttled to the cell surface allowing naive cells to received IL-7-mediated survival signals. Accordingly, the short term response of IL-7Rα^{low} naive T cells to IL-7, monitored by the ability to activate STAT5, was equivalent to the one IL-7Rα^{high} counterparts (Fig. 5*D*). Thus, we reason that the IL-7Rα locus is not completely suppressed by IL-7 signaling. Analyses of the recovery of IL-7Rα expression following IL-7 withdrawal substantiate this hypothesis (Fig. 2*B*). Yet, the biological significance of IL-7Rα down-regulation remains undetermined. Our previous data suggest that the proliferative response of cells to IL-7 is more limited, in terms of number of divisions, as compared with other homeostatic cytokines as IL-15 (8, 15). From this perspective, a continuous expression of IL-7Rα in T cells may favor unwarranted IL-7-driven proliferation. Thus, the down-regulation of IL-7Rα by IL-7 may have evolved as a homeostatic mechanism to dampen IL-7-driven expansion. It is worth noting that the ectopic expression of IL-7Rα on TCR-activated T cells (Fig. 7) did not confer an enormous proliferative advantage to IL-7, suggesting that the responsiveness to IL-7 may also be regulated at different level, apart of the expression of the cognate receptor.

Compared with IL-7, the impact of TCR/CD28 triggering was more prominent on the expression of IL-7Rα (Fig. 2*A*). Additionally, the recovery of IL-7Rα expression was inhibited in TCR/CD28-activated cells during the periods analyzed (7–10 h) (Fig. 2*B*). Similar observations were obtained 24 h after activation (data not shown). In retrovirally transduced cells, we observed that IL-7Rα expression was partially retained 3 days after TCR/CD28 activation in cells cultured in low doses of IL-2 and IL-15 (Fig. 7*C*). Our results are in line with the data by Xue et al. (13) demonstrating that the expression of IL-7Rα can be partially recovered on murine T cells following prolonged culture. A closer look at the

data showed that the levels of the re-expressed IL-7Rα were 2-fold lower as compared with naive cells (13), suggesting that TCR/CD28-activated cells do not have the ability to fully recover IL-7Rα, again contrasting with IL-7 stimulation. Although strong TCR stimulation of T cells provokes down-regulation of IL-7Rα (Fig. 2), it may be difficult to generate cells that remain completely IL-7Rα negative in vitro. The situation appears to be different in vivo, in which IL-7Rα is lost on fully differentiated $CD27^{-}CD45RA^{+/-}$ $CD8^{+}$ T cells and $CD27^{-}CD45RA^{+}$ $CD4^{+}$ T cells, the last a small subset that is present in around 20% of the donors analyzed (Fig. 4) (11, 28, 29). The percentage of $CD27^{-}CD45RA^{+}$ $CD8^{+}$ T cells is significantly increased in the peripheral blood of CMV-infected individuals (20, 30). Interestingly, during latency CMV-specific CD8 T cells remained for the majority IL-7Rα negative (Fig. 4*B*) (11), an observation linked to presumed frequent Ag contact provoked by persistent CMV infection (11, 31). The exclusive impaired capacity of both effector and CMV-specific $CD8^{+}$ T cells to recover IL-7Rα expression once removed from their microenvironment (Fig. 4) may be a consequence of the strength, quality, and/or duration of stimulation received by T cells that result in epigenetic modifications in the IL-7Rα locus. Accordingly, Kim et al. have shown that terminally differentiated IL-7Rα low $CD8^{+}$ T cells have increased methylation in the IL-7Rα gene promoter compared with IL-7Rα high $CD8^{+}$ T cells (32). However, the methylation of the IL-7Rα locus is not augmented via prolonged in vitro TCR activation (32), which suggest that other signals, possibly costimulatory or cytokine mediated, contribute to the cessation of the IL-7Rα promoter in vivo.

Activated T cells differentially express several cytokine receptors during the course of an immune response. Concomitantly with the loss of IL-7Rα, activated cells up-regulate the expression of other cytokine-receptors as IL-2Rα, IL-2Rβ, and IL-15Rα (4). It is accepted that these changes in the repertoire of cytokine receptors consign activated cells under the influence of other homeostatic cytokines. Our data demonstrate that, in line with a strong suppression of IL-7Rα gene (Fig. 2), TCR/CD28-activate cells possess an inadequate response to IL-7. Yet, absence of IL-7Rα will not impact the survival of activated cells as long as other homeostatic cytokines are available, such as IL-15 (Fig. 6). In murine models, it has been postulated that the expression of IL-7Rα on the small subset of activated T cells during a primary immune response defines the population of cells that constitute the pool of long-lived memory cells (12, 14). Yet, it is not completely clear whether these memory cell precursors re-express de novo or have maintained the IL-7Rα expression and if the same occurs in $CD4^{+}$ T cells compartment. Regardless of this uncertainty, the expression of IL-7Rα expression on activated CD8 T cells appears to be mandatory, yet not sufficient, to mediate their transition into the stable Ag-independent memory pool (33, 34). As one may expect, the formation of a stable memory pool is an intricate and integrated process regulated at distinct levels apart from the expression of a cell surface receptor. Our data suggest that ectopic expression of IL-7Rα *o*n activated T cells confers a selective advantage in the response to IL-7 (Fig. 7). These differences may have a considerable importance at biological level. Interestingly, a recent paper by Lefrancois and colleagues has demonstrated that both IL-7Rα regulation and CD8 T cell memory formation occurs in IL-7-independent manner (35). We do not exclude the possibility that sustained IL-7Rα expression in activated T cells confers a competitive advantage to other homeostatic factors, such as thymic stromal lymphopoietin (TSLP). IL-7Rα can form heterodimers with the TSLP receptor to specifically binds TSLP (36). Deciphering the precise role of IL-7Rα in the generation of memory T cells constitutes an upcoming challenge.

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Disclosures

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