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IL-7 Is Essential for Homeostatic Control of T Cell Metabolism In Vivo

Sarah R. Jacobs, Ryan D. Michalek, and Jeffrey C. Rathmell

It has become apparent that T cells require growth signals to maintain function and viability necessary to maintain proper immune homeostasis. One means by which cell extrinsic signals may mediate these effects is by sustaining sufficient basal cell metabolism to prevent cell atrophy. The role of metabolism and the specific growth factors essential to maintain metabolism of mature T cells in vivo, however, are poorly defined. As IL-7 is a nonredundant cytokine required for T cell development and survival and can regulate T cell metabolism in vitro, we hypothesized it may be essential to sustain metabolism of resting T cells in vivo. Thus, we generated a model for conditional expression of IL-7R in mature T cells. After IL-7R deletion in a generally normal lymphoid environment, T cells had reduced responses to IL-7, including abrogated signaling and maintenance of antiapoptotic Bcl-2 family expression that corresponded to decreased survival in vitro. T cell survival in vivo was also reduced after loss of the IL-7R in a T cell-intrinsic manner. Additionally, IL-7R deletion resulted in delayed growth and proliferation following stimulation. Importantly, in vivo excision of IL-7R led to T cell atrophy that was characterized by delayed mitogenesis and reduced glycolytic flux. These data are the first to identify an in vivo requirement for a specific cell extrinsic signal to sustain lymphocyte metabolism and suggest that control of glycolysis by IL-7R may contribute to the well-described roles of IL-7 in T cell development, homeostatic proliferation, and survival. The Journal of Immunology, 2010, 184: 3461–3469.

Control of T cell homeostasis is critical to maintain proper immunity and to avoid autoimmunity or immunodeficiency. The social control model for cell and tissue homeostasis posits that cell extrinsic signals are required for cell survival during development and to maintain cellular homeostasis of mature tissues (1). In the absence of these signals or growth factors, cells undergo a spontaneous programmed cell death via the intrinsic apoptotic pathway. T cells are highly dependent on cell extrinsic signals for survival and function both during development and when mature in the periphery. One mechanism by which extrinsic signals may allow cells to evade apoptosis is through the maintenance of cellular metabolism (2, 3). Acquisition of energy by individual cells in the form of sugars, lipids, or amino acids can be regulated by growth factors (4–6) and is critical to perform housekeeping functions required for survival and production of essential molecules (7). If cell metabolism decreases, the ability of cells to grow and proliferate when stimulated may be diminished, and apoptosis may ensue (2). Although it is clear that cell extrinsic signals are required for evasion of apoptosis, it remains undetermined if the same signals are responsible to sustain basal cell metabolism in vivo and how these pathways may influence T cell physiology and homeostasis.

Among the many signals received by T cells in vivo that may provide survival and growth signals, the cytokine IL-7 has been established as necessary for T cell development, homeostatic proliferation, and survival (8). The absence of IL-7 or any of its proximal signaling components leads to an SCID (9). IL-7 is produced by stromal cells and detected by a two-part receptor on lymphocytes consisting of the common γ-chain that is shared by multiple cytokines and a more specific receptor, IL-7Rα (IL-7R). IL-7R signals through the Jak/STAT and PI3K/Akt signaling pathways, both of which are known to have effects on cell survival, growth, and metabolism (10, 11). The specific role and mechanism by which IL-7R may influence each of these processes in vivo, however, has not been fully determined.

IL-7 may promote cell survival and growth through several mechanisms. One important pathway involves regulation of Bcl-2 family members. Specifically, IL-7 signaling results in increased expression of the antiapoptotic protein Bcl-2 (12), and overexpression of Bcl-2 can partially rescue T cell development in IL-7R−/− animals (13, 14). The antiapoptotic Bcl-2 family member Mcl-1 has also been connected to IL-7−/−-induced cell survival, and IL-7−/− dependent cell survival was eliminated in the absence of Mcl-1 (15). However, no single modification in apoptotic regulatory genes has completely restored survival or repaired functional defects associated with the loss of IL-7. This and evidence that IL-7 can inhibit cell death even in Bcl-2−/−cells (16) suggests that IL-7 may also control cell function and survival through other pathways. An additional function of IL-7 that is potentially essential for T cell development and homeostasis may be regulation of basal T cell metabolism. T cells cultured in the absence of normal environmental signals have decreased glucose uptake and glycolysis. Culture of T cells in the presence of IL-7−/−, however, can partially maintain glucose uptake and surface levels of the glucose transporter Glut1 and can wholly maintain T cell glycolytic flux (11, 17, 18). Glucose metabolism is critical for T cell activation and likely also plays a role in naïve T cell homeostasis, survival, and ability to become effector cells (19–21). No evidence, however, has yet emerged to show that this regulation has a functional role in vivo with endogenous levels.
and localization of IL-7, nor have other cell extrinsic signals been identified that may play this crucial role in vivo.

Identification of signals that regulate T cell metabolism in vivo may provide insight into fundamental T cell homeostatic mechanisms and suggest possible new approaches to immunomodulation. We hypothesized that IL-7 and IL-7R may play essential roles as homeostatic social control signals in vivo to regulate basal T cell metabolism and elicit in this study a model for inducible deletion of the IL-7R to test this notion. In this conditional transgene-mediated rescue system, excision of IL-7R in resting mature T cells by Cre-recombinase resulted in decreased T cell size, number, survival, and growth. Importantly, IL-7R–deficient T cells showed a marked decrease in glycolytic flux. Other hematopoietic cell lineages were also restored by transgenic expression of IL-7R yet were unaffected by the acute loss of IL-7R expression, supporting a T cell intrinsic effect of IL-7R loss on T cell metabolism. This study establishes for the first time that, in addition to preventing apoptosis, homeostatic or social control signals are required in vivo to maintain cell metabolism and identifies IL-7 as an essential in vivo homeostatic signal to prevent atrophy and sustain the basal rate of metabolism and glycolysis in resting T cells.

Materials and Methods

Mice

IL-7Rflx transgenic mice were produced by cloning a murine cDNA of IL-7R on a plasmid which was then microinjected into 157Bl6/J oocytes by the Duke Transgenic Mouse Facility (Durham, NC). Mice were screened by PCR with the following primers: forward 5’-AGCCGAGGCTCCCTCTGA-3’ and reverse 5’-GTCATCGACCCATTGCAGCTAGGTG-3’. Cecropin receptor (ER) transgenic animals expressed Cre-recombinase fused to ER under a ubiquitin promoter and were a generous gift of Eric Brown (University of Pennsylvania, Philadelphia, PA). IL-7R+/– mice were purchased (The Jackson Laboratory, Bar Harbor, ME) and IL-7R+/- mice were a generous gift of Motonari Kondo (Duke University, Durham, NC). Mice were backcrossed and maintained on a 157Bl6/J background and housed at Duke University, and the appropriate institutional boards approved all procedures. To activate Cre:ER and excise the floxed transgene, mice were treated with 0.15 mg tamoxifen 12 h after the indicated number of cells were introduced via tail vein injection. Animals were injected into Thy1.1 hosts, and animals were treated with mouse anti-Akt1, rabbit anti–phospho-Akt (S473), rabbit anti–phospho-STAT5 (Y694) (Cell Signaling Technology, Beverly, MA), rabbit anti–Bcl-2, mouse anti-STAT5 (BD Pharmingen), rabbit anti–Mcl-1 (Biolegend, San Diego, CA), rabbit anti–Glut1 (Abcam), mouse anti–Pim1 (Santa Cruz Biotechnology, Santa Cruz, CA), or mouse anti-actin (Sigma-Aldrich). Secondary Abs Alexa Fluor 680 anti-rabbit IgG (Invitrogen, Carlsbad, CA) and IRDye 800 anti-mouse IgG (LI-COR, Lincoln, NE) were detected by means of fluorescence using a LI-COR Odyssey infrared detection system (LI-COR). Contrast and brightness were adjusted uniformly for each image.

Adoptive transfers

Adoptive transfer experiments were performed by isolation of splenic T cells from animals on a 157Bl6/J background. T cells injected into IL-7R+/- hosts were stained for CFSE prior to injection and examined 7 d postinjection. For in vivo cell survival assays, Thy1.2 T cells from 157Bl6/J or IL-7Rflx animals were injected into Thy1.1 hosts, and animals were treated with tamoxifen 12 h after the indicated number of cells were introduced via tail vein injection.

T cell purification and culture

T cells were purified from spleen and mesenteric lymph nodes by negative selection (StemSep, StemCell Technologies, Vancouver, British Columbia, Canada) and when indicated cultured in RPMI 1640 (Meditech, Washington, DC) supplemented with 10% FBS (Gemini Bio-Products, Woodland, CA). Where indicated, IL-7 was supplemented into media at a concentration of 10 ng/ml (eBioscience, San Diego, CA). Stimulation was accomplished by culturing T cells on plates coated with anti-CD3e (clone 145-2C11) and anti-CD28 (clone 37.51) (eBioscience) at 5 μg/ml in PBS. Cells were counted, and cell size was determined on a Coulter Z2 particle counter (Beckman Coulter, Fullerton, CA).

Proliferation, survival, and flow cytometry

Cells were stained with fluorescently conjugated Abs against murine CD4, CD8, CD11c, CD25, CD44, CD62L, CD69, B220, Mac1, Gr1, Nk1.1, Thy1.1, and Thy1.2 (eBioscience). IL-7R levels were analyzed with anti–IL-7R conjugated to biotin (clone A7R34 from eBioscience) and a step-tavadin PE-Cy5–conjugated secondary (eBioscience). Isotype control for IL-7R stains was RatIgG2a staining (eBioscience). Intracellular staining for total cellular protein levels were determined by fixation in 1% formaldehyde for 10 min at 37˚C, permeabilization with 100% methanol on ice for 30 min, and anti-Glut1 Ab (Abcam, Cambridge, MA) or anti–Bcl-2 Ab (BD Pharmingen, San Diego, CA), followed by a fluoroscently conjugated anti-rabbit secondary (eBioscience). Proliferation was determined by staining T cells with CFSE (Molecular Probes, Eugene, OR) prior to culture and analyzed flow cytometrically. Survival assays were performed by propidium iodide (PI) (Molecular Probes) exclusion and flow cytometry. Flow cytometry was performed on a FACScan or FACSCanto (BD Biosciences, San Jose, CA) and analyzed with FlowJo software (Tree Star, Ashland, OR).

Immunoblotting

To probe for Glut1, cells were lysed for 1 h on ice in PBS plus 1% Triton X-100 and 0.1% SDS containing protease inhibitors (BD Pharmingen) as previously described (6). When probing for any other protein, cells were lysed in radio immunoprecipitation assay buffer and quantitated as previously described (22). Equivalent protein concentrations were subjected to 4–15% or 4–20% SDS-PAGE (Bio-Rad, Hercules, CA). Abs used were mouse anti-Akt1, rabbit anti–phospho-Akt (S473), rabbit anti–phospho-STAT5 (Y694) (Cell Signaling Technology, Beverly, MA). rabbit anti–Bcl-2, mouse anti-STAT5 (BD Pharmingen), rabbit anti–Mcl-1 (Biolegend, San Diego, CA), rabbit anti–Glut1 (Abcam), mouse anti–Pim1 (Santa Cruz Biotechnology, Santa Cruz, CA), or mouse anti-actin (Sigma-Aldrich). Secondary Abs Alexa Fluor 680 anti-rabbit IgG (Invitrogen, Carlsbad, CA) and IRDye 800 anti-mouse IgG (LI-COR, Lincoln, NE) were detected using a LI-COR Odyssey infrared detection system (LI-COR). Contrast and brightness were adjusted uniformly for each image.

Membrane assays

Glucose uptake was performed as previously described (19). Glycolytic flux analysis was also performed as previously described (17). Briefly, glycolysis was determined in freshly isolated 2 x 10^6 viable T cells by washing cells in PBS followed by incubation in glucose free Kreb’s buffer for 30 min prior to addition of 10 μM of D-[5-3H]-glucose. PerkinElmer, Wellesley, MA) and non–radio-labeled glucose to bring total glucose concentration to 10 mM prior to culture for 1 h. Each reaction was stopped by addition of an equal volume of 0.2 N HCl. [3H]H_2O was separated from [1H]glucose by evaporated equilibrium in a sealed environment. Levels of [3H]H_2O produced during glycolysis were measured on a scintillation counter, and glycolytic flux was determined.

Results

IL-7 and T cell atrophy

Based on the ability of IL-7 to regulate T cell survival (23, 24), size, and metabolism in vitro (11, 17), we proposed that IL-7 may play an essential role in sustaining T cell metabolism in vivo to prevent T cell atrophy and apoptosis. To test this hypothesis, purified T cells were adoptively transferred into IL-7–/- (wild-type [WT]) or IL-7+/+ hosts, and T cells were observed after 7 d. Consistent with prior studies showing a role for IL-7 in T cell survival, recovery and survival of transferred T cells was reduced in IL-7–/- hosts (Fig. 1). The number of transferred cells remaining in the spleen was determined by flow cytometric analysis of CFSE-positive cells. A, Cell size of adoptively transferred cells was determined by mean forward light scatter. Each panel is representative of three independent experiments. *p < 0.05.
1A). Importantly, the size of surviving CD4 and CD8 cells was also decreased, suggesting that T cells also require IL-7 to prevent atrophy (Fig. 1B) and possibly to maintain cell metabolism. This decrease in cell size in vivo mimicked in scale that seen following growth factor withdrawal in vitro (2). The relationship between cell size and glucose metabolism established from in vitro work suggested that this decrease in cell size may have been due to a decrease in available energy and glucose uptake or metabolism. Nevertheless, it was not technically possible to recover sufficient T cells following adoptive transfer to perform biochemical glucose uptake assays to test this notion. To address IL-7 regulation of glucose metabolism in vivo more directly, an inducible IL-7R Cre/Lox transgenic system was produced to allow isolation of large numbers of T cells deprived IL-7 in vivo.

Model for conditional expression of IL-7R in vivo

T cells have been previously deprived IL-7 in vivo through adoptive transfer assays or by treatment with IL-7 neutralizing Ab (23–26). Due to the altered lymphoid environment and whole animal loss of IL-7, however, these studies did not prove a T cell intrinsic requirement for IL-7. Nor, due to the low number of recoverable cells after adoptive transfer, did they allow detailed biochemical and metabolic analyses to determine the metabolic state of T cells deprived of IL-7 in vivo.

To overcome these limitations, we generated a genetic model for expression levels of CD25, CD44, CD62L, and IL-7R expression on CD4 and CD8 T cells were measured for expression levels of CD25, CD44, CD62L, and CD69 flow cytometrically in splenocytes. G, B220, Mac1, Nk1.1, CD11c, and Gr1 expression were determined flow cytometrically in splenocytes. Each panel is representative of two or three independent experiments. *p < 0.01.

FIGURE 2. An inducible IL-7R knockout system. A, Schematic of the IL-7Rlox transgene, rescue of IL-7R−/− with the transgene, and incorporation of Cre:ER to allow for in vivo excision. B, DNA extracted from animal tail snips was subjected to PCR and agarose gel electrophoresis to amplify a portion of the IL-7R transgene. C, IL-7R expression on CD4 and CD8 splenic T cells was determined by flow cytometry. D, Numbers of thymocytes and splenocytes from WT, IL-7R−/−, and IL-7Rlox animals were determined. E, CD4 and CD8 expression was determined flow cytometrically in thymocytes and splenocytes. F, CD4 and CD8 T cells were measured for expression levels of CD25, CD44, CD62L, and CD69 flow cytometrically in splenocytes. G, B220, Mac1, Nk1.1, CD11c, and Gr1 expression were determined flow cytometrically in splenocytes. Each panel is representative of two or three independent experiments. *p < 0.01.
suggestions that although numbers were modestly reduced, expression of the IL-7R under an ectopic promoter did not negatively impact T cell development (28, 29). In addition, the surface expression levels of activation markers CD25, CD44, CD62L, and CD69 were similar between WT and IL-7R<sup>flox</sup> when compared with IL-7R<sup>−/−</sup> CD4 or CD8 T cells (Fig. 2F). As the transgene promoter was intended to be T cell specific and should result in restored IL-7R expression levels in T cells alone, the effects on the levels of other immune cells were investigated. Interestingly, the splenic numbers and phenotype of B cells, macrophages, NK cells, granulocytes, and dendritic cells more closely resembled WT in IL-7R<sup>flox</sup> animals than IL-7R<sup>−/−</sup> (Fig. 2G, Table I). Some expression of IL-7R was detectable on these cells in IL-7R<sup>flox</sup> animals (Table I). It is unclear if this restoration of numbers of other hematopoietic lineages was due to leaky expression of the transgene within these cell types or indirect effects through the presence of T cells. It is important to note, however, that this phenotype was observed in multiple transgenic founder mice (data not shown), demonstrating that rescue of these hematopoietic lineages was not due to an insertional effect of the transgene. Together, these data demonstrate that although not wholly restored to WT, expression of the IL-7R<sup>flox</sup> transgene effectively rescued the T cell developmental defect of IL-7R<sup>−/−</sup> animals and allowed generation of mature T cells and other hematopoietic cell lineages that appeared normal.

**IL-7R<sup>flox</sup> transgene largely rescues IL-7R<sup>−/−</sup> phenotype**

The rescue of normal T cell numbers and phenotypes by expression of the IL-7R<sup>flox</sup> transgene suggests that IL-7R signaling and function were reconstituted on an IL-7R<sup>−/−</sup> background. To test this, the ability of the IL-7R<sup>flox</sup> transgene to promote phosphorylation of the transcription factor STAT5, induction of the STAT5 target gene Pim1, and activation of the PI3K/Akt pathway were determined (11). Similar to WT T cells, addition of IL-7 to purified IL-7R<sup>flox</sup> transgenic T cells led to phosphorylation of STAT5 and induction of the STAT5 target gene Pim1 (Fig. 3A). The PI3K/Akt pathway was also activated by the IL-7R<sup>flox</sup> transgene, although to a lesser extent than that observed in WT T cells. Each of these signaling pathways can regulate cell metabolism (6, 11, 30), and the glycolytic rates of resting WT and IL-7R<sup>flox</sup> T cells were tested and found to be indistinguishable (Fig. 3B). Additionally, IL-7R<sup>flox</sup> T cells exhibited a similar level of glucose uptake ex vivo and maintenance of glucose uptake in response to IL-7 compared with WT (Fig. 3C). The IL-7R<sup>flox</sup> transgene also promoted cell survival similar to WT cells. Endogenous and IL-7R<sup>flox</sup> rescued T cells cultured in the presence of IL-7 maintained an ~80% survival rate, whereas without IL-7, only 10% survival was achieved in both WT and IL-7R<sup>flox</sup> cells after 3 d (Fig. 3D). Together, these data suggest that expression of the IL-7R<sup>flox</sup> transgene rescued the T cell developmental defect of IL-7R<sup>−/−</sup> animals and allowed generation of normal mature T cells capable of inducing canonical IL-7 signaling and survival.

**IL-7R<sup>flox</sup> is efficiently excised**

We evaluated the T cell intrinsic role for IL-7R by treatment of IL-7R<sup>flox</sup> transgenic mice with tamoxifen to activate Cre:ER and excise the IL-7R<sup>flox</sup> transgene. Three days postinjection, IL-7R<sup>flox</sup> was excised in ~80–90% of CD4 and CD8 T cells, whereas no loss of IL-7R expression was detected on IL-7R<sup>flox</sup> T cells that did not express the Cre:ER transgene (Fig. 4A). To better characterize the deletion of IL-7R<sup>flox</sup> transgene, surface IL-7R levels were measured over time posttreatment with tamoxifen (Fig. 4B). One day after tamoxifen injection, IL-7R levels were decreased, with the most significant loss of IL-7R expression on days 2 and 3. By day 7 posttreatment, IL-7R<sup>−/−</sup> T cells that likely avoided deletion of the transgene appeared to outgrow, and IL-7R<sup>+</sup> T cells were readily detected. To most efficiently isolate T cells rendered IL-7R<sup>null</sup> in vivo, therefore, animals were examined 3 d after tamoxifen treatment in the following experiments unless otherwise noted. Acute loss of IL-7R, therefore, did not appear to alter T cell activation phenotype or provide an immediate selective advantage to a particular subset, although it is possible such a selective advantage may occur over longer time periods, and other hematopoietic lineages appeared unaffected.

Decreased IL-7R expression after tamoxifen treatment also resulted in a functional loss of IL-7R. Phosphorylation of STAT5 and induction of the STAT5 target gene Pim1 were reduced in IL-7–<sup>−/−</sup> and data not shown). Acute loss of IL-7R, therefore, did not appear to alter T cell activation phenotype or provide an immediate selective advantage to a particular subset, although it is possible such a selective advantage may occur over longer time periods, and other hematopoietic lineages appeared unaffected.

**Hematopoietic phenotype of IL-7R<sup>flox</sup> mice**

Table I. Hematopoietic phenotype of IL-7R<sup>flox</sup> mice

<table>
<thead>
<tr>
<th>Cell Number (Millions)</th>
<th>IL-7R Expression (MFI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WT</strong></td>
<td><strong>IL-7R&lt;sup&gt;flox&lt;/sup&gt;</strong></td>
</tr>
<tr>
<td>B220*</td>
<td>49.9 ± 10.7</td>
</tr>
<tr>
<td>Mac1*</td>
<td>3.5 ± 3.0</td>
</tr>
<tr>
<td>NK1.1*</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>CD11c&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.6 ± ND</td>
</tr>
<tr>
<td>Gr1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

Number of positive splenocytes for a given stain for WT, IL-7R<sup>flox</sup>, and IL-7R<sup>−/−</sup> animals is shown on the left in millions. Mean fluorescence intensity for IL-7R is indicated for each stain. MFI, mean fluorescence intensity; ND, not determined.
Loss of IL-7R effects cell survival

In previous studies, IL-7 signaling has been shown to play a critical role in survival of resting mature T cells, but it has not been possible to establish this dependence as T cell intrinsic due to whole-animal loss or inhibition of IL-7 (23–26). Our model also results in whole-animal genetic loss of IL-7R, although all hematopoietic lineages appear phenotypically normal prior to IL-7R\textsuperscript{flox} deletion and did not undergo rapid cell death or appear to change phenotype after IL-7R\textsuperscript{flox} deletion. To test the T cell dependence on IL-7, IL-7R\textsuperscript{flox} mice with or without expression of Cre:ER were treated with tamoxifen, and T cell numbers were observed (Fig. 6A). Three days posttreatment, recovery of viable CD4 and CD8 T cells was reduced approximately one third, suggesting that T cell viability required intrinsic expression of IL-7R. Although loss of IL-7R\textsuperscript{flox} in vivo appeared to result in T cell death, IL-7R\textsuperscript{flox} and IL-7R\textsuperscript{null} T cells exhibited only slight decreases in Mcl-1 or Bcl-2 levels ex vivo (Fig. 6B, 6C). Nevertheless, IL-7R\textsuperscript{null} cells were prone to rapid apoptosis and demonstrated an increased rate of cell death compared with IL-7R\textsuperscript{flox} cells in culture in the absence of cytokine (Fig. 5C). IL-7 can regulate Bcl-2 and Mcl-1 expression (15, 31), and it is possible that T cells that lose expression of these antiapoptotic Bcl-2 family proteins undergo rapid cell death in vivo and were thus not able to be isolated for inclusion in these analyses.

To directly measure cell survival upon acute loss of IL-7R\textsuperscript{flox} in an otherwise normal lymphoid environment, mature resting T cells from IL-7R\textsuperscript{flox} animals or WT animals were adoptively transferred into allelically marked normal hosts. All recipients were treated with tamoxifen, and T cell numbers were observed (Fig. 6A). Three days posttreatment, recovery of viable CD4 and CD8 T cells was reduced approximately one third, suggesting that T cell viability required intrinsic expression of IL-7R. Although loss of IL-7R\textsuperscript{flox} in vivo appeared to result in T cell death, IL-7R\textsuperscript{flox} and IL-7R\textsuperscript{null} T cells exhibited only slight decreases in Mcl-1 or Bcl-2 levels ex vivo (Fig. 6B, 6C). Nevertheless, IL-7R\textsuperscript{null} cells were prone to rapid apoptosis and demonstrated an increased rate of cell death compared with IL-7R\textsuperscript{flox} cells in culture in the absence of cytokine (Fig. 5C). IL-7 can regulate Bcl-2 and Mcl-1 expression (15, 31), and it is possible that T cells that lose expression of these antiapoptotic Bcl-2 family proteins undergo rapid cell death in vivo and were thus not able to be isolated for inclusion in these analyses.

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Loss of IL-7R and T cell atrophy in vivo

In addition to cell survival, T cells require cell extrinsic signals to prevent atrophy, which is characterized by decreased cell size and rate of growth. Treatment with rIL-7 can prevent T cell atrophy in vitro (17), and consistent with a role for IL-7 in regulation of T cell atrophy, excision of the IL-7R\textsuperscript{flox} transgene in vivo resulted in a significant decrease in T cell size (Fig. 7A) that was not due to increased isolation and measurement of dead cells, as viability of purified T cells was equivalently high regardless of IL-7R status (Fig. 7B). Interestingly, this cell size decrease was similar in scale to that observed following ectopic expression of a Bcl-xL transgene, which prevents apoptosis of growth factor-deprived T cells and results in accumulation of atrophic T cells in vivo (2). To determine if IL-7R\textsuperscript{null} T cells also had a reduced ability to grow when stimulated, as described for other atrophic cells (2, 32), IL-7R\textsuperscript{flox} and IL-7R\textsuperscript{null} T cells were stimulated with anti-CD3 and anti-CD28, and cell size and proliferation were observed. At early time points, IL-7R\textsuperscript{null} T cells remained smaller than IL-7R\textsuperscript{flox} T cells, as shown by size measurement by flow cytometry and particle size analyzer (Fig. 7C). However, IL-7R\textsuperscript{null} T cells ultimately grew to the same extent (Fig. 7D), demonstrating that the small cell size at early time points was due to a decreased rate of growth rather than diminished capacity for growth. Additionally, the decrease in cell growth was associated with delayed cell division, as loss of IL-7R in vivo prior to stimulation resulted in decreased proliferation as measured by CFSE dilution (Fig. 7E). Loss of IL-7 signaling in vivo, therefore, results in atrophy and an impaired ability of T cells to grow and proliferate when stimulated.

Glycolysis is regulated by IL-7 signaling in vivo

T cell atrophy in vitro results in decreased glucose uptake and metabolism and ability to survive and grow (2). Conversely, transgenic expression of Glut1 to elevate glucose uptake can promote increased T cell size and activity (19). Cellular atrophy and delayed growth of IL-7R\textsuperscript{null} T cells, therefore, suggested that IL-7R may have an essential role as a homeostatic control signal to maintain basal resting T cell glucose metabolism. Indeed, IL-7R signaling has the capacity to sustain T cell glucose uptake and glycolysis in vitro (11, 17, 18). It remained unclear if IL-7 was essential to maintain cell metabolism in vivo. Loss of IL-7 signals could potentially affect glucose metabolism at the level of the glucose transporter, rate of glucose uptake, or flux through glycolysis. Unexpectedly, purified IL-7R\textsuperscript{null} T cells had higher total levels of Glut1 protein 3 days postexcision compared with IL-7R\textsuperscript{flox} T cells, although 7 d postexcision, Glut1 protein levels returned to starting IL-7R\textsuperscript{flox} levels (Fig. 8A, 8B). Nevertheless, loss of IL-7R in vivo did not affect glucose transport even at day 3
and CD69 and analyzed by flow cytometry. Treatment splenocytes were stained for CD4, CD8, CD25, CD44, CD62L, and without the Cre:ER transgene were treated with tamoxifen, and 3 d post-treatment splenocytes were stained for CD4, CD8, CD25, CD44, CD62L, and CD69 and analyzed by flow cytometry. D. IL-7Rfox animals both with and without the Cre:ER transgene were treated with tamoxifen, and 3 d posttreatment splenocytes were stained for B220. Each panel is representative of two independent experiments.

posttreatment, as ex vivo glucose uptake was unchanged in IL-7Rfoxnull T cells compared with IL-7Rfox T cells (Fig. 8C). This apparent discrepancy may reflect a decreased proportion of Glut1 protein on the cell surface due to loss of IL-7R signals and Akt activation (6, 11), yet a compensatory increase in Glut1 protein may have resulted in maintenance of overall glucose uptake. In addition to glucose uptake, glucose metabolism is regulated by a number of proteins and additional control mechanisms, including hexokinase and phosphofructokinase (33, 34) that may have affected metabolism despite maintenance of glucose uptake in the absence of IL-7R. Consistent with these additional control mechanisms and in support of a key metabolic role for IL-7, IL-7Rnull T cells failed to maintain a basal rate of glycolytic flux compared with IL-7Rfox T cells after 3 d in vivo lacking IL-7 signals (Fig. 8D). This finding suggests that IL-7 provides a key homeostatic signal for T cell metabolism, and, following the loss of IL-7R, a decrease in glycolytic flux may result in an inability of T cells to produce the energy required to prevent atrophy and maintain survival.

Discussion

Together, these data show that mature resting T cells have an intrinsic requirement for IL-7R signaling to maintain cell survival, growth rates, and glucose metabolism. Through the development of an inducible IL-7R knockout system, we identified IL-7 as a homeostatic control signal for the regulation of metabolism via maintenance of glycolysis. Additionally, our data support a T cell-intrinsic dependence on IL-7 to maintain T cell survival and metabolism in an...
FIGURE 7. Loss of IL-7R and cell growth. A, Purified T cells expressing or lacking expression of IL-7R were examined for cell size by particle size analyzer. \( p < 0.01. \) B, Percentage of live cells was determined following purification by PI exclusion and flow cytometry. C, IL-7R\textsubscript{fox} and IL-7R\textsubscript{null} purified T cells were cultured on control or plates coated with 5 \( \mu \)g/ml anti-CD3 with or without 5 \( \mu \)g/ml anti-CD28 Abs and assayed for cell size by mean forward scatter after 18 h in culture (left panel) \( (p < 0.05) \), by particle size analyzer after 20 h in culture (right panel) \( (p < 0.05) \), or by mean forward scatter after 48 h in culture (D). E, Proliferation was measured after 48 h by CFSE dilution. Each panel is representative of two to six independent experiments.

otherwise apparently normal lymphoid compartment. However, the mechanism of cell survival maintenance remains elusive, as levels of Bcl-2 family members appeared unchanged in vivo, possibly due to death of cells with decreased levels of protein. Additionally, IL-7 signaling was important prior to T cell activation, as the ability of cells to stimulate was decreased in the absence of IL-7 signaling. Most critically, IL-7R was found to be essential to maintain homeostatic control over T cell metabolism and glycolytic flux, with significantly decreased glycolysis in T cells lacking IL-7R in vivo, despite myriad other microenvironmental signals. Taken together, this work identifies a novel feature of IL-7 control of naive T cell metabolism that may have broad import for control of T cell homeostasis and survival.

The transgenic model for conditional deletion of IL-7R employed in this study led to rescue of multiple hematopoietic cell types, yet showed an apparent specific role for IL-7R in resting mature T cells. It is not clear why numbers of non-T cells were near normal in IL-7R\textsubscript{fox} mice, but this phenotype was observed in several transgenic founder lines, and it is likely that leaky transgene expression may have rescued IL-7–dependent developmental checkpoints. Nevertheless, treatment of mice with tamoxifen did not appear to lead to significant loss of mature cells of these lineages. T cell development and phenotype also appeared normal in this model despite constitutive transgene-driven IL-7R expression, and loss of IL-7R did not detectably alter T cell phenotype or the presence of distinct T cell subsets. It appears, therefore, that resting mature T cells have an intrinsic dependence on IL-7–R signaling for metabolism and survival, although a subtle shift in T cell development and status or a secondary effect on T cell metabolism of altered signaling and IL-7R deficiency due to somewhat reduced numbers or phenotype of other cell types cannot be formally excluded.

Although regulation of glucose metabolism suggests a novel means by which IL-7R may affect cell fate, several questions remain. It is

FIGURE 8. Loss of IL-7R and glycolysis. IL-7R\textsubscript{fox}– and IL-7R\textsubscript{null}– purified T cells were permeabilized and stained with anti-Glut1 Ab followed by flow cytometry 3 or 7 d post tamoxifen treatment (A) or lysed and 10 \( \mu \)g immunoblotted 3 d post tamoxifen treatment (B). IL-7R\textsubscript{fox}– and IL-7R\textsubscript{null}–purified resting T cells were analyzed for ability to uptake glucose 3 d post tamoxifen treatment (C) or exposed to \( ^{3} \)Hglucose to measure glycolytic flux (D). Each panel is representative of two to six independent experiments. \( * p < 0.001. \)
unclear how IL-7 regulation of Bcl-2 family members contributes to cell survival relative to effects on cell metabolism. Cells must maintain a minimal rate of glucose metabolism to survive (19, 35), yet Bcl-2 family proteins can slow or prevent cell death even in very low glucose conditions (36). IL-7 may prevent cell death by simultaneously promoting Bcl-2 and Mcl-1 expression and stimulating glycolysis. Thus, loss of IL-7 or IL-7 deficiency may lead to decreased expression of antiapoptotic Bcl-2 family proteins and decreased rates of glycolysis, resulting in a cumulative sensitization of T cells to apoptosis. Nevertheless, we failed to detect large decreases in Bcl-2 or Mcl-1 protein levels postexcision of the IL-7R in vivo. These proteins may be maintained through the action of other growth factors present in a normal lymphoid environment, and IL-7 is potentially not essential in vivo for their expression. Alternatively, decreased levels of these proteins may lead to rapid cell death, preventing isolation of Bcl-2 and Mcl-1 low-expressing cells for ex vivo analysis. It is also unclear how IL-7R signals regulate glycolysis. Both STAT5 and PI3K/Akt signaling pathways can promote glucose uptake (11), and IL-7 can promote both pathways, although the PI3K/Akt pathway is poorly activated by IL-7 relative to other cytokines. It will be important in future work to clarify the role of these signaling molecules in the control of cell metabolism.

Glucose metabolism is controlled through coordinated regulation of glucose uptake and glycolytic flux. Glucose uptake is limiting in T cell activation (19) and must be tightly regulated, yet, surprisingly, IL-7R was not essential to maintain glucose uptake in resting T cells. This may have been due to the balanced effects of decreased Glut1 trafficking to the cell surface and compensatory increase in Glut1 protein level. Similarly, glycolysis can be controlled at many levels, and it is likely IL-7–mediated regulation of glycolytic flux occurs downstream of glucose uptake. For example, Akt has been implicated in the control of phosphofructokinase-2 to maintain glycolytic flux and cell survival (37), as well as the localization of hexokinase to the mitochondria (38). Alternative splice variants of glycolytic enzymes have also been shown to regulate glucose metabolism, such as the M2 variant of pyruvate kinase, which increases aerobic glycolysis (39), and similar regulations may occur as a consequence of IL-7R signaling. As glucose uptake is not altered by the loss of IL-7R, it is possible that these alternate regulations of metabolism may mediate IL-7–dependent glycolysis.

IL-7 regulation of glycolysis may be a critical component of IL-7–dependent cell survival in vivo. This may arise by providing energy to prevent metabolic stress or by metabolic regulation of apoptotic proteins. In developing thymocytes, deprivation from Notch signaling decreased glucose uptake and resulted in rapid apoptosis (35), and expression of a constitutively active Akt can maintain glucose uptake to prevent cell death in thymocytes and apoptosis proteins Puma and Bim are induced when cells are deprived of sufficient glucose (40). Glucose metabolism in hematopoietic cells is, therefore, capable of regulating Bcl-2 family proteins and cell survival, and IL-7 may regulate T cell homeostasis in part through control of glycolysis and similar signaling pathways. An important implication of this finding is that modulation of T cell metabolism either directly or through control of metabolic regulatory mechanisms may provide a useful means to control T cell homeostasis and trophic state.

The role of the social control model and cell extrinsic signals to maintain cell viability and function has been widely accepted (1), yet evidence for how such extrinsic signals impact cell physiology has been limited. Findings presented in this study demonstrate for the first time that T cells require IL-7–induced signals in vivo to maintain basal resting glucose metabolism. Maintenance of T cell homeostasis is critical for proper immunity, and these findings show that IL-7 provides a homeostatic signal essential for T cell glycolytic flux. This control of cell metabolism likely influences a wide array of cell phenotypes. It will be important in future work to further characterize and establish how this plasticity in basal T cell glycolytic flux influences T cell size, survival, and growth in normal immunity as well as in immune pathology.

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Disclosures

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