CD8 lineage specific regulation of interleukin-7 receptor expression by the transcriptional repressor Gfi1

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Background: Expression of the IL-7Rα gene is up/downregulated during T/B-lymphocyte development in the mammalian immune system.

Results: IL7Rα gene transcription is repressed by the transcription factor Gfi1, specifically in CD8+ T-lymphocytes.

Conclusion: Treatment by dexamethasone downregulates Gfi1 which contributes to glucocorticoid receptor mediated upregulation of IL7R expression.

Significance: The mechanism by which genes get turned on and off during development is a critical issue in biology.

SUMMARY

Interleukin-7 receptor alpha (IL-7Rα) is essential for T cell survival and differentiation. Glucocorticoids are potent enhancers of IL-7Rα expression with diverse roles in T cell biology. Here we identify the transcriptional repressor, Growth factor independent-1 (Gfi1), as a novel intermediary in glucocorticoid-induced IL-7Rα upregulation. We found Gfi1 to be a major inhibitory target of dexamethasone by microarray expression profiling of 3B4.15 T-lymphoma cells. Concordantly, retroviral
transduction of Gfi1 significantly blunted IL-7Rα upregulation by dexamethasone. To further assess the role of Gfi1 in vivo, we generated bacterial artificial chromosome (BAC) transgenic mice, in which a modified Il7r locus expresses GFP to report Il7r gene transcription. By introducing this BAC reporter transgene into either Gfi1-deficient or Gfi1-transgenic mice, we document in vivo that IL-7Rα transcription is upregulated in the absence of Gfi1 and downregulated when Gfi1 is overexpressed. Strikingly, the in vivo regulatory role of Gfi1 was specific for CD8+, and not CD4+ T cells or immature thymocytes. These results identify Gfi1 as a specific transcriptional repressor of the Il7r gene in CD8 T lymphocytes in vivo.

A critical issue in biology is the mechanism by which genes get turned on and off during development and differentiation. Because IL-7 receptor (IL-7R) proteins provide critical survival signals to developing lymphocytes, the expression of the Il7r gene that encodes the IL-7Rα receptor protein is tightly regulated at different stages of T and B lymphocyte development and precisely timed to stages when selection and programmed cell death occur in the immune system (1-3). The expression of IL-7Rα follows an on-off-on pattern in the thymus at the CD4⁻CD8⁻ double negative (DN), CD4⁺CD8⁻ double positive (DP) and CD4⁺CD8⁺ or CD4⁻CD8⁺ single positive (SP) stages, respectively (4). Thus, developmental cues during thymocyte differentiation control IL-7Rα expression. During CD8⁺ memory cell generation in the peripheral immune system, Il7r gene expression again correlates with developmental outcome, in that long-lived memory cell precursors upregulate IL-7Rα expression and short lived CD8⁺ cells lose IL-7Rα expression (5). Notably, upregulated IL-7Rα expression is not sufficient to drive long-lived memory CD8⁺ T cell generation, even though IL-7Rα upregulation clearly marks progenitors of this T cell subset (6,7). Importantly, the differentiation signals that match IL-7Rα expression to CD8 T cell fate remain unknown.

In T cells, IL-7Rα expression is thought to be primarily regulated at the transcriptional levels through an array of nuclear factors whose expression is also tightly controlled during development and activation. Several transcription factors that control Il7r gene expression have been identified. The promoter of Il7r contains binding sites for the PU.1 transcription factor, which is necessary for the IL-7Rα expression in developing B cells (8,9). The same site is occupied in T cells by another ETS family transcription factor, GABP (10). Promoter occupancy by these factors likely prevents CpG methylation of promoter sequences and subsequent downregulation of expression in mature T cells (11). Additionally, in human thymopoiesis, Notch may be complementing these ETS family proteins by acting through a conserved RBP-Jk/CSL binding site close by in the promoter of the Il7r gene (12). Therefore, downregulation of Notch expression at the DP stage may be causative of the complete loss of Il7r gene transcription in murine DP thymocytes. Also, the potential role of microRNAs acting on the Il7r gene locus, specifically at the DP stage has not been addressed and needs to be tested. Furthermore, the zinc finger protein Gfi1, for which a regulatory role was originally proposed in T cells and more recently confirmed in pro-B cells, was shown to bind to a putative intronic silencer (13-15). Additionally, glucocorticoid receptor (GR), Runx1/3, FoxOA1/3, and FoxP1 were all shown to bind to a putative enhancer in an evolutionarily conserved region 3.5 kb upstream of the gene (16-21). Finally FoxP3 was found to bind near the promoter in Treg cells to suppress IL-7Rα transcription (22). Importantly however, how these factors interact with each other and what controls the mechanism of developmental stage-specific differences in Il7r gene transcription remains ill defined.

In the present study, we addressed this issue first by profiling gene expression in 3B4.15 T hybridoma cells that respond to dexamethasone (Dex) treatment by upregulating IL-7Rα expression (23). We identified Gfi1 as a novel target of Dex and we further documented that either Gfi1 overexpression or treatment with the glucocorticoid receptor (GR) inhibitor RU486
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(Mifepristone) in 3B4.15 cells prevented IL-7Rα upregulation by Dex. These results indicate that Gfi1 is either controlled by GR or cooperates with it to downregulate IL-7Rα expression. To further assess the role of Gfi1 in vivo, we then generated a novel BAC transgenic mouse that reports transcriptional activity of the Il7r gene locus. We show that Gfi1 is a transcriptional repressor of the Il7r gene locus, only in CD8 lineage cells, by assessing Il7r reporter activity in Gfi1-deficient and Gfi1-transgenic thymocytes and T cells. Our observations place Gfi1 as a lineage specific and developmental stage dependent transcriptional repressor of IL-7Rα in vivo.

EXPERIMENTAL PROCEDURES

Animals- C57BL/6 and RAG2-deficient mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Gfi1-deficient (Gfi1KO) and Gfi1-transgenic (Gfi1Tg) mice have been previously described (24, 25). Animal experiments were approved by the National Cancer Institute Animal Care and Use Committee, and all mice were cared for in accordance with National Institutes of Health (NIH) guidelines.

Generation of 7RIG-BACTg transgenic mice- A BAC clone (RP23-365P6) containing the Il7r gene locus was modified by recombineering an IRES-EGFP cassette into the 3' UTR region of the gene in E.coli (26). Briefly, a targeting vector was generated containing (1) an HincII fragment of the pIRES2EGFP plasmid (Clontech), (2) an SV40 late poly(A) signal sequence PCR amplified from the pGL3Basic plasmid (Promega), (3) a KpnI fragment of the pLTM260 plasmid containing an Frt and a loxP flanked Neomycin resistance gene with a PGK promoter and a bGH poly(A) signal and (4) two flanking regions (210bp and 300bp long) homologous to the Il7r 3'UTR region of the gene in E. coli. Originally, the IL-7Rα-deficient (IL-7RαKO) mouse was generated by inserting a 1-kb MC1neo cassette into a HindIII site within the third exon of the Il7r gene, around position 90 of the 180 amino acid long extracellular domain (1). The originally inserted neo' gene is a modified neo' gene from pMC1Neo as originally described in Thomas and Capecci (29). In this altered neomycin resistance gene, a synthetic translation initiation sequence “5'-gccaatatgggatcggcc-3’” is introduced. We used the reverse sequence of this synthetic translation initiation sequence in combination with an Il7r exon3-specific primer to amplify a short PCR fragment. The exon 3-specific primer corresponds to the amino acid sequence “GSSNICV” of the IL-7Rα extracellular domain. Copy numbers of the IL-7Rα KO allele was determined by real time PCR using primers IL7Rex3GSSNICV and MC1neo-R.

Cell culture and flow cytometric analysis- Thymocytes or LN cells were prepared by processing thymus and LN into single cell suspensions and filtering through a 0.70 micron cell strainer (Becton Dickinson). For cell culture or stimulation, processed cells were incubated at 5 X 10⁶ cells/ml in 7.5% CO₂ at 37 C in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 unit/ml penicillin/streptomycin, 2 mM L-glutamine, 1x MEM vitamins/non-essential amino acids, and 50 μM β-mercaptoethanol. For dexamethasone treatment, cells were incubated with 10 μM of water soluble-dexamethasone (Cat# D4902, Sigma) for 18 hours in the presence or absence of 10 μM Mifepristone (RU486; Cat# M8046, Sigma). For flow cytometry, one million 3B4.15 hybridoma, thymocytes or lymph node cells
were used per staining with the corresponding antibodies and incubated for 45 min on ice. After washing with FACS buffer (1x HBSS, 0.5% Na-azide, 0.5% BSA), cells were analyzed on LSRII, ARIAII, FACS Canto or FACS Calibur flow cytometers (Becton Dickinson). Dead cells were excluded by forward light scatter gating and propidium iodide or 7AAD staining. Antibodies with the following specificities were used for staining: Qa-2 (clone 69H1-9-9), CD44 (clone IM7), HSA (clone M1/69), IL-7Rα (clone A7R34), IL-2Rα (clone PC61.5), IL-21R (clone ebio4A9), CD8α (clone 53-6.7); all from eBioscience; $\alpha$-c-chain (clone 4G3), IL-4Rα (clone mIL4R-M1), CD4 (clone GK1.5), TCRβ (clone H57-957), B220 (RA3-6B2) (all from Becton Dickinson); IL-2Rα (clone 5H4); from Biolegend. Data were analyzed with software designed by the Division of Computer Research and Technology at the NCI or with FlowJo 9.4.3 software (Treestar).

Adoptive transfer- Purified LNT cells from 7RIG-BACTg and Gfi1Tg7RIG-BACTg mice were labeled with CellTrace Violet (Invitrogen) and adoptively transferred into RAG2 -deficient host mice. 4 x 10^6 labeled cells were intravenously injected, and spleen and lymph node cells from host mice were harvested 5 days later. Single cell suspensions were stained for surface IL-7Rα and TCRβ expression and analyzed by flow cytometry.

RNA isolation and Northern blot analysis- Total RNA was isolated using TriZol (Invitrogen). Equal amounts of RNA were resolved in a 1.5% agarose gel under denaturing conditions and blotted onto Hybond-N+ nylon membranes (Amersham). Radioactive probes for detecting specific gene expression were generated using the EZ- strip DNA kit (Ambion) and used to hybridize with RNA-blotted membrane in UltraHyb hybridization solution (Ambion) at 42°C. Next day, membranes were washed two times with 2xSSC/0.1% SDS for 30 min and two more times with 0.1xSSC/0.1% SDS at 55°C. Membranes were exposed to a PhosphorImager screen (Amersham) and analyzed.

Expression plasmids and gene transfer- Full length and truncation mutants of murine Gfi1 cDNAs were C-terminally FLAG epitope tagged and cloned into the pBluescript II plasmid using oligonucleotides incorporating a 5’ XhoI and 3’ NotI restriction site. All cDNAs were transferred from pBluescript II to a retroviral expression plasmid, LZRSBMN-linker-IRES-EGFP, using XhoI and NotI restriction enzymes. This resulted in bicistronic expression of FLAG epitope tagged Gfi1 cDNA variants with an EGFP reporter gene. The following oligonucleotide pairs were used to amplify Gfi1 truncations: D-ZF: M13+dZFsrev; mGfi1-ZF: ZFsfor+T7; D-SNAG: dSNAGfor+T7. LZRSBMN-linker-IRES-EGFP with full length or truncated Gfi1 cDNAs were transfected into Phoenix-Eco retroviral packaging cell lines with a plasmid encoding ecotropic retrovirus envelope proteins (pCL-Eco Addgene plasmid 12371) (30) and supernatants were collected for two days, pooled and filtered through 45 µm filters. 3B4.15 cells were infected by spin infection in the presence of 6 µg/ml Polybrene (Sigma).

Oligonucleotides used in this study- The following oligonucleotides were used to PCR amplify Gfi1 cDNA constructs. Restriction enzyme sites (XhoI and NotI) used for cloning are shown in bold lettering, FLAG epitope tag sequence is shown in italics, and the start and stop codons are underlined. M13: 5’-CGC CAG GGT TTT CCC AGT CAC GAC -3’; T7: 5’-TAA TAC GAC TCA CTA TAG GG -3’; dSNAGfor: 5’- ATC TCG AGG CCA CCA TG -3’; ZFsfor: 5’-ATC TCG AGG CCA CCA TGT CCT ACA AAT GCA TCA AAT G- 3’; dZFsrev: 5’-ATG CGG CCG CTA TTT ATC GTC ATC TGT TTC TTT GTA GGA GCC GCC G -3’; dSNAGrev: 5’-ATG CGG CCG CTA TTT ATC GTC ATC TGT TTC TTT GTA GGA GCC GCC G -3’; dSNAGrev: 5’-ATG CGG CCG CTA TTT ATC GTC ATC TGT TTC TTT GTA GGA GCC GCC G -3’; IL7Re3GSSNICV: 5’- GGT AGC AGC AAT ATA TGT GTG -3’; MC1neo-R: 5’-GCC CGA TCC CAT ATT GCC G -3’.

Microarray analysis- Expression analysis was performed on 3B4.15 T hybridoma cells, either untreated or treated with 1 µM dexamethasone (Sigma) for 16 hours. Total RNA was extracted using Tri-Reagent (Sigma), RNA quality was confirmed on an RNA 6000 Nano chip (AGT-5067-1511) in an Agilent
Bioanalyzer. Double stranded cDNA was generated using SuperScript cDNA Synthesis Kit (Invitrogen), and the cDNA was then labeled with Cy-3, cleaned, quantified and hybridized according to the manufacturer’s protocols (Roche-Nimblegen). Nimblegen full genome Mouse Expression arrays (12X135K RO5543797) were washed and scanned at the Sabanci University Nanotechnology Research and Application Center-SUNUM. Results were processed using the ANAIS software (31). Array quality was assessed at the probe level. Values for 3 probes for each gene in each array were combined to summarize gene expression from probe sets. RMA background normalization and quantile normalization were performed for intra- and inter-array normalization respectively. Genes with signal intensities above a 95% random threshold were chosen for further studies. Differentially expressed genes were obtained based on the following criteria: Fold-change ≥ 2.5 and ANOVA P-value ≤ 0.01. Hierarchical clustering was applied to the top 500 differentially expressed genes with Genesis software (32). The data discussed in this publication have been deposited in NCBI’s Gene Expression Omnibus (33) and are accessible through GEO Series accession number GSE39296 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE39296).

RESULTS

Glucocorticoids induce IL-7Rα expression by downregulating expression of Gfi1

Treatment of primary T lymphocytes and T cell lines with glucocorticoids, such as dexamethasone (Dex), results in the upregulation of surface IL-7Rα expression (17,34). T cells normally express high levels of surface IL-7Rα but the I-Eα-restricted, PCC-specific T cell hybridoma 3B4.15 expresses only low levels of IL-7Rα (23). Nevertheless, when treated with glucocorticoids such as Dex, 3B4.15 cells dramatically upregulate both IL-7Rα mRNA and cell surface protein expression (Fig. 1A and B). These results parallel those previously obtained using the transformed murine T cell line KKF, which responds to Dex by upregulating IL-7Rα protein expression (16). Dex treatment induces nuclear localization and binding of the glucocorticoid receptor transcription factor to an evolutionarily conserved region 3.5 kb upstream of the transcriptional start site of the Il7r gene (16). Thus, Dex-induced IL-7Rα upregulation has been considered to be a direct transcriptional effect of activated GRs. To understand other gene regulators that control this phenotypic change, we compared the gene expression profiles of mock treated versus Dex treated 3B4.15 cells by high coverage Nimblegen expression arrays with 135,000 features. Microarray results confirmed that Dex treated 3B4.15 T hybridoma cells indeed upregulated IL-7Rα gene expression (Suppl. Fig. 1).

To confirm the specificity of Dex signaling in 3B4.15 hybridomas, first, we examined the expression profiles of the known Dex-regulated genes, GILZ (Tsc22d3) and GITR (Tnfrsf18) and found that these genes were positioned in the top 520 differentially expressed genes (Suppl. Fig. 1) (35-37). Next, we further analyzed the expression profiles of all transcription factors that have previously been reported to regulate Il7r transcription. Among these were: Glucocorticoid receptor, Gfi1, its close homolog Gfi1b, GABPα and its partners GABPβ1 and GABPβ2, PU.1 (Sfpi1), Runx1/3, NF-kB, FoxOA1/3, FoxP1, and FoxP3. Notably, we found that among these transcription factors, Gfi1 was the only one that passed our differentially expressed gene criteria of Fold-change ≥ 2.5 and ANOVA P-value ≤ 0.01. Thus, Dex treatment results in an upregulation of Il7r transcription and a dramatic downregulation of the zinc finger repressor protein Gfi1 mRNA expression (Fig. 1B and Suppl. Fig. 1).

Gfi1 was previously proposed to repress IL-7Rα transcription during lymphocyte development (14). Consequently, we wished to assess whether Gfi1 downregulation would contribute to IL-7Rα upregulation in 3B4.15 cells. To test this idea, we retrovirally overexpressed Gfi1 in 3B4.15 cells. Strikingly, Gfi1 overexpression inhibited IL-7Rα upregulation by dexamethasone, and Gfi1 overexpressing 3B4.15 cells remained IL-7Rα low (Fig. 1C). Thus, Dex induces the downregulation of endogenous Gfi1 expression,
but retroviral over-expression of Gfi1 is maintained even in the presence of Dex, and results in these cells remaining IL-7Rα low. This effect was indeed directly dependent on GR, because Dex treatment in the presence of RU-486, which is a competitive inhibitor of Dex for GR binding, completely inhibited IL-7Rα upregulation (Fig. 1D).

Next, to understand the mechanism of Gfi1-mediated repression of IL-7Rα expression, we retrovirally overexpressed a series of truncated Gfi1 cDNAs and assessed their effects on the Dex response of 3B4.15 cells (Suppl. Fig. 2 and B). While expression of full length Gfi1 significantly suppressed IL-7Rα re-expression, truncated Gfi1 lacking the demethylase-recruiting Snail-Gfi (SNAG) domain or the DNA binding zinc finger (ZF) domain failed to do so (Fig. 1F). Thus, repression of IL-7Rα expression by Gfi1 requires both its transcriptional repressor and the DNA binding domains and uncover Gfi1 to be a potent transcriptional inhibitor of IL-7Rα expression that acts downstream of Dex signaling which de-represses Il7r transcription.

**Assessing IL-7Rα transcription in vivo using a novel IL-7Rα reporter mouse**- Untreated 3B4.15 cells express high levels of Gfi1 and low levels of IL-7Rα. Mature resting T cells, on the other hand, express only low levels of Gfi1 and high levels of IL-7Rα (38). Therefore, Gfi1 levels in T cells in vivo do not correspond to those in 3B4.15 T hybridomas. To test whether Gfi1 can also control IL-7Rα expression in vivo, we generated a novel IL-7Rα transcriptional reporter transgenic mouse. We used a 210 kb BAC fragment containing the Il7r gene locus and inserted an IRES-EGFP cassette in the 3’ untranslated region (UTR) of the Il7r gene. We used this construct in pronuclear injections to generate transgenic mice (7RIG-BAC\textsuperscript{Tg}) (Suppl. Fig. 3).

Because the BAC construct contained the full Il7r gene locus, including putative transcriptional control regions, we expected the transgenic Il7r gene to faithfully reproduce expression of endogenous Il7r. To test this, we assessed IL-7Rα surface levels on freshly isolated 7RIG-BAC\textsuperscript{Tg} thymocytes. Endogenous IL-7Rα displays a characteristic on-off-on pattern during the progression through the DN-DP-SP stages of thymocyte development (3,39). Indeed, 7RIG-BAC\textsuperscript{Tg} thymocytes showed the expected on-off-on pattern for IL-7Rα expression in the thymus. These data suggest that transgenic IL-7Rα transcription is appropriately controlled in 7RIG-BAC\textsuperscript{Tg} thymocytes (Fig. 2A). Notably, IL-7Rα expression by 7RIG-BAC\textsuperscript{Tg} differed from that of a human CD2 promoter/enhancer driven IL-7Rα transgene (IL-7Rα\textsuperscript{Tg}), in its ability to downregulate IL-7Rα expression on DP thymocytes (Fig. 2A). These results support the expectation that the BAC transgene retained all endogenous regulatory elements for correct IL-7Rα expression. Notably, IL-7Rα surface levels on 7RIG-BAC\textsuperscript{Tg} transgenic cells were significantly higher compared to WT controls, presumably because Il7r gene transcription was active both from the endogenous and the transgenic Il7r locus. To demonstrate that the 7RIG-BAC\textsuperscript{Tg} transgene faithfully reports Il7r gene transcription, we assessed GFP expression in thymocytes. GFP expression also followed the on-off-on pattern of IL-7Rα expression in DN, DP, SP thymocytes respectively, indicating that transcription from the transgenic locus was correctly inhibited in DP thymocytes (Fig. 2B). We also assessed IL-7Rα reporter expression in peripheral LN cells. Mature B cells do not express IL-7Rα. Accordingly, we found that 7RIG-BAC\textsuperscript{Tg} DN lymph node cells, which include all mature B cells, were negative for GFP expression (Fig. 2C). CD4 and CD8 lymph node T cells, on the other hand, correctly expressed high levels of GFP. Interestingly, CD8 T cells reported higher levels of Il7r transcription than CD4 T cells based on their GFP levels (Fig. 2C). These data confirm that 7RIG-BAC\textsuperscript{Tg} reporter mice faithfully represent endogenous IL-7Rα expression in vivo and reveal a hitherto unappreciated difference in IL-7Rα transcription levels in CD4 and CD8 T cells.

**Cytokine-induced regulation of 7RIG-BAC\textsuperscript{Tg} expression**- To further document distinct IL-7Rα expression in CD4 and CD8 T cells, we quantified surface IL-7Rα and intracellular GFP levels in freshly isolated 7RIG-BAC\textsuperscript{Tg} LN T cells. We confirmed statistically significant
higher levels of both IL-7Rα expression and transcription in CD8 T cells in multiple experiments (Fig. 3A and Fig. 3B). Moreover, such lineage specific IL-7Rα expression was developmentally set as CD4 and CD8 T cells incubated overnight in medium in the absence of \textit{in vivo} signals still displayed distinct levels of IL-7Rα expression (data not shown).

The \textit{Il7r} gene locus is exquisitely sensitive to cytokine signaling. For instance, \textit{in vivo} IL-7 signaling potently downregulates \textit{Il7r} transcription and the steady-state levels of IL-7Rα mRNA in T cells (13). To determine if the transgenic IL-7Rα gene locus also responds to cytokine treatment, we incubated LN T cells from either WT or 7RIG-BACTg mice overnight in medium or in the presence of IL-7. The next day, total RNA was extracted and IL-7Rα mRNA signals were assessed and compared to those from freshly isolated T cells. In both WT and 7RIG-BAC\textsuperscript{Tg} T cells, overnight release from the cytokine-rich \textit{in vivo} environment highly upregulated IL-7Rα mRNA expression (Fig. 3C). Furthermore, overnight IL-7 signaling potently suppressed IL-7Rα mRNA expression in both WT and transgenic T cells. These results suggest that both the endogenous and the transgenic \textit{Il7r} loci are regulated in a cytokine-dependent manner. More importantly, GFP mRNA levels also faithfully replicated IL-7Rα expression, which confirms the validity of this transgenic model as an \textit{in vivo} IL-7Rα reporter (Fig. 3).

Restoring T cell development in IL-7Rα-deficient mice by 7RIG-BAC\textsuperscript{Tg}. IL-7Rα-deficiency results in severely impaired T cell development and peripheral T cell homeostasis. Because 7RIG-BAC\textsuperscript{Tg} replicates expression of endogenous \textit{Il7r}, we wished to know if the BAC transgene could restore T cell defects in IL-7Rα\textsuperscript{KO} mice. To test this, we crossed 7RIG-BAC\textsuperscript{Tg} into IL-7Rα\textsuperscript{KO} mice to generate IL-7Rα\textsuperscript{KO}7RIG-BAC\textsuperscript{Tg} mice (Suppl. Fig. 4A). In these mice, thymic αβ T cell development was largely restored as demonstrated by thymocyte CD4 versus CD8 profiles and TCRβ surface expression (Fig. 4A). Also, thymic NKT cell development and γδ T cell generation was dramatically improved compared to IL-7Rα\textsuperscript{KO} mice (Suppl. Fig. 4B and 4C). Notably, total thymocyte numbers were also restored compared to IL-7Rα\textsuperscript{KO} mice, but they did not fully recover to WT levels (Fig. 4B). To further understand this, we analyzed surface IL-7Rα levels on thymocytes, and we found that IL-7Rα\textsuperscript{KO}7RIG-BAC\textsuperscript{Tg} mice expressed significantly higher levels of IL-7Rα than WT mice (Fig. 4C). This is presumably caused by insertion of multiple copies of the BAC transgene into the genome as usually observed in BAC transgenesis. Elevated levels of IL-7Rα, however, have been shown to increase IL-7 consumption and competition for IL-7, which results in an overall decrease in thymocyte numbers (40,41). Thus, decreased total thymocyte numbers might reflect quantitative differences in surface IL-7Rα expression between WT and IL-7Rα\textsuperscript{KO}7RIG-BAC\textsuperscript{Tg} thymocytes. Interestingly, however, no major differences were observed in peripheral LN T cell numbers and CD4/CD8 profiles (Fig. 4D), despite surface IL-7Rα levels on transgenic LN T cells being still higher than WT counterparts (Fig. 4E). Thus, thymocytes and LN T cells are differently affected by IL-7Rα levels, likely because of the different mechanisms of proliferation and homeostasis in these two organs. Collectively, we find that BAC transgenic IL-7Rα is expressed and regulated in a developmentally correct fashion, and consequently restores T cell development and maintenance in IL-7Rα-deficient mice.

\textit{Gfi1} overexpression suppresses \textit{Il7r} gene transcription in CD8 T cells- Using these reporter mice, next we asked whether \textit{Gfi1} can suppress IL-7Rα transcription and expression \textit{in vivo}. To this end, we introduced the 7RIG-BAC\textsuperscript{Tg} onto mice expressing a T lineage-specific, human CD2 promoter driven \textit{Gfi1}-transgene (25) to generate \textit{Gfi1\textsuperscript{Tg}7RIG-BAC\textsuperscript{Tg}} mice and determined their IL-7Rα transcription by analyzing GFP expression. Strikingly, in \textit{Gfi1\textsuperscript{Tg}7RIG-BAC\textsuperscript{Tg}} thymocytes, we found that GFP levels were significantly downregulated by \textit{Gfi1}, but that this was specific and restricted to CD8SP thymocytes (Fig. 5A). These results suggest that transgenic \textit{Gfi1} expression fails to suppress IL-7Rα transcription in any other thymocyte subpopulation than in CD8SP cells. Such a specific effect of \textit{Gfi1} on CD8 lineage...
cells was further confirmed in peripheral CD8 T cells. We found both surface IL-7Rα and intracellular GFP levels selectively downregulated in CD8, but not in CD4 T cells (Fig. 6B). Thus, these data document that Gfi1 only downregulates IL-7Rα gene transcription and surface protein expression in CD8 T lymphocytes, and that all other thymocyte subpopulations and CD4 T cells are not susceptible to repression of this gene by Gfi1.

To further assess the specificity of Gfi1 transcriptional repression, we examined expression of other members of the γc cytokine receptor family. The γc cytokine family is composed of IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 (42), and antibodies are available for surface staining of each member of the γc receptor family with the exception of IL-9. Consequently, we examined expression of γc cytokine receptors for IL-2Rα, -2R, γc, IL-4Rα, IL-7Rα and IL-21R on WT and Gfi1Tg CD8 T cells (Fig. 5C). Strikingly, IL-7Rα was the only cytokine receptor that was significantly affected by Gfi1 overexpression (Fig. 5D), which revealed a highly selective effect of Gfi1 on IL-7Rα and reaffirmed its specificity for CD8 T cells.

*Gfi1-deficiency upregulates *Il7r transcription in CD8 T cells- Such a CD8 lineage-specific effect of Gfi1 was intriguing. One potential explanation could be that Gfi1 is expressed in all thymocytes and T cells, but that its repressor activity is only limited to CD8 T cells. Consequently, we wished to test whether removal of Gfi1 would upregulate IL-7Rα expression in T cells other than in CD8 lineage cells. To this end, we generated Gfi1KO-7RIG-BACTg mice and analyzed the GFP levels in thymocytes and in mature LN T cells (Suppl. Fig 5A and Fig. 6A) (24). Notably, Gfi1-deficiency failed to de-repress IL-7Rα transcription in most thymocytes, with the exception of CD8SP thymocytes (Suppl. Fig. 5A). DP thymocytes, which express high levels of Gfi1 and which are completely silent for IL-7Rα transcription, were still negative for GFP expression, when Gfi1 expression was ablated. These results indicate that Gfi1 is not required to suppress IL-7Rα expression in this particular subset. Rather, the effect of Gfi1 was highly restricted to post-selection CD8 lineage cells as GFP expression was quantitatively increased in both CD8SP thymocytes and LN CD8 T cells only (Suppl. Fig 5A and Fig. 6B).

Because absent Gfi1 was sufficient to upregulate IL-7Rα expression on CD8 T cells, next, we wished to know whether this would correlate with the Dex effect that we observed in 3B4.15 cells (Fig. 1A and Fig. 1B). To this end, we stimulated WT and Gfi1Tg T cells with Dex and assessed their surface IL-7Rα expression after overnight culture (Fig. 6C). While WT CD8+ T cells significantly upregulated IL-7Rα as previously observed (17), IL-7Rα levels on Gfi1KO CD8+ T cells were unaffected by stimulation with Dex (Fig. 6C). Notably, IL-7Rα expression on Gfi1KO CD4+ T cells was still upregulated by Dex indicating that Gfi1 effect is CD8 lineage specific (Suppl. Fig. 5B). These data strongly suggest that a major role for Dex is to suppress Gfi1, and that Gfi1 directly controls IL-7Rα expression in CD8 T cells.

Gfi1-deficiency has been proposed to promote CD8 memory phenotype cell generation (43). Because memory CD8 T cells express high levels of IL-7R (42), we wished to test whether increased IL-7Rα is a consequence of memory cell differentiation or a direct effect of Gfi1-deficiency. GFP levels in CD8 T cell subsets demonstrated that IL-7Rα transcription was significantly increased in both naïve (CD44low) and activated/memory (CD44high) phenotype CD8 T cells (Fig. 6D). Thus, we conclude that absent Gfi1 expression de-represses IL-7Rα transcription and expression in all CD8 T cells independently of their differentiation status.

In vivo analysis of IL-7Rα transcription using 7RIG-BACTg on a single cell basis- We wished to know if using the 7RIG-BACTg could provide us with new insights on IL-7Rα expression that so far has not been experimentally feasible to assess. Adoptive transfer of CD8 T cells into chronic lymphopenic mice results in lymphopenia-induced homeostatic proliferation (LIP). Slowly dividing homeostatic proliferation is dependent on IL-7 whereas rapid proliferation is IL-7 independent and driven by commensal antigens (44). To further understand the role of IL-7 in this process, it would be important to assess how
IL-7Rα expression is regulated during LIP. To do so, we assessed surface IL-7Rα and intracellular GFP expression on day 5 adoptively transferred 7RIG-BAC<sup>Tg</sup> CD8 T cells. Analyzing IL-7Rα transcription in adoptively transferred cells on a single cell basis has not been possible so far. IL-7 signaling suppresses IL-7Rα expression under steady-state conditions (13). Surprisingly, however, surface IL-7R levels remained largely unchanged during IL-7-driven LIP as assessed on slowly dividing cells (Fig. 7A left and Fig. 7B). Strikingly, in the same cells, Il7r transcription was dramatically downregulated upon cell division, as demonstrated by reduced GFP expression in Cell trace-diluted cells (Fig. 7A right and Fig. 7B). These data indicate that surface IL-7Rα expression is not a reliable marker for Il7r transcription, at least during homeostatic proliferation. They further suggest the operation of a transcription-independent mechanism of surface IL-7R expression, which could be either increased recycling of endocytosed IL-7Rα, stabilization of pre-existing IL-7Rα proteins or a yet unknown post-transcriptional mechanism. We are currently in the process of addressing these possibilities.

Because Gfi1 suppresses Il7r transcription, next we wished to assess the effect of Gfi1 on IL-7Rα expression during LIP. Day 5 adoptively transferred Gfi1<sup>Tg</sup>7RIG-BAC<sup>Tg</sup> cells displayed a comparable pattern of surface IL-7Rα and intracellular GFP expression to WT cells, in that GFP levels steadily decreased upon proliferation (Fig. 7C right) but surface IL-7Rα levels remain largely unaffected (Fig. 7C left). Thus, the dichotomy of IL-7Rα transcription and surface protein expression during LIP still remained distinct in Gfi1<sup>Tg</sup> cells. Moreover, LIP further downregulated IL-7Rα transcription in Gfi1<sup>Tg</sup> cells, which is presumably mediated by a mechanism independent of Gfi1. Importantly, Gfi1 overexpression significantly impaired CD8 T cell LIP which correlated with lower IL-7Rα levels on Gfi1<sup>Tg</sup> CD8 T cells (Fig. 5B). Thus, Gfi1 suppresses IL-7Rα expression and also IL-7-dependent proliferation. Taken together, these results uncover Gfi1 as a critical regulator of IL-7Rα transcription and expression in vivo, but exclusively in CD8 lineage thymocytes and T cells.

DISCUSSION

To understand the molecular mechanisms of IL-7Rα expression during T cell development and activation, here we generated a novel BAC GFP-reporter transgene and utilized this tool to assess IL-7Rα transcription in vivo. The BAC transgene was constructed by inserting a GFP-reporter and an IRES element into the 3′ UTR of the murine Il7r gene. Consequently, BAC reporter mice overexpressed full length IL-7Rα proteins in addition to GFP. We affirmed the developmentally correct expression of reporter transgenes by assessing lymphocyte differentiation in IL-7Rα-deficient mice reconstituted with the BAC construct (IL-7Rα<sup>KO</sup>7RIG-BAC<sup>Tg</sup>). In these mice, development of all IL-7Rα-dependent lymphoid cell populations, including αβ-, γδ-T cells, B-cells, and NKT cells, were restored. Thus, the transgenic Il7r gene locus in 7RIG-BAC<sup>Tg</sup> mice is correctly and lineage specifically regulated, and it equipped us with a new tool to assess IL-7Rα transcription and expression in vivo.

IL-7Rα is the ligand-specific subunit of the functional IL-7 receptor, which is composed of the IL-7Rα chain and the γc-chain (4). In contrast to the γc-chain, IL-7Rα expression is dynamic and is actively regulated during T cell development and differentiation. All developing thymocytes and all mature T cells express IL-7Rα, albeit at varying degrees. However, immature DP thymocytes are unique in that they have completely terminated IL-7Rα expression. Such peculiar absence of IL-7Rα on pre-selection DP cells was proposed to reflect a critical thymic selection mechanism that ensures a random but self-MHC-specific TCR repertoire (39,45,46). Accordingly, absent IL-7Rα expression renders DP thymocytes dependent on selecting TCR signals and not on non-discriminatory IL-7 signaling for survival. However, the molecular mechanisms that terminate IL-7Rα expression in DP cells remain unclear. Along this line, the molecular circuitry that re-induces IL-7Rα expression upon positive
selection also remains unmapped. This is even more intriguing as TCR signaling in mature T cells downregulates IL-7Rα expression, but while in immature DP thymocytes, TCR signaling induces IL-7Rα expression. Recent studies have suggested that re-expression of IL-7Rα in DP cells is dependent on positive selecting TCR signals in a NFAT and MAPK-dependent fashion and to a lesser degree on Akt (47). However, the detailed mechanism and molecular events remain unknown. Using the 7RIG-BAC Tg mice, here we establish that absent IL-7Rα expression in DP cells is a transcriptionally regulated event. Pre-selection DP cells failed to express GFP, while positive selection and maturation resulted in re-expression of GFP. With the 7RIG-BAC Tg reporter mouse, it is now feasible to test nuclear factors that regulate IL-7Rα transcription in vivo. We anticipate further applications of these reporter mice in dissecting the thymic signals that lead to termination as well as re-induction of IL7r transcription under various in vitro and in vivo settings.

In this regard, we were able to assess the effect of a transcriptional repressor on Il7r gene expression in vivo. Gfi1 has been considered as a potential Il7r regulator in immature thymocytes because of its high level of expression in pre-selection DP thymocytes and its potent suppression of IL-7Rα in pre-B cells. As such, Gfi1KO7RIG-BACTg mice offered a unique opportunity to assess Gfi1’s effect on IL-7Rα transcription in DP thymocytes in vivo. The complete absence of GFP signals in Gfi1KO7RIG-BAC Tg DP thymocytes strongly suggested that transcriptional silencing of Il7r gene expression does not require Gfi1, at least in pre-selection DP thymocytes. In mature T cells, Gfi1 clearly plays a more critical role. In fact, Gfi1 was first reported to suppress IL-7Rα expression by analyzing peripheral mature T cells (13). Nevertheless, a definitive and direct role of Gfi1 in IL-7Rα transcription has remained highly controversial. In some cases, surface IL-7Rα expression on Gfi1KO T cells was found to be not – or insignificantly-different (38), and also increased IL-7Rα levels in Gfi1KO mice had been suggested to be indirect, i.e. due to high proportions of memory cells under Gfi1-deficiency (43). Moreover, increased Gfi1 mRNA levels in human IL-7Rα high effector memory CD8 T cells contradicted a repressor role for Gfi1 in IL-7Rα expression (48). Our current data showing significantly increased GFP expression in Gfi1KO CD8 T cells, however, clearly documents Gfi1 as an in vivo repressor for IL-7Rα transcription.

Nevertheless, contradictory observations still keep open the possibility of a more complex regulatory network, with several Gfi1 co-factors being involved. As such, the cis-elements participating in the Gfi1 control of Il7r gene transcription are still not well defined. In B lymphocytes, Gfi1 can bind to the second intron of the Il7r gene to suppress transcription (14). In myeloid cells, Gfi1 can also associate with PU.1, an ETS family transcription factor that binds to the promoter of IL-7Rα and activates its transcription (49). However, PU.1 is not expressed in T lymphocytes. Rather another ETS family transcription factor, GABP is thought to occupy the ETS site in the promoter of the Il7r gene (50). Whether Gfi1 can interact with GABP protein is not known. But in T cells, GABP and Gfi1 were shown to have opposing roles so that a direct interaction and mutual suppression can not be excluded (51). Thus, in addition to its role in binding the Il7r gene promoter, GABP may control Gfi1 expression as GABP-deficient splenocytes lack Gfi1 expression, presumably due to the positively acting GABP binding sites in the Gfi1 promoter (52).

With Gfi1 emerging as a key control factor in IL-7Rα expression and also in many aspects of T and B cell immunology, it is critical to know what controls Gfi1 expression. So far, an auto-regulatory role for Gfi1 as well as a trans-regulatory role for Gfi1b has been documented (53). Also, downstream signaling of the GTPase Cdc42 has been proposed to suppress Gfi1 expression in resting T cells (54). The current study now proposes that glucocorticoids are a new family of molecules that can control Gfi1 expression. Glucocorticoids have been known to take part in thymocyte development and selection (55-58). Recently, conditional deletion of GR in pre-selection thymocytes documented significantly reduced thymus cellularity with an
altered TCR repertoire and increased negative selection (59). Mechanistically, glucocorticoids have been shown to intersect with TCR signaling and increase TCR signaling thresholds to promote thymic selection (60). Our current data now also proposes a role for Gfi1 downstream of GR signaling, and it would be informative to assess the contribution of Gfi1 to the GR-deficient phenotype.

In peripheral T cells, glucocorticoids are immunosuppressive, but they also upregulate IL-7R transcription and expression. The identification of Gfi1 as a novel target of Dex sheds new light to pre-existing observations on the effects of glucocorticoids and in this regard, the discovery of Gfi1 as an intermediary of Dex in IL-7Rα expression suggests that other Dex-induced events might also employ such a mechanism. While the upregulation of IL-7Rα expression in Dex-treated B cells, which normally do not express IL-7Rα, could be another case of an Gfi1-mediated Dex effect (34), inhibition of cytokine production in Dex-treated cells also could be explained along this line. Further studies are planned to address these possibilities and to delineate a direct Dex effect versus Gfi1-mediated Dex effects.

Although Gfi1 expression is downregulated by Dex signaling, and overexpression of Gfi1 can overcome Dex-mediated upregulation of IL-7Rα, we found, as expected, that GR is critical for Dex-mediated IL-7Rα upregulation. GR binds to a putative Il7r enhancer in an evolutionarily conserved sequence (ECR) 3.5 kb upstream of the transcriptional start site (16). The GR site in this ECR is 50 base pairs away from a FoxO1 transcription factor binding site which positively regulates gene expression in both naive CD4⁺ and CD8⁺ T cells (20,21). Whether this enhancer, the PU.1/GABP binding promoter and the putative Gfi1-binding intronic silencer are the only functional control elements in the Il7r gene locus is not known. In fact, a series of nuclear factors are known to regulate IL-7Rα expression. In addition to Gfi1, two other transcriptional repressors have been identified that downregulate Il7r gene expression in T cells. The forkhead family transcription factor FoxP1 suppresses IL-7Rα expression by competing with the Il7r transactivator FoxO1 for enhancer occupancy (19). On the other hand, FoxP3, which is specifically expressed in regulatory T cells, directly suppress IL-7Rα expression (22).

Overexpression of a Gfi1 family member, Gfi1b can also repress IL-7Rα expression in T lymphocytes, but presumably this effect is through the same intronic Gfi1 binding site, because the DNA binding domains of these two factors are very similar (61). Notably, in our expression profiling experiments, Gfi1 was the only nuclear factor that showed a significant difference in expression upon Dex treatment.

Collectively, the present study identified and tested a novel Il7r transcriptional control mechanism in vivo using a newly established IL-7Rα reporter mouse. We validated these reporter mice by complementing IL-7Rα-deficiency, and we utilized this tool to assess Il7r transcription downstream of Gfi1. Using a lymphopenia-induced homeostatic proliferation model, we further documented the superiority of these reporter mice in assessing IL-7Rα gene transcription in T cells to traditional methods. Finally, the current study not only resolves the controversies surrounding Gfi1’s role as a transcriptional repressor of IL-7Rα in CD8 T cells but also demonstrates that IL-7Rα expression in any other T cell population is independent of Gfi1. The molecular basis for such lineage specific regulation of cytokine receptor expression is intriguing and important, and we think that our findings will provide a new venue to identify critical players for controlling IL-7Rα expression.

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FOOTNOTES
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FIGURE LEGENDS

FIGURE 1. Glucocorticoids upregulates IL-7Rα expression while downregulating Gfi1.
(A) Dexamethasone (Dex) induces surface IL-7Rα expression on 3B4.15 hybridoma. Single parameter histograms of IL-7Rα expression on 3B4.15 hybridoma cells incubated overnight in medium or with Dex. Isotype staining controls are shown in dotted line.
(B) Dex-induced IL-7Rα expression inversely correlates with Gfi1 expression. Northern blot analysis of total RNA from overnight medium or Dex-treated 3B4.15 cells with probes indicated on the left.
(C) Gfi1 overexpression inhibits Dex-induced IL-7Rα expression. Histograms show surface IL-7Rα expression on control retrovirus infected 3B4.15 cells incubated for 16 hr, either in medium (filled histogram) or with Dex (dotted line). Solid histogram shows IL-7Rα expression on Dex treated, Gfi1-expressing retrovirus infected 3B4.15 cells.
(D) Dex-induced IL-7Rα expression is a glucocorticoid receptor dependent event. Histograms show IL-7Rα expression on 3B4.15 cells incubated for 16 hr in medium (filled histogram), with dexamethasone (solid histogram), or with Dex in the presence of Mifepristone (RU486) (dotted line).
(E) Zinc finger and SNAG domains of Gfi1 are required for inhibiting Dex-induced IL-7Rα expression. IL-7Rα surface expression on 3B4.15 cells was quantified into linear fluorescence units, with IL-7Rα expression on empty LZRS retrovirus infected cells set equal to 100. Inhibition of IL-7Rα expression by retroviruses expressing either full length (mGfi1), or domain deletions of Gfi1 were compared. Zinc finger domain deleted (Δ-ZF), N-terminal truncation containing only the ZF domain (mGfi1-ZF) and SNAG domain deleted (Δ-SNAG).

FIGURE 2. 7RIG-BAC^{Tg} faithfully reports IL-7Rα expression in vivo.
(A) Cell surface IL-7Rα expression during thymocyte development. IL-7Rα expression on gated thymocyte subpopulations from WT mice (upper panel), 7RIG-BAC^{Tg} (middle panel), and a human CD2 mini-cassette driven IL-7Rα^{Tg} mice (lower panel) are shown (solid line) over isotype control staining (dotted line).
(B) Assessing IL-7Rα transcription using GFP reporter activity in thymocytes subpopulations. Total thymocytes from WT and 7RIG-BAC^{Tg} mice were stained for CD4 and CD8 surface markers, and GFP expression was determined in individual subpopulations. Data are representative of four independent experiments.
(C) Lineage specific IL-7Rα transcription in LNT cells. Total LN cells from WT and 7RIG-BAC^{Tg} mice were stained for CD4 and CD8 surface markers, and GFP expression was determined in CD8 and CD4 LN T cells. Mean fluorescence intensity (MFI) of surface IL-7Rα are shown for CD8 and CD4 T cells, respectively. Data are representative of four independent experiments.
**FIGURE 3. Transcriptional regulation of GFP reporter expression.**
(A) Relative surface IL-7Rα expression on CD4 and CD8 LNT cells. Surface IL-7Rα levels on 7RIG-BACₜₛ T cells were quantified in MFI and normalized to IL-7Rα levels on CD4 cells. Bar graph shows mean +/- S.E.M. from three independent experiments.
(B) Relative GFP expression in CD4 and CD8 LN T cells. Intracellular GFP levels in 7RIG-BACₜₛ T cells were quantified in MFI and normalized to GFP levels in CD4 cells. Bar graph shows mean +/- S.E.M. from three independent experiments.
(C) Purified LN T cells from WT or 7RIG-BACₜₛ mice were assessed for IL-7Rα and GFP mRNA expression by Northern blot analysis with probes indicated on the left. Total RNA was isolated from fresh, overnight medium incubated, or overnight IL-7 treated LNT cells.

**FIGURE 4. 7RIG-BACₜₛ restores thymocyte development and T cell homeostasis in IL-7RαKO mice.**
(A) Thymocyte development in IL-7RαKO7RIG-BACₜₛ. Total thymocytes from WT and IL-7RαKO7RIG-BACₜₛ mice were assessed for CD4, CD8, and TCRβ surface marker expression (Top and middle). Mature TCRβhi thymocytes were analyzed for CD4 and CD8 profiles (bottom).
(B) Total thymocyte numbers in IL-7RαKO7RIG-BACₜₛ mice. Thymocytes numbers from WT, IL-7RαKO7RIG-BACₜₛ, and IL-7RαKO mice were determined. Bar graph shows mean +/- S.E.M. from three independent experiments.
(C) Surface IL-7Rα on WT and 7RIG-BACₜₛ thymocytes. IL-7Rα expression was determined on WT and IL-7RαKO7RIG-BACₜₛ thymocyte subpopulations. Bar graph shows mean +/- S.E.M. from three independent experiments.
(D) Peripheral T cell homeostasis in IL-7RαKO7RIG-BACₜₛ mice. LN cells were isolated, counted and phenotyped for CD4 and CD8 expression (top). Bar graph shows total LN numbers (bottom). Data show mean +/- S.E.M. from four independent experiments.
(E) Surface IL-7Rα on WT and 7RIG-BACₜₛ LNT cells. IL-7Rα expression was assessed and quantified on WT and IL-7RαKO7RIG-BACₜₛ LN cell subpopulations. Data show representative histograms from three independent experiments.

**FIGURE 5. Gfi1 suppresses IL-7Rα expression and transcription in vivo.**
(A) Suppression of GFP reporter activity by overexpression of Gfi1. IL-7Rα transcriptional activities in individual thymocyte subpopulations were determined using the 7RIG-BACₜₛ on WT or Gfi1Tg backgrounds. Contour plots are representative of four independent experiments with 4 WT and 5 Gfi1Tg mice transgenic for 7RIG-BACₜₛ. Bar graph shows mean +/- S.E.M. four independent experiments.
(B) IL-7Rα surface expression and transcription in Gfi1Tg transgenic CD4 and CD8 LNT cells. Gfi1’s effect was assessed in WT or Gfi1Tg mice transgenic for 7RIG-BACₜₛ. Cell surface IL-7Rα and GFP expression were determined by flow cytometry. Bar graph shows mean +/- S.E.M. from three independent experiments with 3 WT and 5 Gfi1Tg 7RIG-BACₜₛ mice.
(C) Expression of γc cytokine receptor families on Gfi1Tg CD8⁺ LNT cells. Surface expression of indicated γc cytokine receptors were determined on gated CD8 T cells from WT and Gfi1Tg mice. Data are representative of three independent experiments.
(D) Gfi1Tg specifically suppresses IL-7Rα expression on CD8⁺ T cells. Surface cytokine receptor levels on Gfi1Tg CD8⁺ T cells were quantified and normalized to levels on WT CD8⁺ T cells. Bar graph shows mean +/- S.E.M. of three independent experiments.
FIGURE 6. Gfi1-deficiency de-represses IL-7Rα transcription and expression in CD8 lineage cells.
(A) Increased GFP reporter activity in Gfi1KO7RIG-BAC Tg CD8 T cells. GFP expression was assessed in CD8 and CD4 T cells from WT or Gfi1KO mice expressing 7RIG-BAC Tg. Data are representative of four independent experiments.
(B) Quantification of GFP expression in Gfi1KO7RIG-BAC Tg T cells. MFI of intracellular GFP levels were determined from 7RIG-BAC Tg and Gfi1KO7RIG-BAC Tg LNT cells. Bar graph shows the mean +/- S.E.M. of two independent experiments.
(C) Dex-effect on CD8' LNT IL-7Rα expression. Surface IL-7Rα expression was determined on WT and Gfi1KO CD8 T cells incubated overnight in medium or in Dex.
(D) Gfi1’s effect on IL-7Rα transcription is independent of activation/differentiation status in CD8 T cells. GFP reporter expression was assessed in freshly isolated CD44lo and CD44hi CD8 T cells from 7RIG-BAC Tg and Gfi1KO7RIG-BAC Tg mice. Data are representative of four independent experiments.

FIGURE 7. In vivo and in vitro effects of Gfi1 on IL-7Rα expression.
(A) IL-7Rα expression during lymphopenia-induced homeostatic proliferation. Surface IL-7Rα expression and intracellular GFP expression were assessed on day 5 adoptively transferred 7RIG-BAC Tg CD8+ T cells in RAG-2-deficient host mice. Cell division was monitored by Cell trace Violet dye dilution. Dot plots are representative of three independent experiments.
(B) IL-7Rα expression and transcription in proliferating donor cells. MFI of surface IL-7Rα (open circle) and intracellular GFP (closed circle) were assessed for each cell division (D1 to D5), and normalized to non-dividing cells (D0), which was set to 100 (%). Graph shows the mean +/- S.E.M. from three independent experiments.
(C) IL-7Rα expression on proliferating Gfi1Tg7RIG-BAC Tg CD8+ T cells. Surface IL-7Rα expression and intracellular GFP expression was assessed on day 5 adoptively transferred Gfi1Tg7RIG-BAC Tg CD8+ T cells in RAG-2-deficient host mice. Cell division was monitored by Cell trace Violet dye dilution. Dot plots are representative of three independent experiments.
(D) Gfi1 impairs lymphopenia-induced homeostatic proliferation. Cell trace Violet dilutions on slow dividing donor 7RIG-BAC Tg or Gfi1Tg7RIG-BAC Tg were quantitated. Numbers indicated percentage of cells that have undergone one or more divisions during homeostatic proliferation. Data are representative of three independent experiments.
Figure 2

A

WT

IL-7Rα^Tg

7RIG-BAC^Tg

IL-7Rα

DN  DP  CD8SP  CD4SP

B

WT

7RIG-BAC^Tg

DN  DP  CD8SP  CD4SP

GFP

C

WT

7RIG-BAC^Tg

DN  CD8  CD4

MFI=224  MFI=126

GFP
Figure 3

A. Relative IL-7Rα expression (%)

B. Relative GFP expression (%)

C. Northern blot

- IL-7Rα mRNA
- GFP mRNA
- 18S rRNA

WT and 7RIG-BAC^Tg
Figure 5

A

7RIG-BACTg  
Gfi1Tg7RIG-BACTg

CD4

CD8

5.3 87.9
1.6 1.9
5.4 88.4
3.2 1.7

GFP (MFI)

DN  DP  CD8SP  CD4SP

NS  NS  NS  NS

B

7RIG-BACTg  
Gfi1Tg7RIG-BACTg

P<0.01

IL-7Rα (MFI)

CD8  CD4

NS  NS

GFP (MFI)

CD8  CD4

NS  NS

C

Gated on CD8+ LNT

IL-2Rα  IL-2Rβ  γc

WT  Gfi1Tg  Control Ab

IL-4Rα  IL-7Rα  IL-21R

Cytokine receptor expression

D

Gated on CD8+ LNT cells

Relative expression (%)

2Rα  2Rβ  γc  4Rα  7Rα  21R

P<0.05
Figure 7

A

7RIG-BACTg
LNT cells

i.v.

RAGKo host

IL-7Rα expression (surface staining)

IL-7Rα transcription (intracellular GFP)

Cell trace

GFP

Cell trace

B

Relative expression (%)

0

50

100

150

D6
D5
D4
D3
D2
D1

O IL-7Rα

GFP

C

Gfi1Tg7RIG-BACTg
LNT cells

i.v.

RAGKo host

IL-7Rα expression (surface staining)

IL-7Rα transcription (intracellular GFP)

Cell trace

GFP

Cell trace

D

7RIG-BACTg

Gfi1Tg7RIG-BACTg

77.4

52.8
CD8 lineage specific regulation of interleukin-7 receptor expression by the transcriptional repressor Gfi1


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