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Formation of IL-7Rα^{high} and IL-7Rα^{low} CD8 T Cells during Infection Is Regulated by the Opposing Functions of GABPα and Gfi-1

Anmol Chandele,* Nikhil S. Joshi,* Jinfang Zhu,† William E. Paul,† Warren J. Leonard,‡ and Susan M. Kaech²‡

IL-7 is essential for the survival of naive and memory T cells, and IL-7 receptor α-chain (IL-7Rα) expression is dynamically regulated in activated CD8 T cells during acute viral and bacterial infections. Most virus-specific CD8 T cells become IL-7Rα^{low} and are relatively short-lived, but some escape IL-7Rα repression (referred to as IL-7Rα^{high} memory precursor effector cells) and preferentially enter the memory CD8 T cell pool. How antiviral effector CD8 T cells regulate IL-7Rα expression in an “on and off” fashion remains to be characterized. During lymphocytic choriomeningitis virus infection, we found that opposing actions of the transcription factors GABPα (GA binding protein α) and Gfi-1 (growth factor independence 1) control IL-7Rα expression in effector CD8 T cells. Specifically, GABPα was required for IL-7Rα expression in memory precursor effector cells, and this correlated with hyperacetylation of the II/7ra promoter. In contrast, Gfi-1 was required for stable IL-7Rα repression in effector CD8 T cells and acted by antagonizing GABPα binding and recruiting histone deacetylase 1, which deacetylated the II/7ra promoter. Thus, I/7ra promoter acetylation and activity was dependent on the reciprocal binding of GABPα and Gfi-1, and these data provide a biochemical mechanism for the generation of stable IL-7Rα^{high} and IL-7Rα^{low} states in virus-specific effector CD8 T cells. The Journal of Immunology, 2008, 180: 5309–5319.

Interleukin-7 plays an essential role in lymphocyte development and survival and has been shown to be a key regulator of memory T cell homeostasis (1, 2). IL-7 signals are transduced through the high-affinity IL-7 receptor α-chain (IL-7Rα) and the common cytokine receptor γ-chain (γc, CD132) (1, 3). IL-7Rα signals downstream through at least two major signal transduction pathways, the JAK/STAT and PI3K/AKT pathways, which together induce expression of several genes that promote T cell growth and proliferation and survival (4–7).

The expression of IL-7Rα is dynamically controlled at many different stages during the life of T and B cells. For example, in the development of mature naive B and T cells IL-7Rα is expressed by common lymphoid progenitors, but then is down-regulated during

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3 Abbreviations used in this paper: γc, common cytokine receptor γ-chain; CD132, common γ-chain; GABPα, GA binding protein α; Gfi-1, growth factor independence 1; HDAC, histone deacetylase; H3K9, histone 3 acetylated at lysine 9; LCMV, lymphocytic choriomeningitis virus; MFI, mean fluorescence intensity; MPEC, memory precursor effector cell; p.i., postinfection; qRT-PCR, quantitative real-time PCR; RNAi, RNA interference; SLEC, short-lived effector cell; TSA, trichostatin A; TRAIL, TNF-related apoptosis-inducing ligand; VSV, vesicular stomatitis virus; YFP, yellow fluorescent protein; ZAP-70, ζ-chain-associated protein kinase 70; BCR, B cell receptor; Fas, cell death-inducing ligand; FasL, Fas ligand; ITAM, immunoreceptor tyrosine-based activation motif; LCMV, lymphocytic choriomeningitis virus; MHC class I, major histocompatibility complex class I; HIV, human immunodeficiency virus; HIV-1, human immunodeficiency virus type 1; HIV-1 ENcorr, down-regulated memory CD4 T cells (11, 14, 15). However, a small subset of the effector CD8 T cells express higher amounts of IL-7Ra (referred to as IL-7Rα^{high}) and these two subsets have been shown to have different cell fates (12, 14). The IL-7Rα^{high} effector CD8 T cells generated during infection have been considered memory precursor effector cells (MPECs) because they preferentially survive and develop into a stable long-lived memory CD8 T cell population that can protect against secondary infection and self-renew through homeostatic turnover (12, 16). These MPECs require functional IL-7Ra to become long-lived memory CD8 T cells (2, 12, 17), and a similar process likely occurs in memory CD4 T cell development (18, 19). In contrast, the IL-7Rα^{low} CD8 T cell subset is relatively short lived and forms memory T cells poorly; these cells have been referred to as short-lived effector CD8 T cells (SLECs) (12, 16, 20). The majority of the antiviral IL-7Rα^{high} effector cells do not appear to convert to an IL-7Rα^{low} state, indicating that stable repression of the IL-7Ra locus is maintained over time in the absence of antigenic stimulation (20). Although it is likely that the steady IL-7 deprivation
contributes to the finite lifespan of IL-7Rαlow cells, recent data on the overexpression IL-7Rα on these cells show that IL-7Rα down-regulation is symptomatic of, but not causal to, the bulk effector cell death that occurs after viral clearance (21). These IL-7Rαlow cells become dependent on IL-15, but this does not sustain them long term (20).

Considering that fairly stable changes in IL-7Rα expression accompany the decision to become a memory T cell or not and that memory T cell longevity is functionally dependent on IL-7Rα expression, it is important to understand how IL-7Rα expression is regulated in Ag-specific T cells during immune responses. Currently, most evidence suggests that control of IL-7Rα expression in T cells is mediated primarily through changes in Il7ra gene transcription (12, 13, 22, 23), but very few of the mechanistic details of this process are known. Multiple signals can affect Il7ra transcription in T cells, including IL-7 (23), IL-2 (24), and TCR signaling (12, 13, 25) and glucocorticoids (13, 26) that induce Il7ra transcription. In addition, a few transcription factors have been identified that directly bind to and control Il7ra expression. Two transcription factors of the Ets family, PU.1 and GABPα binding protein α (GABPα; also known as NRF-2 and E4TFI–60), bind to the same site in the Il7ra promoter and activate its transcription (27–29). GABPα is part of a heterodimeric complex that includes GABPβ (also known as NRF1) that can activate transcription and recruit histone acetyltransferases (30). In B cell progenitors, both GABP and PU.1 drive IL-7Rα expression (28, 31), but in thymocytes and naive T cells GABP alone performs this function (29). The reason behind the separation of functions of Gfi-1 and GABP in the different lymphocyte lineages is not clear. Moreover, it is not known whether GABP functions in memory T cells or their precursors to maintain IL-7Rα expression. To date, growth factor independence 1 (Gfi-1) is the only known IL-7Rα transcriptional repressor in T cells; it binds to introns 2 and 4 of the Il7ra gene and down-regulates expression following IL-7 signaling (23). Gfi-1 has been shown to recruit G9a histone lysine methylase, ETO proteins, and histone deacetylases (HDACs) to mediate transcriptional repression of other genes (32). Whether Gfi-1 acts at other stages of T cell development or following TCR activation to silence the IL-7Rα locus remains to be determined.

The aims of this study were to determine how Il7ra transcription is transcriptionally regulated in Ag-specific effector and memory CD8 T cells following LCMV infection in mice. In particular, we examined whether epigenetic modifications of chromatin and the binding of GABPα or Gfi-1 were associated with different transcriptional states of Il7ra in effector and memory CD8 T cells. Our data suggest that Gfi-1 and GABPα function in a “yin-yang” relationship during effector CD8 T cell development. Gfi-1 is necessary and sufficient to maintain hypoacetylation of histones and stable Il7ra repression in late-stage effector CD8 T cells. Conversely, GABPα helps to maintain histone acetylation and Il7ra transcription in IL-7Rαhigh MPECs. Together, these data provide a mechanistic outline of Il7ra transcriptional regulation as effector and memory CD8 T cells differentiate during viral infection.

Materials and Methods

Mice and infections

C57BL/6 mice were purchased from National Cancer Institute (Frederick, MD) and The Jackson Laboratory. Thy1.1P14 TCR transgenic (tg) mice have been described previously (33). Gfi-1tg mice were generously provided by Dr. S. Orkin (Children’s Hospital, Boston, MA) (34). For experiments involving P14 tg CD8 T cells, ~1 × 106 Gfi-1tg or Gfi-1+P14 C57BL/6 mice were transferred to make “P14 chimeric mice”. Mice were infected i.p. with 2 × 10⁸ PFU of LCMV-Armstrong strain (33).

Cell isolations and separations

Populations of Thy1.1P14 C57B1/6 mice were purified by magnetically depleting non-CD8 T cells using Abs to CD4 (clone GK1.5), B220 (TIB164), and H-2d (TIB120) obtained from K. Bottomly (Yale University, New Haven, CT), followed by anti-rat IgG and mouse-IgM magnetic beads (Polysciences) and sorted using FACSaria (BD Biosciences) or MoFlo (DakoCytomation) cell sorters. Equal numbers of sorted cells were transferred i.v. into C57BL6/J recipient mice.

Retrovirus (RV) constructs and transduction

P14tg mice were directly infected with 2 × 10⁸ PFU of LCMV-Armstrong i.v. and 1 day later P14 tg splenocytes were spin transduced for 90 min at 37°C with fresh viral supernatants from 293T cells (transfected 48 h prior with pVSVG-MigR1 helper and either MigR1 control, MigR1-Gfi-1–35, MigR1-Mir (containing the microRNA (Mir) cassette from the LMP vector (Open Biosystems) in MigR1) or MigR1-Mir-shGaba expressing a short hairpin oligomer for RNAi against GABPα in the presence of 8 μg/ml Polybrene and 10 μg/ml IL-2 and then immediately transferred i.v. into C57BL6/J mice that were subsequently infected with 2 × 10⁸ PFU LCMV. For experiments involving transfer into naive mice, ~10 × 10⁶ P14 CD8 T cells were cultured for 24 h with 20 μg/ml GF-3 proliferation peptide and spin transduced as described above to avoid the transfer of LCMV into naive mice. Mice containing RV-transduced P14 CD8 T cells were sacrificed at day 5 or day 8 posttransduction and GFP+ P14 CD8 T cells were analyzed by flow cytometry or sorted using FACSaria (BD Biosciences) or MoFlo (DakoCytomation) cell sorters.

Real-time PCR analyses

For real time analysis ~2 × 10⁶ naive P14 CD8 T cells, day-5 to day-8 P14 effector CD8 T cells, and day-8 IL-7Rαhigh and IL-7Rαlow cells were sorted by FACS. Total RNA was extracted by the TRIzol method using the manufacturer’s instructions and cDNA was synthesized using SRTTI (Invitrogen Life Technologies). Real-time PCR analysis was performed as previously described (36). Primers for Il7ra were 5’-GCAAGCGCAGCCA TCACCT-3’ and 5’-ATTTTTGCGAGGCTTCAGG-3’ and primers for Gfi-1 were 5’-CTCATTTGACAGACG-3’ and 5’-CATGACTAGGGCTTGAAA-3’. Because of the extensive homology between Gfi-1 and its homologue Gfi1b, real-time PCR primers were chosen in the nonhomologous regions of the Gfi1 coding sequence: L9, 5’-TGAAAGATCTCGTG GGTCCAAG-3’ and 5’-GCACTACGCCGACTAACT-3’. L9 is a ribosomal protein gene that is expressed at a relatively constant level in naive, effector, and memory CD8 T cells and serves as an internal control. All PCR products were run on an annealing temperature of 55°C. Relative fold differences were calculated as described (37).

Western blotting

Protein lysates from 1 × 10⁶ sorted naive cells (CD8+CD44low) or day-5 and day-8 sorted total effector CD8 T cells were used to detect IL-7Rα expression. Abs to GABPα (29) were used for Western blotting at 1/1000 dilution. The blots were stripped and reprobed with Grp94 (Cell Signalling Technology) at a dilution of 1/5000 as a normalization control.

Chromatin immunoprecipitation

ChIP assays were performed with 5–8 × 10⁶ CD8 T cells with standard procedures (Upstate Biotech). Briefly, DNA was crosslinked with formaldehyde (1% final concentration) and chromatin was sheared by sonication until an average length of DNA ladder of 500-bp increments was obtained. Salmon sperm DNA/protein agaroase (Upstate Biotechnology) was used for preclearing and immunoprecipitation. Abs against acetyl H3K9 and H3DA1 (Upstate Biotechnology), GABPα (29), and Gfi-1 (gift from Dr. H Bellen that is the nonhomologous regions between Gfi-1 and Gfi-1B and is not reactive in Gfi1-/- mice) (38) were used for precipitating the protein–DNA complex. Precipitated protein–DNA complexes were washed in low-salt buffer (20 mM Tris-HCl (pH 8.1), 2 mM EDTA, 150 mM NaCl, 0.1% SDS, and 1% Triton X-100) and with TE (10 mM Tris-HCl (pH 8.1) and 1 mM EDTA) buffer twice. The precipitated protein–DNA complexes were eluted and crosslinks were reversed with NaCl (200 mM final concentration) incubated at 65°C overnight. After treatment with RNase A and proteinase K, DNA was extracted with phenol/chloroform and precipitated with ethanol. Purified DNA was then subjected to 40 cycles of PCR amplification. Primers spanned the ~294 to ~59 region within the Il7ra promoter and the reported binding site for Gfi-1 in intron 2. The primer sequences for the Il7ra promoter were 5’-CCAGCTTGCAG GAGCTTCAGG-3’ (forward) and 5’-GAAGACCGGTTGATGTCGAG TG-3’ (reverse), and those for Il7ra intron 2 were 5’-CCAGCTATCTCA GTAATGG-3’ (forward) and 5’-TTCAAGTCCACAGATAAAT-3’.
DNA concentration was calculated from a genomic DNA standard 8-fold dilution series, and in most cases the fold differences between samples were calculated by normalizing to naive CD8 T cells set at 1 or calculated as relative DNA concentration with respect to the genomic DNA curve and the initial input DNA.

Statistical analyses

Where indicated, p values were determined using a two-tailed unpaired Student’s t test. p < 0.05 was considered significant. All graphs show averages of the mean ± SEM.

Results

Transcriptional repression of Il7ra in LCMV-specific effector CD8 T cells is associated with histone deacetylation and HDAC1 recruitment

To more thoroughly characterize the kinetics and mechanism(s) of Il7ra repression during effector CD8 T cell differentiation, we first measured IL-7Ra protein and mRNA levels as well as histone acetylation and HDAC1 binding to the Il7ra promoter region (see schematic in Fig. 1A; Ref 23). Two experimental systems using...
endogenous and P14 TCR tg LCMV-specific CD8 T cells were used to measure IL-7Ra expression of effector CD8 T cells as they clonally expand and differentiate in response to LCMV infection. The use of P14 CD8 T cells facilitated earlier detection and isolation of large numbers of the LCMV-specific CD8 T cells; for these experiments, a small number of congenically marked Thy1.1 naive P14 CD8 T cells (<10,000 cells) specific for the LCMV epitope D<sup>9</sup>GP33–41 were adoptively transferred into C57BL/6 mice (Thy1.2<sup>+</sup>) that were subsequently infected with LCMV. The precursor frequency of P14 CD8 T cells in this experimental set-up does not have a major effect on IL-7Ra expression or effector and memory CD8 T cell differentiation (20).

The surface expression of IL-7Ra was measured using flow cytometry and the gates for IL-7Ra<sup>high</sup> and IL-7Ra<sup>low</sup> T cells were created based on two criteria: 1) the IL-7Ra staining pattern of total lymphocytes, because the two populations of IL-7Ra<sup>high</sup> and IL-7Ra<sup>low</sup> cells were clearly identifiable (Fig. 1B, top row, left plot); and 2) the treatment of effector CD8 T cells with IL-7, because this treatment virtually abolishes detection of IL-7Ra on T cells (23) (Fig. 1B, top row, right plot). Naive CD8 T cells are for the most part uniformly IL-7Ra<sup>high</sup> (median fluorescence intensity (MFI) = 50 ± 5), but within ~4 days of infection IL-7Ra levels dropped considerably on most of the activated CD8 T cells in the spleen (Fig. 1B, bottom row). Both the frequency and number of IL-7Ra<sup>high</sup> cells steadily increased with each passing day of infection until days 7 or 8 postinfection (p.i.), when the virus was cleared and effector CD8 T cell expansion peaked. Although a population of IL-7Ra<sup>high</sup> cells was consistently present at each time point (based on staining after IL-7 treatment), the IL-7Ra<sup>high</sup> effector cell population became more discernible by day 8 p.i. (Fig. 1B, bottom row). A similar pattern of IL-7Ra expression was observed in endogenous populations of LCMV-specific CD8 T cells (data not shown). Previous studies have shown that these IL-7Ra<sup>high</sup> effector CD8 T cells present at day 8 p.i. preferentially develop into long-lived memory CD8 T cells and, hence, are referred to as memory precursor effector cells or MPECs (12, 20). Consequently, the majority of memory P14 CD8 T cells found at days 50<sup>+</sup> p.i. were IL-7Ra<sup>high</sup> (12) (Fig. 1B, bottom row). Although the proportion of IL-7Ra<sup>low</sup> effector CD8 T cells was larger in the lung and smaller in the inguinal lymph node relative to the spleen, liver, and blood, the overall kinetics of IL-7Ra repression on effector CD8 T cells occurred similarly in these tissues, indicating that the formation of IL-7Ra<sup>low</sup> CD8 T cells was not compartmentalized to a particular tissue (data not shown).

To determine whether a decrease in IL-7Ra mRNA mirrored the reduction of surface IL-7Ra expression, the P14 CD8 T cells were purified at days 5 and 8 p.i. by FACS and IL-7Ra mRNA levels were measured using quantitative real-time PCR (qRT-PCR). Compared with naive CD8 T cells, IL-7Ra mRNA levels dropped ~5–9-fold in the effector CD8 T cells, consistent with significant transcriptional repression in these cells (Fig. 1C). Further comparison of day-8 effector CD8 T cells that were separated based on IL-7Ra<sup>high</sup> and IL-7Ra<sup>low</sup> expression revealed that the IL-7Ra<sup>high</sup> cells contained ~41-fold more IL-7Ra mRNA than IL-7Ra<sup>low</sup> cells (Fig. 1C). Also, the mRNA expression of IL-7Ra<sup>high</sup> CD8 T cells was comparable to that of memory CD8 T cells. This indicated that IL-7Ra mRNA levels directly correlate with surface protein levels and that, for the most part, IL-7Ra down-regulation during effector cell development is transcriptionally regulated as has been previously shown in activated T cells both in vivo and in vitro (12, 13).

Next, we determined whether the repression of IL-7Ra was associated with chromatin modifications such as deacetylation of histone amino-terminal tails. Acetylation of histone 3 at lysine residue 9 (H3K9) is one of the most common chromatin modifications associated with active gene expression and, therefore, it was analyzed by ChIP using Abs specific for acetylated histone H3K9 in naive, day-5, and day-8 effector P14 CD8 T cells. This ChIP assay revealed that the Il7ra promoter was hypacetylated in day-5 effector cells compared with naive T cells (Fig. 1D). Interestingly, acetylation of the promoter region was regained and perhaps somewhat increased in day-8 effector CD8 T cells. This result was primarily due to the increased acetylation preferentially found in the IL-7Ra<sup>high</sup> effector CD8 T cell subset, which had ~4–fold greater acetylated H3K9 than did the IL-7Ra<sup>low</sup> subset (Fig. 1D). Furthermore, hypacetylation of Il7ra promoter correlated with the recruitment of HDAC1 to the promoter, which was maximal at day 5 p.i. and remained elevated at day 8 p.i., albeit at a level significantly lower than that observed at day 5 (Fig. 1E). Lastly, to verify whether Il7ra repression could be mediated through HDAC1 recruitment via deacetylation of Il7ra locus, naive P14 CD8 T cells were stimulated with their cognate peptide (GP<sub>33–41</sub>) in the presence or absence of trichostatin A (TSA), an HDAC inhibitor (39), and analyzed for surface IL-7Ra expression 24 h later. This experiment showed that TSA inhibited peptide-induced Il7ra repression of activated (CD69<sup>+</sup>) CD8 T cells, confirming the involvement of HDACs in Il7ra repression (Fig. 1F). In summary, these data showed that CD8 T cells repress IL-7Ra as they expand and differentiate into effector CD8 T cells, and this is associated with the recruitment of HDAC1 and histone deacetylation of the promoter region.

**IL-7Ra down-regulation is accompanied with the loss of GABPα binding in vivo**

Expression of GABPα is necessary for IL-7Ra expression in lymphocytes, but it is unclear whether GABPα regulates IL-7Ra expression in effector and memory CD8 T cells. Therefore, we examined whether alterations in GABPα binding to the Il7ra promoter were associated with IL-7Ra mRNA down-regulation in the effector CD8 T cells at days 5 and 8 p.i. LCMV-specific P14 CD8 T cells were purified and the amount of GABPα bound to the Il7ra promoter was measured using ChIP with anti-GABPα Abs. There was no significant difference in GABPα binding at day 5 p.i. as compared with naive CD8 T cells (Fig. 2A), but surprisingly the amount of GABPα bound to the Il7ra promoter was ~2- to 3-fold higher in day-8 effector CD8 T cells compared with naive or day-5 effector cells. Further analysis showed that it was primarily the subset of IL-7Ra<sup>high</sup> effector CD8 T cells at day 8 p.i. that contained increased GABPα promotor binding. Strikingly, even more GABPα was associated with the Il7ra promoter in memory CD8 T cells isolated 40–60 days p.i. Together with the data in Fig. 1, these results suggest that the occupancy of GABPα on the Il7ra promoter was important for IL-7Ra expression in MPECs and correlated with increased histone acetylation (Fig. 1D). Moreover, this analysis showed that even though naive and IL-7Ra<sup>high</sup> effector and memory CD8 T cells express similar amounts of IL-7Ra protein and mRNA, the naive CD8 T cells achieve IL-7Ra expression with relatively less histone acetylation and GABPα binding. This may suggest that IL-7Ra<sup>high</sup> effector and memory CD8 T cells require additional posttranscriptional regulatory mechanisms or relatively higher levels of histone acetylation and GABP binding to achieve similar IL-7Ra expression as naive T cells.

**GABPα is necessary for increased IL-7Ra expression in MPECs**

To directly test the requirement of GABPα for increased IL-7Ra expression in IL-7Ra<sup>high</sup> MPECs, we used shRNAi to knock down GABPα in LCMV-specific P14 effector CD8 T cells, similar to that done previously (29). Naive P14 CD8 T cells were activated...
whether Gfi-1 functions in effector CD8 T cell differentiation during infection to repress IL-7Ra. To explore this possibility, we analyzed the expression of Gfi-1 mRNA in naive and effector CD8 T cells and found that, as previously reported (40, 41), Gfi-1 was not expressed in naive CD8 T cells. Early after T cell activation Gfi-1 mRNA was induced and maintained at relatively high levels throughout effector cell expansion and memory cell formation (data not shown). However, there was no significant difference in Gfi-1 mRNA levels in IL-7Ra<sup>high</sup> and IL-7Ra<sup>low</sup> effector CD8 T cells at day 8 p.i. (data not shown). Thus, Gfi-1 and IL-7Ra expression do not appear to be inversely correlated as one might have predicted.

To determine whether Gfi-1 bound to the Il7ra locus during effector CD8 T cell differentiation, we performed ChIP using anti-Gfi-1 Abs (that do not cross-react with Gfi-1b) and analyzed the binding of Gfi-1 within intron 2 of IL-7Ra as demonstrated previously (23) (see schematic in Fig. 1A). The level of Gfi-1 binding to Il7ra was nearly below detection in naive Gfi1<sup>−/−</sup> CD8 T cells and Gfi1<sup>−/−</sup> effector CD8 T cells, confirming both the qRT-PCR data in naive T cells and the specificity of the anti-Gfi-1 Ab used in these experiments. By day 5 p.i., the amount of Gfi-1 bound to the Il7ra locus increased (data not shown), and this correlated with the repression of Il7ra transcription and recruitment of HDAC1 (Fig. 1). Surprisingly, Gfi1 binding to Il7ra was lower in day-8 effector CD8 T cells even though IL-7Ra expression was significantly repressed in >75% of the cells at this time (data not shown). However, when day-5 and day-8 IL-7Ra<sup>high</sup> and IL-7Ra<sup>low</sup> CD8 T cells were separated and analyzed directly, Gfi-1 was preferentially bound to the Il7ra gene in the IL-7Ra<sup>low</sup> effector cells, demonstrating a strong correlation between Gfi-1 binding and IL-7Ra repression in virus-specific effector CD8 T cells. As the surviving IL-7Ra<sup>high</sup> MPECs matured into memory CD8 T cells, the amount of Gfi-1 bound to the Il7ra remained low even though the levels of Gfi-1 mRNA in these cells was comparable to those in day-8 effector CD8 T cells (Fig. 3A). These data suggested that Gfi-1 is predominantly recruited to the Il7ra locus in effector CD8 T cells that will maintain stable repression of Il7ra and subsequently decline in number following infection.

To determine whether the Gfi-1 binding to IL-7Ra was functionally relevant in effector CD8 T cells, the Gfi1<sup>−/−</sup> mice were crossed to the P14 TCR tg strain to obtain Gfi1<sup>−/−</sup> P14 CD8 T cells, and then small numbers of Gfi1<sup>−/+</sup> or Gfi1<sup>−/−</sup> P14 CD8 T cells were adoptively transferred into wild-type mice that were subsequently infected with LCMV. The levels of IL-7Ra protein and mRNA were examined on the donor cells at various times p.i. (Fig. 3B). Both naive Gfi1<sup>−/+</sup> and Gfi1<sup>−/−</sup> P14 CD8 T cells expressed similar amounts of IL-7Ra at the protein and mRNA (Fig. 3B, left plots). At day 5 p.i., IL-7Ra expression was substantially reduced in LCMV-specific Gfi1<sup>−/−</sup> cells compared with naive T cells, but not as profound as the reduction seen in Gfi1<sup>−/−</sup> CD8 T cells (Fig. 3B, middle plots). By day 8 p.i., a small subset (~15–25%) of IL-7Ra<sup>high</sup> effector CD8 T cells formed in the Gfi1<sup>−/+</sup> population as expected. In contrast, a substantially larger percentage (~70–80%) of IL-7Ra<sup>high</sup> effector CD8 T cells formed in Gfi1<sup>−/−</sup> effector CD8 T cells (Fig. 3B, right plots). The MFI of IL-7Ra in the two groups of effector CD8 T cells is shown (Fig. 3C, upper line graph). Accordingly, the amounts of IL-7Ra mRNA followed a similar trend and was slightly elevated in day-5 Gfi1<sup>−/−</sup> effector CD8 T cells compared with Gfi1<sup>−/+</sup> cells, but the day-8 Gfi1<sup>−/−</sup> effector CD8 T cells contained significantly more IL-7Ra mRNA (Fig. 3C, lower graph).

To determine whether the increased IL-7Ra expression in day-8 Gfi1<sup>−/−</sup> effector CD8 T cells correlated with decreased HDAC1 binding and/or increased histone acetylation, we performed ChIP

and 24 h later transduced with either a control RV or one that expressed shGapa. The RV-transduced cells could be identified by GFP expression. First, the efficiency of Gapa knock-down was verified by sorting on day-8 GFP<sup>+</sup> P14 effector CD8 T cells and Western blotting for Gapa; this showed very efficient knockdown because the GFP<sup>+</sup> CD8 T cells expressing shGapa contained little to no detectable Gapa protein (Fig. 2B). Next, the RV-transduced P14 CD8 T cells were adoptively transferred into LCMV-infected wild-type mice, and then a week later the donor GFP<sup>+</sup> cells were examined for IL-7Ra expression. As expected, ~15–25% of the day-8 GFP<sup>+</sup> P14 CD8 T cells transduced with control RV were IL-7Ra<sup>high</sup> (Fig. 2C, left plot). In contrast, the frequency of IL-7Ra<sup>high</sup> effector cells was substantially reduced in the GFP<sup>+</sup> P14 CD8 T cells transduced with shGapa RV (Fig. 2C, right plot). These results demonstrated that the increased binding of GAPPa to the IL-7Ra promoter in MPECs is functionally required for their ability to express IL-7Ra.

Gfi-1 regulates formation of IL7Ra<sup>low</sup> effector CD8 T cells

Next, we turned our attention to the transcription factor Gfi-1 because it has been shown to negatively regulate IL-7Ra expression in response to IL-7 signaling (23). However, it is not known
assays in Gfi1+/+ and Gfi1−/− P14 CD8 T cells. This analysis showed that significantly less (~7-fold) HDAC1 was bound to Il7ra in Gfi1−/− vs Gfi1+/+ day-8 LCMV-specific CD8 T cells (Fig. 3D), correlating with the increased IL-7Ra expression in Gfi1−/− CD8 T cells. Moreover, the abundance of acetylated H3K9 on the IL-7Ra promoter in Gfi1−/− P14 effector CD8 T cells was nearly twice that of the Gfi1+/+ cells (Fig. 3E). Together, these observations suggested that Gfi-1 was not absolutely required for the early IL-7Ra repression that occurs when viral infection was present (~day 5 p.i.) but may be needed subsequently to maintain stable IL-7Ra repression as antigen stimulation declines by preserving HDAC1 recruitment and hypoacetylation of the promoter in the majority of terminally differentiated effector cells.

Gfi-1 is required for stable repression of Il7ra in late effector CD8 T cells

The analysis above suggested that Gfi-1 was not sufficient for TCR-riven repression of IL-7Ra (day 5 p.i.) but was needed for stable silencing of the locus in the absence of antigenic signaling (day 8 p.i. and later). To examine this point more closely, naive Gfi1+/+ or Gfi1−/− P14 CD8 T cells were stimulated with peptide in vitro for 12–24 h. Similar to previous data (13, 25), IL-7Ra was rapidly down-regulated within 24 h in activated Gfi1−/− CD8 T cells (Fig. 4A), and Gfi-1 was recruited to the Il7ra locus (data not shown). Interestingly, the Gfi1−/− CD8 T cells exhibited a similar reduction in IL-7Ra expression in response to TCR activation.
(Fig. 4A). Together, these results corroborate those observed by Pargmann et al. (42) and suggest that even though Gfi-1 was recruited to Il7ra in activated T cells, a Gfi-1-independent mechanism largely operates downstream of TCR signaling early on to repress Il7r. This helps to explain the nearly normal decrease in IL-7Rα observed in Gfi1−/− LCMV-specific effector CD8 T cells at day 5 p.i. when the virus was still prevalent (Fig. 3, B and C).

Next, we tested whether the abnormally swift transition from IL-7Rαlow to IL-7Rαhigh found in Gfi1−/− effector CD8 T cells between days 5–8 p.i. was due to direct conversion (i.e., the inability to sustain Il7ra silencing in late effector Gfi1−/− CD8 T cells) or to the preferential death of IL-7Rαlow Gfi1−/− cells. At day 5 p.i., Gfi1+/+ and Gfi1−/− P14 CD8 T cells (which were congenically marked Thy1.1+) were sorted by FACS based on IL-7Rα expression and then equal numbers were adoptively transferred separately into congenic (Thy1.2) recipients that were infected 5 days previously (Fig. 4B). At day 8 p.i. (3 days later), the IL-7Rαlow donor cells were examined for IL-7Rα expression. As expected, the majority of the Gfi1+/+ effector CD8 T cells maintained repression of IL-7Rα over this time. In contrast, most of the donor Gfi1−/− effector CD8 T cells could not sustain IL-7Rα transcriptional repression and 70% of the cells converted to an IL-7Rαhigh state within 3 days (Fig. 4, B and C). This result was most certainly due to direct conversion from an IL-7Rαlow→IL-7Rαhigh state in the Gfi1−/− cells rather than selective survival on small numbers of contaminating IL-7Rαhigh donor cells at the time of transfer, because equal numbers of donor cells were recovered from both populations (Fig. 4D). These data indicated that at least two mechanisms of IL-7Rα repression exist in effector CD8 T cells: one form being driven by TCR signaling that does not require Gfi-1 and another that stably maintains Il7ra repression in the absence of antigenic signals and requires Gfi-1.

**Gfi-1 overexpression represses Il7ra by reducing GABPα binding to Il7ra**

To further investigate the role of Gfi-1 in IL-7Rα expression in effector CD8 T cells, Gfi-1 was overexpressed in LCMV-specific CD8 T cells using RV transduction. P14 CD8 T cells were activated and transduced with control or Gfi-1 RV and transferred into either LCMV-infected or naive recipients; the RV-transduced cells were marked by GFP expression. This experiment revealed that overexpression of Gfi-1 inhibited the development of a subset of IL-7Rαhigh effector cells during infection (Fig. 5A, right plot). Moreover, when P14 CD8 T cells were briefly stimulated with peptide for 24 h in vitro (under low inflammatory conditions), IL-7Rα repression was transient for the first 24–36 h and was then regained as the activated CD8 T cells continued to clonally expand (Fig. 4A and data not shown). Using this system, Gfi-1 RV overexpression was sufficient to repress IL-7Rα in nearly all of the activated CD8 T cells found 7 days later (Fig. 5B, right plot).

To assess the effects of Gfi-1 overexpression on histone acetylation of the Il7ra promoter, GFP+ (Gfi-1 RV) and GFP− (no RV) P14 CD8 T cells were sorted and the amount of acetylated H3K9 was measured using ChIP assays. This analysis showed that Gfi-1 overexpression resulted in profound hypoacetylation of the Il7ra promoter (Fig. 5C). These data, in combination with those shown

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**FIGURE 4.** Gfi-1 is required for stable repression of IL-7Rα in effector CD8 T cells during infection. A. IL-7Ra repression following in vitro peptide stimulation is Gfi-1 independent. Splenocytes containing Gfi1+/+ (upper panel) and Gfi1−/− (lower panel) P14 CD8 T cells were stimulated with GP33–41 peptide for ~12 h and IL-7Ra and CD69 expression was measured using flow cytometry. The plots are gated on total CD8 T cells and the percentage of unstimulated (CD69low) and stimulated (CD69high) cells that are IL-7Rαhigh is indicated. Data in plots are representative of four independent experiments. B and C. Gfi1−/− effector CD8 T cells cannot maintain stable repression of IL-7Ra as infection wanes. On day 5 p.i., Gfi1+/+ (upper panels) or Gfi1−/− (lower panels) P14 CD8 T cells were purified based on IL-7Rαlow staining by FACS and transferred separately into day-5 LCMV-infected recipients (left plots in B show sort purity). B. Three days later (day 8 p.i.) donor P14 CD8 T cells in the spleen were analyzed for IL-7Ra expression by flow cytometry (right plots). Contour plots are gated on P14 CD8 T cells and the percentage of IL-7Rαhigh cells is indicated. C. Line graph shows the percentage of IL-7Rαhigh Gfi1+/+ (filled squares) and Gfi1−/− (open circles) donor effector P14 CD8 T cells in the blood each day after transfer. Data are mean ± SEM (n = 4). D. Bar graph shows absolute numbers of donor Gfi1+/+ or Gfi1−/− CD8 T cells recovered from spleen 3 days after transfer.
followed by qRT-PCR. In IL-7R

Discussion
This study was designed to understand the molecular mecha-
nism(s) that control IL-7Rα expression in CD8 T cell with expan-
sion and differentiation of IL-7Rαhigh MPECs and IL-7Rαlow
SLECs in response to an acute viral infection. We found that IL-
7Rα expression is regulated in these two effector cell populations
by a balance between the transcriptional repressor Gfi-1 and the
transcriptional activator GABPα. In this article, our results suggest
that the IL-7Rα locus exists in multiple states as naïve CD8 T cells
differentiate into MPECs and SLECs. In the first state, in resting
naïve CD8 T cells Gfi-1 is not expressed, IL-7Rα is transcriptionally
active, and the promoter is acetylated with moderate levels of
GABPα binding. Upon activation, TCR signaling causes rapid
down-regulation of IL-7Rα expression, and this is correlated with
hydroacetylation of the promoter region and recruitment of
HDAC1. Gfi-1 is induced early in activated T cells and recruited
to the IL-7Rα promoter. To ex-
amine this hypothesis, we measured the amounts of GABPα bound
to IL-7Rα promoter using ChIP when Gfi-1 was absent (i.e., Gfi1−/−
effector CD8 T cells) or overexpressed by RV transduction. This
demonstrates that Gfi-1 is necessary and sufficient (when
overexpressed) to maintain repression of

CD8 T cells (Fig. 5E), indicating that overexpression of Gfi-1 re-
duces GABPα binding to IL-7Rα promoter.

in Fig. 3, demonstrate that Gfi-1 is necessary and sufficient (when
overexpressed) to maintain repression of IL-7Rα, perhaps via histone
H3 deacetylation, during effector CD8 T cell differentiation.

The above data suggested that GABPα and Gfi-1 have opposing
effects on IL-7Rα transcription; GABPα binding enhances promoter
acetylation and transcription whereas Gfi-1 promotes HDAC bind-
ing, promoter deacetylation, and transcriptional silencing. A pos-
sibility could be that overexpression of Gfi-1 inhibits binding of
the transcriptional activator GABPα to the IL-7Rα promoter. To ex-
amine this question, we measured the amounts of GABPα bound
to IL-7Rα promoter using ChIP when Gfi-1 was absent (i.e., Gfi1−/−
effector CD8 T cells) or overexpressed by RV transduction. This
showed that the amount of GABPα bound to the IL-7Rα promoter
in day-8 Gfi1−/− effector CD8 T cells was ∼1.5-2-fold greater than
in Gfi1+/- cells (Fig. 5D). This result suggested that GABPα func-
tion is important for the increased IL-7Rα expression in Gfi1−/−
effector CD8 T cells. Next, the day-8 effector P14 CD8 T cells
overexpressing Gfi-1 were sorted and GFP+ (Gfi-1 RV) and
GFP− (no RV) cells were compared for GABPα expression and
binding. qRT-PCR confirmed that there was no difference in
GABPα mRNA levels (data not shown) in GFP+ and GFP− P14
CD8 T cells, showing that Gfi-1 overexpression did not affect
GABPα expression (data not shown). However, ChIP analysis of
GABPα revealed that GFP+ P14 CD8 T cells had ∼20.5-fold less
GABPα bound to their IL-7Rα locus compared with GFP− P14

High effector CD8 T cells in GFP− P14 CD8 T cells were transduced with Gfi-1 RV (GFP+) or not (GFP−) were purified using FACS and analyzed for GABPα binding to the IL7ra promoter in effector CD8 T cells. Day-8 P14 effector CD8 T cells either transduced with Gfi-1 RV (GFP+) or not (GFP−) were purified using FACS and analyzed for acetylated H3K9 histones using ChIP
followed by qRT-PCR. Effector CD8 T cell samples were compared with naive CD8 T cells set at 1-fold. The data in bar graphs show the mean ± SEM (n = 3; *, p < 0.05). D and E, Gfi-1 inhibits GABPα binding to the IL7ra promoter in effector CD8 T cells. Day-8 P14 effector CD8 T cells in which Gfi-1 was absent (Gfi1−/-) (D) or overexpressed (Gfi-1 RV) (E) were purified using FACS and analyzed for GABPα binding on the IL7ra promoter using ChIP followed by qRT-PCR. In D, GABPα binding to the IL7ra promoter was compared between Gfi1+/- and Gfi1−/- effector P14 CD8 T cells, and in E GABPα binding was compared between Gfi-1 RV transduced with GFP− (GFP+) or not (GFP−). D and E. The amount of GABPα binding in P14 effector CD8 T cells was normalized to naive CD8 T cells. The data in bar graphs show the mean ± SEM (n = 3; *, p < 0.05).
IL-7Rα and are considered terminally differentiated because they have lost the ability to become long-lived memory CD8 T cells. The reduced IL-7Rα expression in this subset was associated with increased HDAC1 recruitment and decreased GABPα binding and histone acetylation of the promoter. Gfi-1 preferentially bound to the Il7ra locus and was required for stable Il7ra repression in these cells. When Gfi-1 was overexpressed, the occupancy of GABPα and promoter acetylation were reduced even further, suggesting that in addition to recruiting HDACs, Gfi-1 also functions to repress Il7ra by inhibiting recruitment of a requisite transcriptional activator, GABPα.

The Il7ra locus existed in the opposite state in the smaller population of IL-7Rαhigh MPECs, where GABPα binding to and histone acetylation of the promoter was abundant and Gfi-1 binding to intron 2 was significantly decreased. Furthermore, GABPα was functionally required to sustain IL-7Rα expression in these IL-7Rαhigh MPECs. Because IL-7Rα expression is functionally required for generation memory CD8 T cells this result denotes a critical role for GABPα in memory CD8 T cell development and maintenance.

These data offer a model for how the "on" or "off" state of Il7ra is mediated by the binding of opposing transcriptional activators and repressors and concurrent epigenetic remodeling of the Il7ra promoter. Interestingly, the primary determinant of Il7ra transcriptional regulation was not necessarily the differential expression of GABPα or Gfi-1 in IL-7Rαhigh or IL-7Rαlow effector CD8 T cells, but rather it was the preferential recruitment of GABPα or Gfi-1 to the Il7ra locus in these respective T cell subsets. How is this reciprocal binding pattern of Gfi-1 and GABPα set up and maintained in the effector CD8 T cells? GABPα is a target of phosphorylation by certain kinases such as ERK and JNK (43) (44) and, perhaps, differential activity of these kinases in IL-7Rαhigh and IL-7Rαlow effector CD8 T cells contributes to Il7ra gene expression. Another possibility is that other signals (aside from TCR activation) stabilize Gfi-1 binding in the majority of effector CD8 T cells and that this directly impairs GABPα docking to the promoter. Evidence for this idea is provided by prior work showing that Gfi-1 can antagonize the transcriptional activation of Ets-family members, ETS-1 and PU.1, by direct binding (45, 46). Another possibility is that the transcription factor T-bet (Tbx21) cooperates with Gfi-1 to antagonize GABPα activity in effector CD8 T cells. Our recent work has shown that certain inflammatory signals during infection, such as IL-12, directly increase T-bet expression in effector CD8 T cells and that this induces development of IL-7Rαlow SLEC (20). Like Gfi-1 RV overexpression, T-bet RV overexpression represses IL-7Rα expression in effector CD8 T cells (20, 47). Therefore, it is possible that increased T-bet stabilizes Gfi-1 recruitment to the Il7ra locus in effector CD8 T cells that were exposed to high levels of IL-12, but this question remains to be tested our recent data supports this hypothesis that T-bet might be instructive to Gfi-1 function (data not shown).

As might be expected for a protein whose expression is critical to naive and memory T cell generation and maintenance, IL-7Rα expression appears to be tightly regulated by multiple signals and mechanisms. TCR activation leads to rapid IL-7Rα down-regulation, but this TCR-directed repression is transient and the mechanism by which this occurs is not known. We show here that TCR-dependent inhibition of IL-7Rα expression is largely independent of Gfi-1 both in vitro and in vivo (when viral Ags are present), and this is similar to another recent report (42). Moreover, this process is also T-bet independent (data not shown). However, we found that Gfi-1 was necessary to sustain a stable repressed state in late effector CD8 T cells (~day 7 onwards) when viral infection and antigenic stimulation were ceasing. Thus, at least two mechanisms, which occur over two phases during infection, exist that inhibit IL-7Rα expression in activated CD8 T cells during viral infection. One is transient repression mediated by TCR signaling and the other is stable repression that occurs primarily in terminally differentiated effector CD8 T cells and requires Gfi-1.

Our analysis showed that Il7ra repression in activated CD8 T cells was also associated with characteristic epigenetic chromatin modifications, namely histone (H3K9) deacetylation via HDAC1. A previous report showed that increased CpG DNA methylation in the promoter of IL-7Rαlow CD8 T cells was likely important for maintenance of the IL-7Rαlow state in resting effector memory T cells (48). However, DNA methylation did not seem to be involved in the rapid TCR- or IL-7-directed repression of Il7ra (48). Thus, the epigenetic control of Il7ra expression appears to involve multiple layers to ensure tight yet long-term regulation of either high and low IL-7Rα expression levels in T cells.

Some γc chain cytokines and steroids have also been found to regulate IL-7Rα expression (13, 23, 24, 26). IL-7 and several other γc chain cytokines induce rapid IL-7Rα down-regulation that is transient in nature because the T cells quickly regain IL-7Rα expression following removal from high-dose cytokine exposure (23, 24). It was hypothesized that this temporary form of IL-7Rα down-regulation was an altruistic way by which T cells could decrease competition for the limiting amounts of IL-7 in the periphery (23). This mode of repression contrasts with the more "permanent" form of IL-7Rα repression found during effector CD8 T cell development (12, 20). However, in CD8 T cells, but not CD4 T cells, this cytokine-dependent silencing is Gfi-1 dependent (23). Glucocorticoids provide another signal that up-regulate IL-7Rα expression via binding of the glucocorticoid receptor to a glucocorticoid receptor binding site in a small evolutionary conserved region (ECR) ~3.6kb upstream of the Il7ra promoter (13, 26). Using rVista 2.0 analysis (49), multiple other transcription factor binding sites (such as Foxo1/3/4, NF-κB, Gata6, Pax3, and Stat1/4) are predicted in this region and serve as additional candidates to regulate IL-7Rα expression. In addition to this ECR, at least two others can be found using this analysis 6 kb upstream of the Il7ra promoter, but the relevance of these ECRs and the potential transcription factors that bind to these regions remains to be determined.

Our data shed light on the biochemical processes by which effector CD8 T cells gain or lose memory CD8 T cell potential. Currently, very low amounts of surface IL-7Rα is one of the best markers for terminally differentiated and/or short-lived virus-specific CD8 T cells during infections (12, 14, 16). Therefore, understanding how the repression of IL-7Rα is initiated and sustained in anti-viral CD8 T cells is key to understanding how this differentiated state is acquired. In addition to serving as a marker, IL-7Rα down-regulation was initially predicted to be the underlying cause of death of the majority of the effector CD8 T cells after infection, because IL-7 deprivation causes T cell apoptosis (1, 7). However, our recent data and those of others have shown that the bulk of effector T cell contraction that occurs following infection is IL-7 independent (21, 50, 51). When IL-7Rα expression was enforced on all virus-specific effector CD8 T cells using an Il7ra transgene, this did not rescue the preferential death of the naturally arising IL-7Rαlow effector CD8 T cell subset (21). Other work showed that this IL-7Rαlow subset is mainly dependent on IL-15 for survival; however, IL-15 does not suffice to maintain the IL-7Rαlow CD8 T cells long term (20). In contrast, the IL-7Rαhigh MPECs functionally require IL-7Rα to persist and develop into long-lived memory CD8 T cells (12, 17, 52). Therefore, elucidating that GABPα is required for expression of IL-7Rα in these cells is an important finding for memory CD8 T cell development.
Numerous target genes have been identified for both GABPa and Gfi-1 (30, 53–55) and, therefore, it is important to consider the additional effects, aside from abnormal IL-7Rα expression, that result in GABPa- and Gfi-1-deficient effector CD8 T cells. Interestingly, in addition to IL-7Rα, a few other genes such as IL-2, TNF-α, and ELA2 are predicted to contain binding sites for both Gfi-1 and GABPa, and it is possible that these two factors play opposing roles on their gene expression (54, 56–58). It is noteworthy that GABPa also controls the expression of several genes involved in mitochondrial function, cellular energy metabolism, and cell cycle progression (30, 59). It is possible that the coregulated expression of these genes with Il7ra (via GABPa) in MPECs is vital to their cellular fitness and ability to become long-lived memory CD8 T cells with a high proliferative potential.

A long-term goal is to elucidate the transcriptional network that controls effector and memory CD8 T cell differentiation and to identify key transcriptional regulators. Beginning with a gene vital to memory CD8 T cell development and survival, IL-7Rα, this work provides inroads into this extremely complex problem. In the future it will be of value to find other genes that are coregulated with IL-7Rα, identify their transcriptional regulators, and identify whether these represent a cassette of genes critical to memory CD8 T cell formation.

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References

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