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Ets-1 Maintains IL-7 Receptor Expression in Peripheral T Cells

Roland Grenningloh,*† Tzong-Shyuan Tai,*, Nicole Frahm,‡±,1 Tomoyuki C. Hongo,‡ Adam T. Chicoine,‡ Christian Brander,†‡±,5 Daniel E. Kaufmann,†‡± and I-Cheng Ho*,†

The expression of CD127, the IL-7–binding subunit of the IL-7 R, is tightly regulated during the development and activation of T cells and is reduced during chronic viral infection. However, the molecular mechanism regulating the dynamic expression of CD127 is still poorly understood. In this study, we report that the transcription factor Ets-1 is required for maintaining the expression of CD127 in murine peripheral T cells. Ets-1 binds to and activates the CD127 promoter, and its absence leads to reduced CD127 expression, attenuated IL-7 signaling, and impaired IL-7–dependent homeostatic proliferation of T cells. The expression of CD127 and Ets-1 is strongly correlated in human T cells. Both CD127 and Ets-1 expression are decreased in CD8+ T cells during HIV infection. In addition, HIV-associated loss of CD127 is only observed in Ets-1low effector memory and central memory but not in Ets-1high naive CD8+ T cells. Taken together, our data identify Ets-1 as a critical regulator of CD127 expression in T cells. The Journal of Immunology, 2011, 186: 969–976.

 interleukin-7 signals are required for T cell development, maintaining the naive T cell pool, mounting proper primary responses, and inducing and maintaining CD4+ and CD8+ T cell memory (1–3). The IL-7R consists of the IL-7Rα–chain (CD127), which binds IL-7, and the common γ-chain (CD132). CD127 expression is dynamically regulated throughout T cell development, activation, and memory formation (2, 4). Many extracellular stimuli can modulate the expression of CD127. For example, TCR signals (5), IL-7 (6), and the HIV Tat protein (7) have been shown to inhibit the expression of CD127. However, the mechanisms governing this dynamic regulation of CD127 are poorly understood. The constitutive expression of CD127 is regulated by members of the Ets family of transcription factors in different lymphoid lineages. Ets proteins are characterized by their DNA-binding Ets domain (8). The B cell-specific PU.1 is essential for the expression of CD127 in lin− hematopoietic progenitors and pro-B cells (9), whereas GABPα is required for the expression of CD127 in early thymocytes (10). Both factors bind to a functionally critical Ets binding site in the CD127 promoter. But whether GABPα or another Ets protein is responsible for maintaining CD127 expression in peripheral T cells is unknown.

Ets-1 (E26 transformation-specific sequence) is the founding member of the Ets family of transcription factors and is expressed at high levels in lymphoid cells (8, 11). Ets-1 has been shown to promote Th1 and inhibit Th17 differentiation (12, 13). Ets-1 is also recruited to the IL-5/IL-13/IL-4 locus and required for the optimal expression of these cytokines (14). T cells express two splice variants, the full-length Ets-1 p51 and the shorter Ets-1 p42 that lacks exon VII (15, 16). The activity of Ets-1 is regulated by activating and inactivating phosphorylation events (11), but the role of these phosphorylation events in regulating the function of Th cells remains controversial (17).

In this study, we show that Ets-1 directly binds to and activates the CD127 promoter. Ets-1-deficient (KO) T cells expressed reduced levels of CD127 and displayed impaired IL-7–dependent survival and homeostatic proliferation. Importantly, the level of CD127 in human T cells strongly correlates with that of Ets-1. Loss of CD127 expression is a hallmark of CD8+ T cells during chronic viral infection (18, 19). The expression of Ets-1 is also reduced in CD8+ T cells of HIV-positive individuals mainly due to an expansion of effector/effector memory cells. Notably, HIV-associated reduction in CD127 occurs only in Ets-1low effector memory and central memory cells but not in Ets-1high naive cells. Thus, our data demonstrate that Ets-1 is a critical regulator of CD127 in peripheral T cells.

Materials and Methods

Study subjects

The study included 10 individuals chronically infected with HIV. Four of these subjects were treated with antiviral therapy, and six were untreated. All but one had plasma viral loads above the limit of detection of the quantitative assays used for clinical monitoring (>50 or >75 viral mRNA copies/ml). Median viral load was 21,200 viral mRNA copies/ml (range, 75 to 233,000). Additionally, 10 HIV-uninfected healthy volunteers were included as controls. Human subject protocols were approved by all participating hospitals and clinics, and all subjects provided written informed consent prior to enrollment.

Mice

Ets-1–deficient mice were described previously (20). Mice were backcrossed to the C57BL/6 background for six generations and maintained...
by mating Ets-1−/− male to Ets-1−/− female mice. Ets-1−/− (Ets-1 wild-type [WT]; designated WT), Ets-1−/+/− (Ets-1 heterozygous; designated HET) and Ets-1−/−/− (KO) littersmates were used throughout the study. Congenic CD45.1 C57BL/6 and CD45.1 Rag2−/− mice were purchased from Ta
conic (Hudson, NY). The animals were housed under specific pathogen-
free conditions, and all experiments were carried out in accordance with
the institutional guidelines for animal care at the Dana-Farber Cancer In-
stitute (Boston, MA).

Cell lysates, Western blot, and Abs

Total cell lysates and nuclear or cytosolic extracts were adjusted for total
protein content and subjected to SDS-PAGE followed by Western blot. The
following Abs were used: anti-STAT5 and anti–phospho-STAT5 (Tyr694)
(Cell Signaling Technology, Danvers, MA) and anti-Hsp90 (Santa Cruz
Biotechnology, Santa Cruz, CA). As secondary Abs, HRP-coupled goat-
anti–rabbit IgG was used (Zymed Laboratories). Proteins were visualized
using an ECL kit (PerkinElmer).

Cell isolation and culture, reagents, flow cytometry

Mouse T cells were isolated from lymph nodes and spleen using magnetic
cell sorting as described (12). For staining of human CD127 expression,
the FACSAria (BD Bio-
technology, Boston, MA).

Bone marrow transduction

Five days before bone marrow harvest, HET or KO donor mice were
injected i.p. with 100 µl 50 mg/ml 5-fluorouracil (Sigma). Bone marrow
cells were isolated, and SCA-1–positive cells were enriched using
magnetic cell sorting. The cells were cultured in media containing 20 ng/
ml 5-fluorouracil (Sigma). Bone marrow

Luciferase assays and chromatin immunoprecipitation assay

Luciferase assays were performed as described (17). The CD127 promoter
construct pGL3-IL-7Rpr, the Ets-1 p51 expression vector pcDNA-Ets-1
p51, and the Runx1 expression vector pcDNA-MyrRunx1 have been de-
cribed elsewhere (12, 21, 22). Chromatin immunoprecipitation (ChIP)
assays were performed as described using in vitro differentiated Th1 cells
(12). Precipitated DNA fragments were amplified by quantitative PCR.
The Abs used for immunoprecipitation were anti–Ets-1 C-20 and control
rabbit IgG (both Santa Cruz Biotechnology). Specific binding was calcu-
lated as 2−(CtEts1 / CtCtrl) . The following primer pairs were used: CD127 pro-
moter Ets binding site, 5′-ACCACGACAGGGAATCTG-3′ and 5′-CAC-
ACTCTACCTCCTGTTT-3′; IFN-γ promoter, 5′-TTTCAAGGAATCTC-
CAAG-3′ and 5′-TTTGAAGTGGTACAGTGGG-3′; TLR7, 5′-TC-
TAGATTTTTGTTGTTGCG-3′ and 5′-TCTGTCTAAATGCTGCTTG-3′.

Quantitative RNA analysis

RNA isolation, reverse transcription, and real-time PCR were performed as
described (13). The following primer pairs were used: mouse CD127, 5′-
GGGACGATCACTCCTTCTG-3′ and 5′-AGCCCCCATATTGTGAAAT-
TTCCA-3′; human CD127, 5′-CCCTCCTGGAAGTAAAGTG-3′ and
5′-CCCTCCCAGAGACGACACTC-3′; human/mouse Ets-1 p51, 5′-CT-
CATTGACGGCTTACT-3′ and 5′-ATTCTCTGTGACTGAAAT-3′;
human β2-microglobulin (β2m), 5′-GTCGCGCTTACTGCTGTTCG-3′
and 5′-ACCTGAATGTTGAGATCCCT-3′; human Actin, 5′-GTGACAG-
CAGTCGGTGGAGG-3′ and 5′-AGGACTGGCAGCTTCTCTC-3′; mouse
Actin, 5′-GGGCGATTCCCCCTCAGG-3′ and 5′-CCAGTGGTAAAC-
AATGCGATG-3′.

Statistical analysis

Statistical analysis was done using Prism Software (GraphPad, La Jolla,
CA). Paired or unpaired t tests were used as indicated in the figure legends
of this article, and p < 0.05 was considered significant.

Results

Ets-1 is required for constitutive CD127 expression in murine peripheral T cells

PU.1 and GABPs have been shown to regulate the expression of CD127 in pro-B cells and early thymocytes, respectively (9, 10).
Because Ets-1 is expressed at high levels in thymocytes and ma-
ture T cells, we hypothesized that Ets-1 might participate in reg-
ulating the expression of CD127. We first analyzed the expression
of CD127 at different stages of thymocyte development in KO mice.
In agreement with a previous publication (23), we found that
KO thymi contained a higher percentage of double-negative (DN)
cells but fewer mature CD8 single-positive (SP) cells (Fig. 1A, 1B) than HET thymi. The expression of CD127 was reduced at the DN1 stage but normal in DN2 and DN3 cells in KO mice (Fig. 1A). CD127 is normally downregulated in the DN4 and double-positive (DP) stage and reappears in mature (TCR\(^{hi}\) CD24\(^{lo}\)) SP cells. However, both KO CD4 and CD8 SP thymocytes displayed significantly reduced CD127 expression (Fig. 1B, 1C) compared with that of HET cells, which expressed a normal level of Ets-1 and, expectedly, CD127 (Supplemental Fig. 1).

We then analyzed CD127 expression in peripheral T cells. Fig. 2A shows the gating strategy used to identify naive (CD44\(^{lo}\) CD62L\(^{hi}\)), effector memory (EM; CD44\(^{hi}\)CD62L\(^{lo}\)), and central memory (CM; CD44\(^{hi}\)CD62L\(^{hi}\)) T cells. As described by others, we observed an increased percentage of CD4\(^{+}\) and CD8\(^{+}\) EM cells in KO mice (23) (Fig. 2A). The expression of CD127 was significantly reduced in naive and CM T cells from KO animals. EM CD4\(^{+}\) and CD8\(^{+}\) T cells expressed a relatively low level of CD127, and there was no difference between Ets-1 HET and KO EM T cells (Fig. 2B, 2C). Thus, Ets-1 is required for constitutive expression of CD127 in DN1 and SP thymocytes, naive and CM T cells, but not DN2 or DN3 thymocytes. The impaired expression of surface CD127 in naive Ets-1KO T cells correlated with a significant reduction in the level of CD127 mRNA (Fig. 2D), suggesting that Ets-1 regulates the expression of CD127 at the transcriptional level.

**Ets-1 binds to and activates the CD127 promoter**

The CD127 promoter contains a conserved Ets binding site that is critical for activation by PU.1 or GABPa (Fig. 3A) (10, 22). We therefore speculated that Ets-1 might bind to this site in mature peripheral T cells. Fig. 2A shows the gating strategy used to identify naive (CD44\(^{lo}\) CD62L\(^{hi}\)), effector memory (EM; CD44\(^{hi}\)CD62L\(^{lo}\)), and central memory (CM; CD44\(^{hi}\)CD62L\(^{hi}\)) T cells. As described by others, we observed an increased percentage of CD4\(^{+}\) and CD8\(^{+}\) EM cells in KO mice (23) (Fig. 2A). The expression of CD127 was significantly reduced in naive and CM T cells from KO animals. EM CD4\(^{+}\) and CD8\(^{+}\) T cells expressed a relatively low level of CD127, and there was no difference between Ets-1 HET and KO EM T cells (Fig. 2B, 2C). Thus, Ets-1 is required for constitutive expression of CD127 in DN1 and SP thymocytes, naive and CM T cells, but not DN2 or DN3 thymocytes. The impaired expression of surface CD127 in naive Ets-1KO T cells correlated with a significant reduction in the level of CD127 mRNA (Fig. 2D), suggesting that Ets-1 regulates the expression of CD127 at the transcriptional level.

**Ets-1 binds to the CD127 promoter and drives CD127 expression.** A. Schematic diagram of the CD127 promoter. The locations of the conserved Ets and Runx1 binding are marked. Arrows indicate the location of primers used for the detection of Ets-1 binding by ChIP TSS, transcriptional start site. B. Binding of Ets-1 to the CD127 and IFN-\(\gamma\) promoters and to the TLR7 gene in CD4\(^{+}\) T cells. Binding of Ets-1 to the indicated genes was determined by ChIP, and binding strength was calculated as enrichment over control IgG. Mean values and error bars from three independent experiments are shown. C. Activation of the CD127 promoter by Ets-1, Runx1, and PU-1 in the human embryonic kidney cell line 293T. 293T cells were transfected with expression plasmids encoding the indicated transcription factors and a reporter construct containing the minimal CD127 promoter shown in A. After 24 h, luminescence was measured. Luminescence was normalized to empty expression vector (pcDNA). Assays were done in triplicate, and means and SEs are shown. D and E. Retroviral expression of Ets-1 p51 restores CD127 expression in peripheral naive T cells. Bone marrow cells from HET or KO mice were isolated, enriched for SCA-1–positive cells, and infected with the indicated retroviruses. The cells were injected into Rag2\(^{-/-}\) mice to allow reconstitution of the lymphoid system, and CD127 expression on naive CD4\(^{+}\) or CD8\(^{+}\) T cells was analyzed 8 wk later. D. Histograms from CD127 staining from one representative mouse are shown. Black lines, CD127; gray lines, isotype control. E. Combined data from three individual mice. An unpaired t test was used to identify significantly different CD127 expression: *p < 0.05.
T cells and directly regulate the transcription of CD127. By ChIP assay, we found that Ets-1 indeed bound to the endogenous CD127 promoter with an affinity even higher than its binding to the IFN-γ promoter, a known target of Ets-1 (Fig. 3B) (12). In contrast, there was no specific binding of Ets-1 to the TLR7 gene, which served as a negative control. In addition, Ets-1 activated a reporter construct containing the 200-bp minimal promoter of mouse CD127, including the Ets site (22) (Fig. 3C). The p42 isoform of Ets-1, which lacks part of the inhibitory domain, was slightly more efficient than the full-length p51 isoform. The degree of activation was comparable with that induced by PU.1, which we used as a positive control (22). The CD127 promoter contains a conserved Runx site next to the Ets site (22) (Fig. 3A). Runx1 is required for CD127 expression in CD4+ T cells (24) and can physically interact with Ets-1 (25). Although Runx1 also transactivated the CD127 promoter, coexpression of Runx1 and Ets-1 only led to additive, but not synergistic, activation. Next, we isolated bone marrow cells from HET or KO mice and transduced the cells in vitro with a control retrovirus expressing only GFP (GFP-RV) or a virus expressing GFP along with Ets-1 p51 (RV-Ets1 p51). We have previously shown that such retroviral transduction can restore the expression of Ets-1 to a near physiological level (17).

The infected bone marrow cells were then transferred to Rag2 knockout (Rag2<sup>−/−</sup>) mice. Peripheral T cells were harvested from the host animals 8 wk later and analyzed. As shown in Fig. 3D and 3E, retrovirally expressed Ets-1 efficiently restored CD127 levels in both peripheral naive CD4<sup>+</sup> and CD8<sup>+</sup> T cells.

**FIGURE 4.** Impaired STAT5 phosphorylation and survival of Ets-1–deficient (KO) T cells in response to IL-7. A. Reduced STAT5 phosphorylation in naive KO CD4<sup>+</sup> T cells in response to IL-7. Naive CD4<sup>+</sup> T cells were sorted based on CD4<sup>+</sup>CD62L<sup>hi</sup> expression and either left untreated (Media) or stimulated with 10 ng/ml IL-7 for 20 min. Total STAT5 levels and phosphorylation of Tyr694 (P-STAT5) were examined by Western analysis. Hsp90 expression was determined to ensure equal loading. B. Naive CD4<sup>+</sup> T cells from congenic CD45.1 C57BL/6 (WT) or KO mice were sorted based on CD45RB<sup>hi</sup> expression. The cells were mixed at a 1:1 ratio (left panel), labeled with CFSE, and CD127 expression was analyzed (black, CD127; gray, isotype control). C. Decreased survival of KO CD4<sup>+</sup> T cells in response to IL-7. The WT (CD45.2<sup>+</sup>) and KO (CD45.2<sup>+</sup>) CD4<sup>+</sup> T cells mixture from B was cultured in media alone or in media containing 10 ng/ml IL-7. After 72 h, the cells were stained for CD45.2, and the percentage of live cells was determined by flow cytometry based on their position in the forward/sideward scatter (left panels). The WT and KO cells within the live populations were further separated according to the expression of CD45.2 (right panels). D. The percentage of WT and KO live cells within the total population was calculated from C and is shown.

**FIGURE 5.** Impaired expansion of Ets-1–deficient T cells in a lymphopenic host. A. A mixture of naive, CFSE-labeled WT (CD45.2<sup>+</sup>), and KO (CD45.2<sup>+</sup>) CD4<sup>+</sup> T cells (at an ~1:1 ratio) was injected intravenously into Rag2<sup>−/−</sup> mice (1 million cells/mouse). At day 7 and 29, inguinal, axillary, and mandibular lymph nodes were isolated. Donor T cells (CD4<sup>+</sup>/TCRβ<sup>+</sup>) were analyzed for the expression of CD44 and content of CFSE (left panels). WT and KO donor cells were distinguished according to the expression of CD45.2 (middle panels). The CFSE<sup>−</sup> and CFSE<sup>+</sup> populations represent cells undergoing endogenous and homeostatic proliferation, respectively. The percentage of CFSE<sup>−</sup> and CFSE<sup>+</sup> WT or KO T cells from one representative mouse is shown in the middle panels. The means and SEs from three mice are shown in the right panels. B and C. The indicated populations were further analyzed for the expression of CD127 on day 7 and day 29. Data from one representative mouse are shown in B. Combined mean values and SD from three mice are shown in C. An unpaired t test was used to detect significant differences between WT and KO: *p < 0.05. D. The cell numbers for each indicated donor population recovered at different time points after transfer were calculated by normalizing the percentage of T cells against the percentage of host NK cells. Mean values and SEs from three individual mice are shown.
Impaired IL-7 signaling and survival of KO T cells

Binding of IL-7 to CD127 leads to phosphorylation of STAT5 and is required for survival and homeostatic proliferation of peripheral T cells. To examine whether the impaired CD127 expression had any impact on these IL-7–induced downstream events in KO T cells, we first tested the ability of KO T cells to phosphorylate STAT5 in response to IL-7. We sorted naive CD4⁺ T cells from HET or KO mice, stimulated the cells with 10 ng/ml IL-7 for 20 min, and analyzed the total protein levels for STAT5 as well as phosphorylation of STAT5 Tyr694 by Western blot. Unexpectedly, we found that KO T cells expressed a higher than normal level of total STAT5. Whereas treatment with IL-7 readily induced Tyr694 phosphorylation in HET naïve CD4⁺ T cells, very little phosphorylated STAT5 was detected in KO T cells despite the increase in the level of total STAT5 (Fig. 4A). We have shown earlier that KO Th cells, once the expression of CD25 is induced, are fully capable of phosphorylating STAT5 in response to IL-2 (13), indicating that the downstream machinery for phosphorylating STAT5 is intact in these cells.

We then examined whether the impaired IL-7 signaling would affect IL-7–dependent survival of KO T cells. To this aim, we set up cocultures of sorted naive CD4⁺ T cells from KO mice or congenic WT C57BL/6 mice expressing CD45.1. Naive T cells were sorted based on high CD45RB expression. WT and KO cells were mixed and labeled with CFSE. Fig. 4B shows the expression of CD127 on WT and KO cells in the starting coculture. The cells were then either cultured in media alone or in media containing 10 ng/ml IL-7 for 72 h. As shown in Fig. 4C, only ~4% of the unstimulated cells fell in the live gate after 72 h of culture, and the ratio between KO and WT cells remained largely unchanged. More live cells (~30%) were detected in the culture containing IL-7. But the ratio between KO and WT cells within the live population was significantly reduced. Thus, whereas IL-7 substan
tially increased the percentage of live HET CD4⁺ T cells, it only had a minimal effect on KO CD4⁺ T cells (Fig. 4D). The change in the ratio was not due to a difference in proliferation between WT and KO T cells because the content of CFSE remained unaltered even in the presence of IL-7 (Fig. 4C, right panels).

It has been shown that naive T cells transferred into lymphopenic hosts will undergo both endogenous and homeostatic proliferation. Endogenous proliferation occurs rapidly and is dependent on TCR signals and possibly IL-6. In contrast, homeostatic proliferation has slower kinetics and requires IL-7 (26, 27). Accordingly, reduced CD127 expression should impair homeostatic, but not endogenous, proliferation of KO T cells. We therefore transferred a mixture of CFSE-labeled naive WT and KO CD4⁺ T cells into Rag2 knockout (Rag2−/−) mice and analyzed cell numbers and CFSE dilution after 7 and 29 d. As described by others (26), by day 7 the transferred T cells had split into two populations undergoing endogenous (fast) and homeostatic (slow) proliferation and expressing high and low CD44 levels, respectively (Fig. 5A, left upper panel). The population dividing faster had completed at least seven divisions and become CFSE-negative (CFSE−). It was made up of KO and WT cells at a near 1:1 ratio (Fig. 5A, middle upper and right upper panels). Thus, the endogenous proliferation of KO T cells was undisturbed. In contrast, whereas WT cells undergoing homeostatic proliferation had divided up to two times and remained CFSE+, their KO counterparts had not divided at all, resulting in a substantial reduction in the ratio between KO and WT populations (Fig. 5A, middle upper and right upper panels). Thus, IL-7–dependent homeostatic proliferation is impaired in the absence of Ets-1. This defect in IL-7–dependent homeostatic proliferation may in part contribute to the reduction in the number of naive peripheral T cells in KO mice (Fig. 2A, Supplemental Fig. 2).

We also found that those T cells that had undergone endogenous proliferation expressed a high level of CD127 compared with those undergoing homeostatic proliferation (Fig. 5B, 5C, day 7). This upregulation of CD127 was independent of Ets-1 as both WT and KO populations expressed comparable levels of CD127. However, Ets-1 was required for maintaining the high level of CD127 in those cells at later time points. When examined at 29 d after transfer, the WT cells that had become CFSE− still expressed a high level of CD127 and the population continued to expand (Fig. 5D). In contrast, the KO CFSE− cells could not maintain the high level of CD127 (Figure 5B, 5C), and their number did not further increase (Fig. 5D), resulting in a significant reduction in

FIGURE 6. Ets-1 and CD127 expression is correlated in human T cells. Total (A, n = 10 individuals) and indicated subsets of peripheral CD8⁺ T cells (C, n = 6 individuals) were purified from peripheral blood of healthy donors according to the gating criteria shown in B. Each population of cells was further stained with anti-CD127 (black lines) or an isotype control (gray lines). Histograms from one representative experiment are shown in B. The transcript level of CD127 and Ets-1 p51 was quantified with real-time PCR and normalized against the level of Actin. The correlation between CD127 and Ets-1 was tested with a Spearman non-parametric test. The r² for naive cells is 0.7927.
the ratio between CFSE− KO and CFSE− WT populations (Fig. 5A, lower panels). At this late time point, the WT CFSE+ cells started to merge with the CFSE− cells (Fig. 5A, lower panels), and their numbers increased slightly despite a reduction in percentage (Fig. 5D). Instead, very few KO CFSE+ cells were detected, and these cells still expressed low levels of CD127 and stayed segregated from the CFSE− population.

The transcript level of Ets-1 correlates with the level of CD127 in human T cells

To determine whether the level of Ets-1 correlates with the expression of CD127 in human T cells, we isolated peripheral blood CD8+ T cells from healthy donors and analyzed their expression of CD127 and Ets-1. We found a strong linear correlation between the transcript level of CD127 and Ets-1 (Fig. 6A). Using live cell sorting of primary cell subsets, we further fractionated CD8+ T cells into naive (CD27−CD45RA−), effector (CD27−CD45RA−), EM (CD27−CD45RA−), and CM (CD27−CD45RA−) populations (Fig. 6B). As expected, naive T cells expressed high levels of CD127 (Fig. 6B, 6C). There was a strong correlation between Ets-1 and CD127 mRNA levels in this subset (Fig. 6C). Effector cells lost both Ets-1 and CD127 expression (Fig. 6C) but partially regained CD127 when they became EM cells. Notably, CM cells fully regained CD127 expression despite a low level of Ets-1. These data suggest that Ets-1 also participates in maintaining the CD127 level in human naive T cells and that additional transcription factors are required for the reexpression of CD127 in human CM cells.

Loss of CD127 expression during HIV infection occurs only in Ets-1low T cells

Loss of CD127 expression in CD8+ T cells is a hallmark of HIV infection (18, 19, 28). We wondered whether the HIV-associated loss of CD127 expression correlated with downregulation of Ets-1. Therefore, we also analyzed peripheral blood CD8+ T cells from HIV-positive donors. As expected, the surface level of CD127 was significantly lower in CD8+ T cells of HIV-positive donors than those of healthy controls (Fig. 7A, 7B). The reduction in the level of surface CD127 was also reflected in the level of CD127 transcript (Fig. 7C, left panel), suggesting that the downregulation of CD127 is mediated mainly by a transcriptional mechanism rather than a posttranscriptional mechanism. Notably, the transcript level of Ets-1 p51 was also significantly reduced in HIV-infected subjects compared with HIV-negative individuals (Fig. 7C, middle panel). In contrast, the transcript level of β2m was comparable between healthy and HIV-infected individuals (Fig. 7C, right panel).

To examine further whether downregulation of Ets-1 may contribute to the loss of CD127 expression during HIV infection, we also fractionated CD8+ T cells of HIV-positive donors into naive, effector, EM, and CM populations and measured the transcript levels of Ets-1 and CD127 in each subset. We found that HIV-associated loss of CD127 was observed only in EM and CM cells (Fig. 7D, left panel), which expressed a low level of Ets-1 (Fig. 7D, right panel). In contrast, no reduction in CD127 expression was detected in naive T cells, which expressed the highest level of Ets-1. We did not detect any difference in the level of CD127 between healthy and HIV-positive effector cells probably because these cells already expressed a nearly undetectable level of CD127. However, there was no difference in the transcript level of Ets-1 in any of the subsets between healthy and HIV-positive individuals (Fig. 7D, right panel), indicating that downregulation of Ets-1 alone is insufficient to induce the loss of CD127 during HIV infection. We also found that peripheral blood of HIV-positive individuals contained more effector and EM cells than that of healthy donors (Fig. 7E). Thus, expansion of Ets-1low effector and EM cells at the expense of Ets-1high naive cells contributes to the reduction in the level of Ets-1 in bulk CD8+ T cells of HIV-positive individuals.

Discussion

The expression of CD127 is tightly regulated and varies swiftly in response to environmental cues. But very little is known regarding the molecular mechanism controlling the dynamic expression of CD127. The current report fills some of the gaps in our knowledge of CD127 regulation and a) identifies Ets-1 as a critical regulator for CD127 expression in mature T cells and b) provides evidence suggesting that downregulation of Ets-1 may contribute to the loss of CD127 expression in CD8+ T cells during HIV infection. Our data expand on the role of Ets members in regulating the expression of CD127 in lymphoid cells. GABPα has been shown to
control the expression of CD127 in early thymocytes (DN1 and DN2 cells) (10). Deficiency of Ets-1 also affects CD127 expression in DN1 but not DN2 thymocytes. In addition, KO naive and central memory T cells also express a low level of CD127, suggesting that Ets-1 is the dominant Ets factor that controls CD127 expression in peripheral T cells. However, because KO peripheral T cells are not completely negative for CD127, other Ets factors such as Ets-2, ELF, ELK, or GABPα may compensate for the loss of Ets-1. It is noteworthy that a recent publication proposes a role for GABPα in regulating CD127 in peripheral T cells (29). We did, however, not detect any significant change in the level of GABPα in KO T cells (data not shown). The role of other Ets factors in maintaining the constitutive expression of CD127 in T cells remains to be determined.

In addition to the Ets family of transcription factors, other transcription factors are also important for the expression of CD127. It was recently reported that Foxo1-deficient T cells also displayed a profound defect in the expression of CD127, did not respond to IL-7, and had impaired homeostatic expansion (30, 31). Foxo1 binds to the promoter of CD127 in a region upstream of the putative Ets-1 binding site and is required for the cytokine withdraw-mediated induction of CD127. Given our findings, it would be very interesting to examine whether there is any functional interaction between Ets-1 and Foxo1. To our knowledge, our data also demonstrate for the first time that the transcriptional regulation of CD127 varies among different peripheral T cells subsets. Human CM T cells still express a high level of CD127 despite a “sub-naïve” level of Ets-1, and the expression of CD127 in murine EM cells is not affected by the absence of Ets-1. Examining the differential expression of the aforementioned transcription factors in each of the subsets will yield important insights into the molecular mechanism regulating CD127 expression in T cells.

KO T cells expanded poorly in lymphopenic hosts. Previous studies have demonstrated that IL-7 is required for homeostatic proliferation, whereas endogenous proliferation depends on TCR-mediated signals and IL-6 (27). We indeed found that homeostatic proliferation but not endogenous proliferation of KO T cells was impaired at day 7. Notably, KO T cells that had undergone endogenous proliferation could not maintain the expression of CD127 and failed to expand beyond day 7. This observation suggests that the survival or continuous proliferation of these cells may also become dependent on IL-7 after the initial expansion through IL-7–independent endogenous proliferation. CD127 has recently been shown to be critical for the development of Th cells–induced colitis in lymphopenic mice (32). We have previously shown that KO Th cells are unable to cause colitis in SCID mice (12). The molecular mechanism mediating this phenomenon is still unclear. It is very likely that the impaired CD127 expression contributes to the incapacity of KO Th cells to induce colitis. This scenario is now being examined.

IL-7 signals were recently shown to induce Runx3 expression and promote CD8 lineage choice in postselected thymocytes (33). Deficiency of Ets-1 leads to abnormal thymic development characterized by a reduced number of mature CD8 SP thymocytes and the presence of TCRβ− DP cells (34). We have shown that this phenotype is caused by impaired Runx3 expression, resulting in incomplete silencing of CD4 gene in CD8 SP thymocytes. Although Ets-1 can be recruited to the runx3 gene and can potentially transactivate Runx3 promoter, our data strongly suggest that Ets-1 can also indirectly regulate the level of Runx3 through controlling CD127 expression and IL-7 signaling.

Our data also provide insights into the molecular mechanism mediating the loss of CD127 expression during HIV infection. Both HIV-derived Tat protein and abnormally high levels of serum IL-7 detected in HIV-positive individuals have been shown to cause downregulation of surface CD127 even in uninfected T cells. We found that HIV-associated loss of CD127 was observed only in EM and CM, but not naive, CD8+ T cells. As naive T cells are also susceptible to the influence of Tat and IL-7, additional mechanisms or signals are required for shutting down the expression of CD127 in EM and CM cells during HIV infection. It is intriguing to notice that both EM and CM, but not naive, cells expressed a low level of Ets-1. This observation raises the possibility that downregulation of Ets-1 is a prerequisite, but insufficient, for the loss of CD127 expression. It will be of great interest to examine whether forced expression of Ets-1 will render T cells resistant to HIV-associated loss of CD127 expression and how Ets-1 is downregulated in Ag-experienced T cells.

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Disclosures

The authors have no financial conflicts of interest.

References


**Supplemental Figure 1.** Thymocytes obtained from 6-week-old WT and HET mice were subjected to FACS analyses. The level of CD127 in CD4SP thymocytes was shown in A. Ets-1 protein level was examined with Western analyses and shown in B. HSP90 was used as a loading control.

**Supplemental Figure 2.** The total number of CD62L+CD44- naïve CD4+ and CD8+ T cells from lymph nodes of WT and KO mice (6 to 8-week-old) were enumerated and shown.