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Interleukin-27 signalling induces stem cell antigen-1 expression in T lymphocytes *in vivo*

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Summary

Stem cell antigen-1 (Sca-1/Ly6A/E) is a cell surface glycoprotein that is often used as a biomarker for stem cells and cell stemness. However, it is not clear what factors can directly induce the expression of Sca-1/Ly6A/E in T lymphocytes *in vivo*, and if induction of Sca-1 is associated with T cell stemness. In this study, we show that interleukin-27 (IL-27), a member of the IL-12 family of cytokines, directly induces Sca-1 expression in T cells *in vivo*. We found that mice-deficient for IL-27 (either P28 or EBI3) or its signalling (IL-27Rα) had profound reduction of Sca-1 expression in naive ($CD62L^+$ $CD44^-$), memory ($CD62L^+$ $CD44^+$) and effector ($CD62L^-$ $CD44^+$) T cells. In contrast, *in vivo* delivery of IL-27 using adeno-associated viral vectors strongly induced the expression of Sca-1 in naive and memory/effector T-cell populations in an IL-27 receptor- or signal transducer and activator of transcription 1-dependent manner. Interestingly, IL-27-induced Sca-1^+ T cells do not express or up-regulate classic stem cell-associated genes such as *Nanog*, *Oct4*, *Sox2* and *Ctnnb1*. However, IL-27-induced Sca-1^+ T cells had increased expression of effector/memory-associated transcription factor *T-bet*, *Eomes* and *Blimp1*. Hence, IL-27 signalling directly induces the expression of Sca-1/Ly6A/E expression in T cells. Direct expansion of Sca-1^+ $CD62L^+$ $CD44^-$ T memory stem cells may explain why IL-27 enhances T-cell memory.

Keywords: interleukin-27; stem cell antigen-1; T-cell memory.

Introduction

Interleukin-27 (IL-27) is a member of the IL-12 cytokine family that consists of two subunits, i.e. Epstein–Barr virus-induced gene 3 (EBI3) and P28 (also known as IL-30).[1] IL-27 is produced by activated antigen-presenting cells such as dendritic cells and macrophages,[2–4] and signals through a heterodimeric receptor (IL-27R) consisting of the WSX-1 and the gp130 subunits in a variety of cell types including T cells.[5] IL-27 activates both the signal transducer and activator of transcription 1 (Stat1) and Stat3 signalling cascade,[6,7] with the activation of Stat1 being dominant.[8–10] Hence, IL-27 has potent activity in regulating T helper types 1, 2 and 17, and FoxP3$^+$ regulatory T cell responses.[11–13] IL-27 is also

known to induce T-cell expression of IL-10[14–16] and programmed death ligand 1 (PD-L1),[17] two inhibitory pathways that are associated with T-cell tolerance.

In addition to the well-appreciated anti-inflammatory effects of IL-27, we and others have shown that IL-27 can also enhance T-cell survival[18–20] and promote T-cell memory.[18,21,22] Our *in vitro* analysis of IL-27-stimulated cytotoxic T lymphocytes revealed that IL-27 induces T-cell expression of stemness-associated molecule Sca-1/Ly6A.[18] Sca-1 is a cell surface glycoprotein that is expressed in all adult mouse haematopoietic stem cells[23] and also in activated T cells.[24] Haematopoietic stems cells deficient for Sca-1 are defective[25] and T lymphocytes deficient for Sca-1 have altered proliferative responses.[24] Recently, we also showed that Sca-1 could be induced in T cells by *in vivo* delivery of IL-27 using adeno-associated viral (AAV) vectors.[26] However, it remains unclear if IL-27 directly induces Sca-1 expression in T cells, and if its induction is associated with T-cell stemness.

Previous studies have revealed a group of $CD62L^+$ $CD44^-$ $Sca\text{-}1^+$ T cells that are termed as T memory stem cells (T_{SCM}). T_{SCM} cells are an early-stage T memory subset that has robust proliferative potential, long-term survival capacity and the ability to mediate superior tumour regression upon adoptive transfer into tumour-bearing mice.[27,28] T_{SCM} cells can be generated by programming naive T cells in the presence of glycogen synthase-3β inhibitors[27,28] or cytokines such as IL-15[29] and IL-21.[30] It would therefore be interesting to determine if IL-27 can induce the expansion of T_{SCM} cells.

In this study, we have examined whether IL-27 signalling directly induces Sca-1 expression in T cells *in vivo* and if induction of Sca-1 is associated with T-cell stemness. We found that mice deficient for IL-27 (either P28 or EBI3) or its receptor (IL-27Rα) had profound reduction of Sca-1 expression in both naive and memory T-cell populations. In contrast, *in vivo* delivery of IL-27 by AAV significantly induced the expression of Sca-1 in naive and memory T-cell populations in IL-27 receptor- and Stat1-dependent manners. Interestingly, IL-27-induced Sca-1 expression is not associated with T-cell stemness, as IL-27-stimulated T cells failed to up-regulate traditionally stemness-associated genes such as *Nanog*, *Oct4*, *Sox2* and *Ctnnb1*. *In vivo* delivery of IL-27 by AAV induced an effector/memory phenotype in T cells characterized by the expression of *T-bet*, *Eomes* and *Blimp1*. Hence, IL-27 signalling directly induces the expression of Sca-1/Ly6A expression in T cells. Direct expansion of $Sca\text{-}1^+$ $CD62L^+$ $CD44^-$ T_{SCM} cells may explain why IL-27 enhances T-cell memory.

Materials and methods

Mice

C57BL/6 mice and IL-27Rα$^{-/-}$ mice were purchased from the Jackson Laboratory (Bar Harbor, ME). EBI3-deficient mice in the C57BL/6[31] and BALB/c background[22] have been described. C57BL/6 mice with a targeted mutation of the P28 gene (IL-27P28$^{-/-}$)[13] were obtained from Dr Daniel J. Cua via a material transfer agreement. Stat1-deficient BALB/c mice[32] have been described previously. All mice were maintained in OSU laboratory animal facilities that were fully accredited by Institutional Animal Care and Use Committee.

Antibodies and flow cytometry

Fluorescein isothiocyanate-, phycoerythrin-, allophycocyanin- or Peridinin chlorophyll protein-labelled antibodies to CD4, CD8a, CD44, CD62L, PD-L1, Sca-1 and isotype control antibodies were purchased from BD Biosciences (San Diego, CA). For staining of cell surface markers, cells (single-cell suspensions of spleen) were stained with various antibodies in staining buffer (PBS with 1% fetal calf serum) on ice for 30 min, after washing with staining buffer, cells were fixed in 1% paraformaldehyde in PBS and were analysed on a FACSCalibur flow cytometer. Data were analysed using FLOWJO software (Tree Star, Inc., Ashland, OR).

Production of adeno-associated viral vectors and mice treatment

We used rAAV vector to express IL-27 and IL-30 *in vivo*. The IL-30 expression plasmids[33] were obtained from Dr Shulin Li (MD Anderson Cancer Center). Briefly, IL-27 or IL-30 cDNA was inserted into an AAV carrier vector under the control of the cytomegalovirus-chicken β-actin hybrid (CAG) promoter.[34,35] The IL-27 or IL-30 carrier AAV vector was compacted with a helper vector in 293K cells into the AAV serotype 8 (AAV8). AAV8 is known to be particularly suitable for expression in muscle cells.[36,37] Intramuscular injection of 2×10^{11} DNAse resistant particle (DRP)/mouse of AAV-IL-27 or AAV-IL-30 achieved high concentrations of IL-27 or IL-30 production in the peripheral blood of mice. Hence, we evaluated the *in vivo* effects of AAV-IL-27 and AAV-IL-30 on T-cell activation in the context of a concanavalin A-induced liver injury model.

ELISA

Blood was drawn from mice treated with AAV-IL-27, AAV-IL-30 and AAV-ctrl vectors at 2 weeks after viral injection. Serum was investigated for the presence of IL-27 and IL-30 using ELISA kits purchased from eBioscience (San Diego, CA) for IL-27 and R&D Systems, Inc. (Minneapolis, MN) for IL-30.

Real-time PCR

Quantitative real-time PCR was performed using an ABI 7900-HT sequence system (PE Applied Biosystems, Foster

City, CA) with the QuantiTect SYBR Green PCR kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. PCR was performed using previously determined conditions.[38] The following primers were used for amplifying specific genes: Actin: 5′-GAG ACCTTCAACACCCCAGC-3′ (forward) and 5′-ATGT-CACGCACGATTTCCC-3′ (reverse); Bcl2: 5′-TGCGGAG-GAAGTAGACTGATA-3′ (forward) and 5′-TGGCATGA GATGCAGGAAA-3′ (reverse); Bcl6: 5′-CATACAGAG ATGTGCCTCCATAC-3′ (forward) and 5′-CCCATTCTC ACAGCTAGAATCC-3′ (reverse); Blimp1: 5′-TCTACCC TCGGGTGGTTTAT-3′ (forward) and 5′-TGAGTTATG-TAGGTGGGTCTCT-3′ (reverse); Ctnnb1: 5′- GCTGCTC ATCCCACTAATGT-3′ (forward) and 5′-CCGCGTCATC CTGATAGTTAAT-3′ (reverse); Eomes: 5′-CGTTCACC-CAGAATCTCCTAAC-3′ (forward) and 5′-GCAGAGAC TGCAACACTATCA-3′ (reverse); Foxo1: 5′-CGTGCCC TACTTCAAGGATAAG-3′ (forward) and 5′-GCACTC-GAATAAACTTGCTGTG-3′ (reverse); ID2: 5′-CTACTCC AAGCTCAAGGAACTG-3′ (forward) and 5′-GATCTGCA GGTCCAAGATGTAA-3′ (reverse); ID3: 5′-AGACTACAT CCTCGACCTTCA-3′ (forward) and 5′-GATCACAAG TTCCGGAGTGAG-3′ (reverse); Klf4: 5′-CCCTTCGGTCA TCAGTGTTAG-3′ (forward) and 5′-GGACCGCCTCTT GCTTAAT-3′ (reverse); Lef1: 5′-AGAACACCCTGAT-GAAGGAAAG-3′ (forward) and 5′-GTACGGGTCGCTGT TCATATT-3′ (reverse); Nanog: 5′-GGCAGCCCTGATT CTTCTAC-3′ (forward) and 5′-GAGAACACAGTCCGCA TCTT-3′ (reverse); NFATc1: 5′-CCGTCCAAGTCAGTT TCTATGT-3′ (forward) and 5′-GTCCGTGGGTTCTGTC TTTAT-3′ (reverse); Oct4: 5′-CCTACAGCAGATCACTCA CATC-3′ (forward) and 5′-GCCGGTTACAGAACCA-TACTC-3′ (reverse); Stat4: 5′-GAAGTGCAGTACTGGGA GTAAA-3′ (forward) and 5′-GGTTAATGGTGAGGCCA-TAGAG-3′ (reverse); Sox2: 5′-TGAACGCCTTCATGG-TATGG-3′ (forward) and 5′-GATCTCCGAGTTGTGCA TCTT-3′ (reverse); TCF1: 5′-CCTTGGTGGAGGAGTGTA ATAG-3′ (forward) and 5′-GTTGGCAAACCAGTTGTA-GAC-3′ (reverse); T-bet: 5′-CCAGGGAACCGCTTATAT GT-3′ (forward) and 5′-CCTTGTTGTTGGTGAGCT

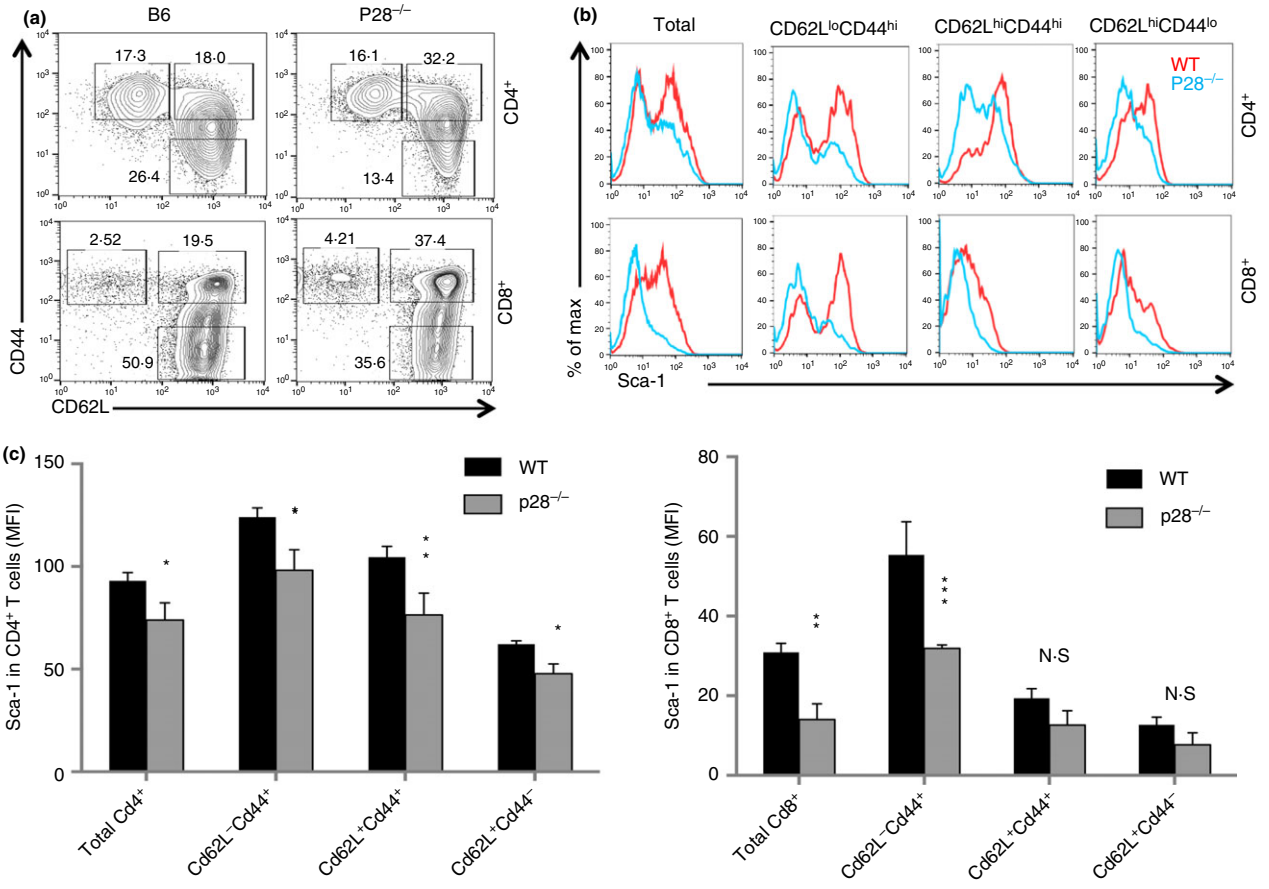

Figure 1. IL-27P28-deficient mice had a reduced Sca-1$^+$ memory pool of T cells. Splenocytes from naive wild-type (WT) B6 and P28$^{-/-}$ mice were analysed by flow cytometry. Spleen CD4$^+$ and CD8$^+$ T cells and their subsets (a), based on the expression of CD62L and CD44, were analysed for the expression of Sca-1 (b). Sca-1 expression in CD4$^+$ and CD8$^+$ T cells and their subsets were quantified (c). Three to five mice were used in each group for this experiment. Data are expressed as means of individual determinations ± SE and represent three experiments using both male and female mice. Statistical analysis was performed using the unpaired Student's *t*-test. *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$. N.S., not significant.

TTAG-3′ (reverse). Each sample was assayed in triplicate and the experiments were repeated twice. The relative gene expression was calculated by plotting the C*t* (cycle number) and the average relative expression for each group was determined using the comparative method ($2^{-\Delta\Delta Ct}$).

Statistics

Data are expressed as means of individual determinations ± SE. Statistical analysis was performed using the unpaired Student's *t*-test.

Results

Reduced Sca-1/Ly6A expression in T cells in IL-27 and IL-27 receptor-deficient mice

Previous studies[18,21,22] have revealed that IL-27 contributes to T-cell memory, and expression of Sca-1/Ly6A, a cell surface glycoprotein, is considered to be a biomarker for T_{SCM}.[28] We therefore examined if the lack of IL-27 or IL-27 receptor signalling affected T-cell expression of Sca-1 in naive and memory T-cell populations. In the peripheral lymphoid organs of naive mice, T cells can be sub-divided into three populations based on their expression of CD62L and CD44, i.e. $CD62L^+$ $CD44^-$ (naive), $CD62L^+$ $CD44^+$ (central memory) and $CD62L^-$ $CD44^+$ (effector memory) T cells. As shown in Fig. 1(a), $CD4^+$ and $CD8^+$ T cells from IL-27P28-deficient and wild-type B6 mice were subdivided into three populations based on their expression of CD62L and CD44. We found that $CD4^+$ and $CD8^+$ T cells and their subpopulations from IL-27P28$^{-/-}$ mice had significantly reduced expression of Sca-1, and the reduction of Sca-1 expression was particularly significant among memory T-cell populations in both $CD4^+$ and $CD8^+$ T cells (Fig. 1b,c). Similarly, $CD4^+$ and $CD8^+$ T cells from IL-27EBI3$^{-/-}$ and wild-type mice were also subdivided into three populations based on

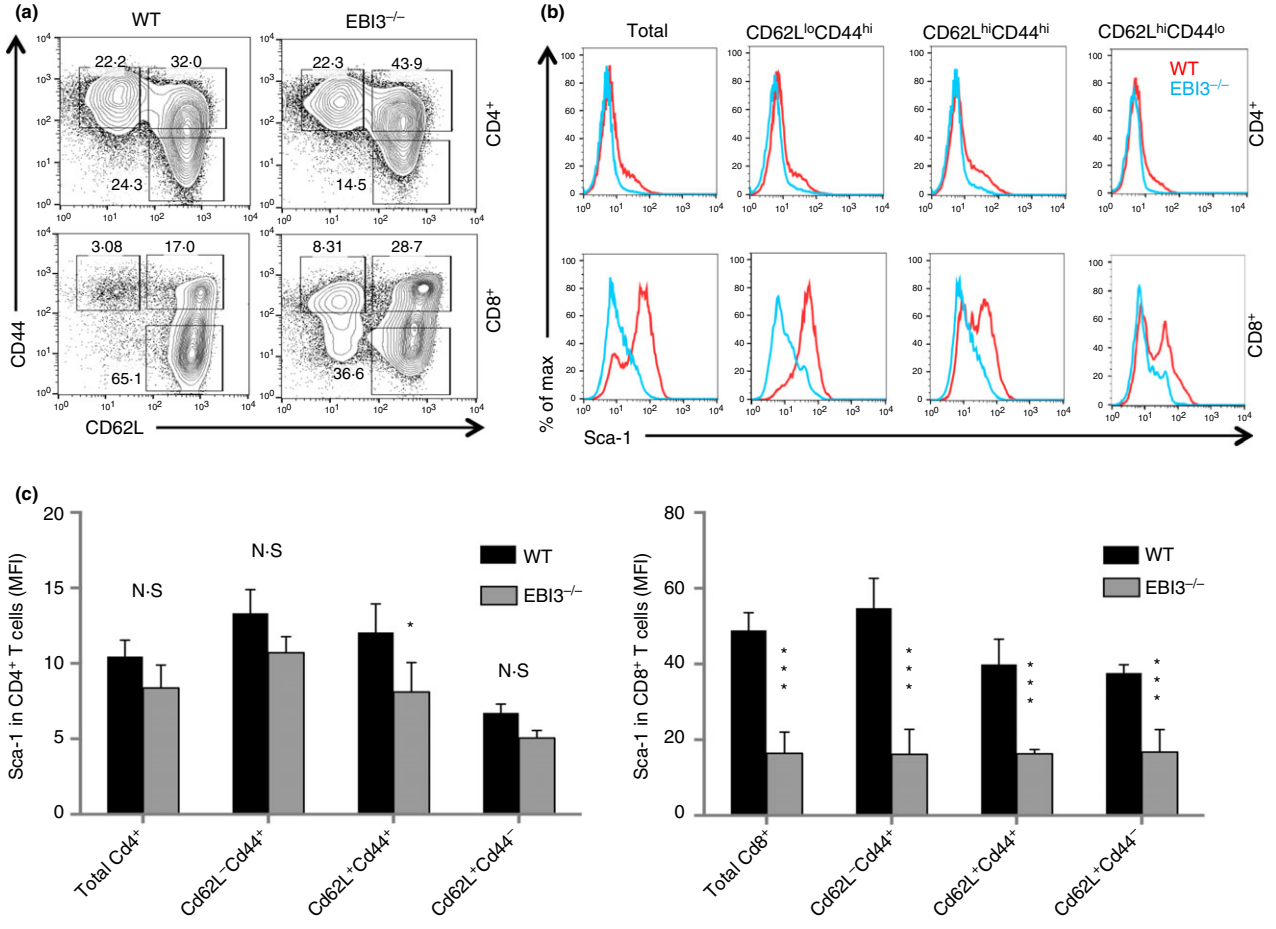

Figure 2. IL-27EBI3-deficient mice had a reduced Sca-1^+ memory pool of T cells. Splenocytes from naive wild-type (WT) and EBI3$^{-/-}$ mice were analysed by flow cytometry. Spleen $CD4^+$ and $CD8^+$ T cells and their subsets (a) were analysed for the expression of Sca-1 (b). Sca-1^+ T cells in $CD4^+$ and $CD8^+$ T cells and their subsets were quantified (c). At least three mice were used in each group for this experiment. Data are expressed as means of individual determinations ± SE and represent three experiments using both male and female mice. Statistical analysis was performed using the unpaired Student's *t*-test. *$P < 0{\cdot}05$; ***$P < 0{\cdot}001$. N.S., not significant.

their expression of CD62L and CD44 (Fig. 2a). We observed reduced Sca-1 expression in all subpopulations of $CD8^+$ T cells in IL-27EBI3$^{-/-}$ mice, whereas a trend of Sca-1 reduction in $CD4^+$ T cells from IL-27EBI3$^{-/-}$ mice was also observed (Fig. 2b,c). Finally, the T-cell subpopulations in IL-27R$\alpha^{-/-}$ mice (Fig. 3a) were also analysed in a similar manner. Significant reduction of Sca-1/Ly6A expression was observed in all subpopulations of $CD4^+$ and $CD8^+$ T cells in IL-27R$\alpha^{-/-}$ mice (Fig. 3b,c). Hence, IL-27-IL-27R signalling appears to be required for the expression of Sca-1/Ly6A in both naive and memory/effector T cells under steady state.

IL-27 directly induce Sca-1/Ly6A in T cells in an IL-27R- or Stat1-dependent manner

We previously showed that cultured T cells stimulated with IL-27 up-regulated Sca-1/Ly6A.[18] We also observed that T cells in AAV-IL-27-treated mice had increased expression of Sca-1/Ly6A.[26] To determine if IL-27 directly induces Sca-1 expression in T cells *in vivo*, we generated AAV-IL-27, AAV-IL-30 (IL-27P28) or AAV-Ctrl viral vectors and injected the vectors intramuscularly into wild-type mice. As shown in Fig. 4(a), 2 weeks after the injection of viral vectors, significant levels of IL-27 or IL-30 were detected only in the serum from mice receiving respective AAV vectors. Multi-coloured flow cytometry analysis was used to determine the expression of Sca-1. As shown in Fig. 4(b) and summarized in Fig. 4(c,d), T cells from AAV-IL-27, but not AAV-IL-30 or AAV-Ctrl viral vector-treated mice had significantly up-regulated expression of Sca-1, essentially in all subpopulations. These results confirm that IL-27, but not its subunit P28 (IL-30), induces Sca-1 expression in all T-cell subpopulations *in vivo*. Moreover, we found that AAV-IL-27-induced Sca-1 expression was IL-27R and Stat1-dependent, as AAV-IL-27-induced Sca-1 expression was only detected in T cells from wild-type, but not IL-27Rα (Fig. 5a) and Stat1-deficient mice (Fig. 5b).

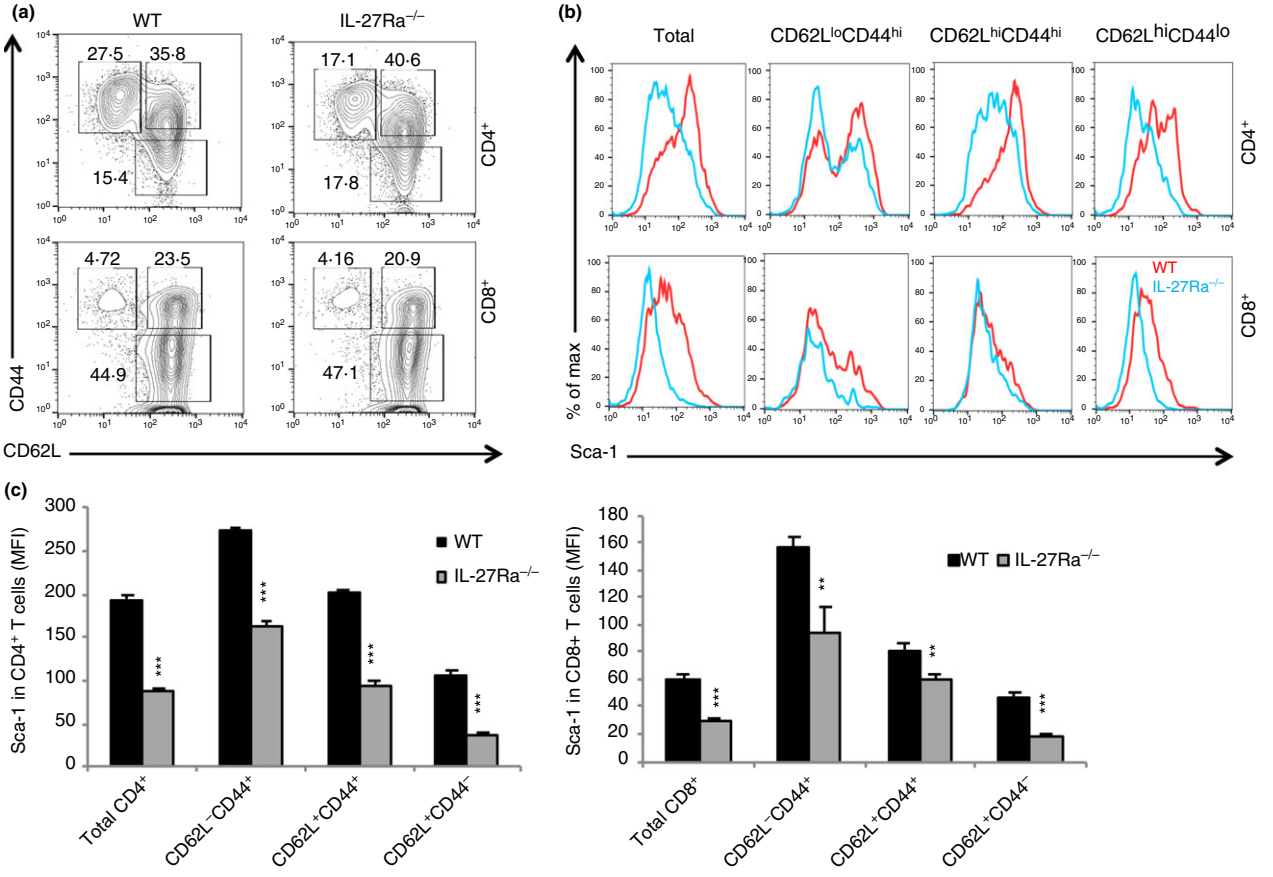

Figure 3. Interleukin-27 receptor α (IL-27Rα) -deficient mice had a reduced Sca-1$^+$ memory pool of T cells. Splenocytes from naive wild-type (WT) B6 and IL-27R$\alpha^{-/-}$ mice were analysed by flow cytometry. Spleen $CD4^+$ and $CD8^+$ T cells and their subsets (a) were analysed for the expression of Sca-1 (b). Sca-1 expression in $CD4^+$ and $CD8^+$ T cells and their subsets was quantified (c). Four to five mice were used in each group for this experiment. Data are expressed as means of individual determinations $\pm$ SE and represent two experiments. Statistical analysis was performed using the unpaired Student's *t*-test. $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

Transcription factors in IL-27-stimulated Sca-1$^+$ T cells

To understand the phenotypes of AAV-IL-27-induced Sca-1$^+$ T cells, we sorted CD4$^+$ and CD8$^+$ T cells from AAV-IL-27 and AAV-ctrl virus-treated mice by FACS, and used quantitative PCR to measure T-cell differentiations and stemness-associated transcription factors. As shown in Fig. 6, AAV-IL-27 treatment significantly up-regulated the expression of T-bet, Eomes and Blimp1, transcription factors typically expressed by effector memory T cells.[39] We failed to detect the expression of stem cell-associated transcription factors[40] such as *Nanog*, *Oct4* and *Sox2*, in T cells from either AAV-IL-27 or AAV-ctrl virus-treated mice. Induction of Ctnnb1 (β-catenin) has been shown to be associated with T-cell stemness.[28] However, we found that Ctnnb1 was slightly reduced in T cells from AAV-IL-27-treated mice. Finally, *Klf4* up-regulation was found in CD4$^+$, but not CD8$^+$, T cells from AAV-IL-27-treated mice. Hence, IL-27-induced Sca-1$^+$ T cells express transcription factors of effector memory T cells but do not show many features of typical stem cells.

Discussion

In this study, we have found that IL-27 directly induces Sca-1 expression in naive and memory T cells *in vivo*. In IL-27 and IL-27 receptor-deficient mice, Sca-1 expression is greatly reduced in all three populations of T cells, i.e. CD62L$^+$ CD44$^-$ naive, CD62L$^+$ CD44$^+$ central memory and CD62L$^-$ CD44$^+$ effector memory T cells. In contrast, in mice treated with AAV-IL-27, Sca-1 expression is greatly increased in naive, central memory and effector memory T cells, and AAV-IL-27-induced Sca-1 expression

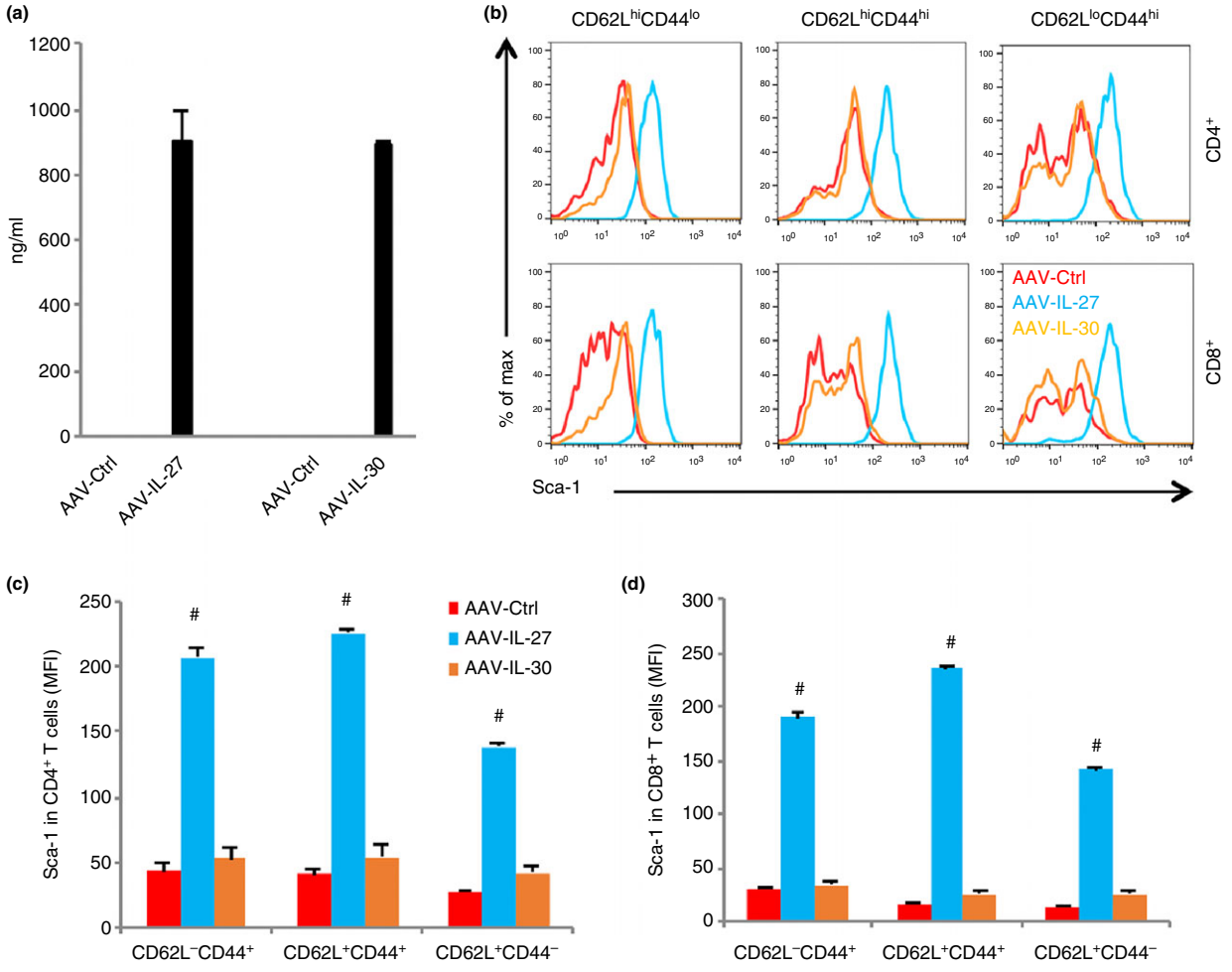

Figure 4. Adeno-associated virus (AAV) vector-delivered interleukin-27 (IL-27) induces Sca-1 expression in T cells. (a) Wild-type (WT) mice were injected with AAV-ctrl, AAV-IL-27 or AAV-IL-30 viral vectors intramuscularly at a dose of 2×10^{11} DNAse resistant particle (DRP)/mouse, and serum IL-27 or IL-30 levels were measured by ELISA 2 weeks later. Spleen CD4$^+$ and CD8$^+$ T cells and their subsets were analysed for the expression of Sca-1 (b). Sca-1 expression in CD4$^+$ (c) and CD8$^+$ (d) subsets was quantified. Three mice were used in each group for this experiment. Data are expressed as means of individual determinations $\pm$ SE and represent three independent experiments using both male and female mice. Statistical analysis was performed using the unpaired Student's *t*-test. #$P < 1 \times 10^{-5}$.

is IL-27R- and Stat1-dependent. Hence, our results suggest that IL-27 signalling directly induces the expression of Sca-1/Ly6A/E in T cells *in vivo*.

Demoulin *et al.*[41] showed that IL-6 and IL-9 induced the expression of Sca-1 in T lymphoma cells and mature T lymphocytes *in vitro*. They found that both IL-6 and IL-9 mediated the transcriptional activation of Sca-1 through a GAS element in the Sca-1 promotor, which was able to bind Stat1 and Stat3. In this study, we found that IL-27 signalling induced Sca-1 expression *in vivo* exclusively through Stat1 (Fig. 5b). Hence, it is likely that IL-27-induced activation of Stat1 directly binds to GAS element in the Sca-1 promoter, leading to the expression of Sca-1 in T cells.

Sca-1/Ly6A/E is a cell surface glycoprotein that is expressed in all adult mouse haematopoietic stem cells[23] and has been shown to be necessary for haematopoietic

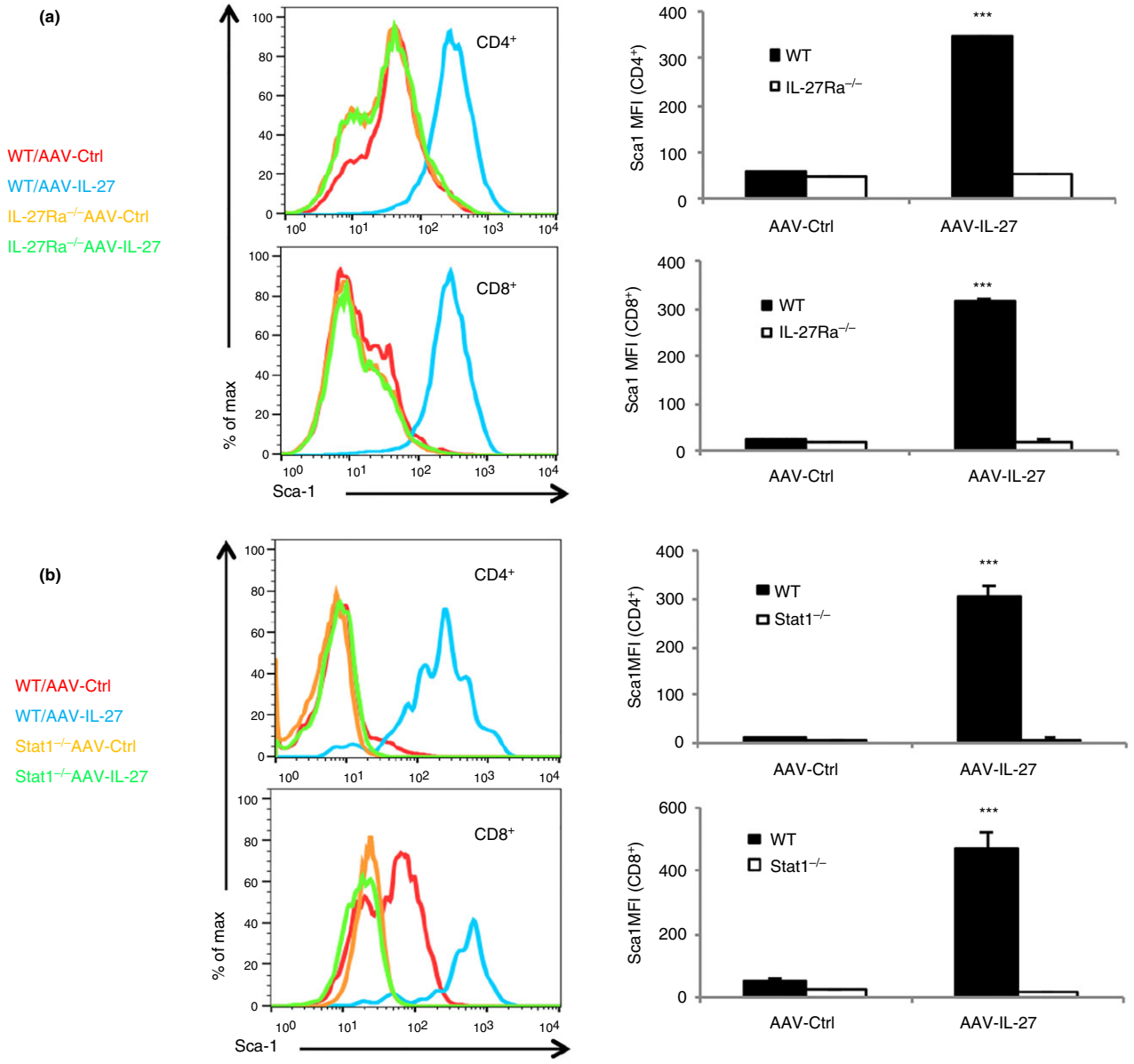

Figure 5. Adeno-associated virus–interleukin-27 (AAV-IL-27) -induced Sca-1 expression in T cells is interleukin-27 receptor (IL-27R) and signal transducer and activator of transcription 1 (Stat1) -dependent. (a) IL-27R$\alpha^{-/-}$ and wild-type (WT) mice were treated with AAV-IL-27 or AAV-Ctrl vectors. Two weeks later, the expression of Sca-1 in spleen was examined by flow cytometry and mean fluorescence intensity (MFI) of Sca-1 was quantified. (b) Stat1$^{-/-}$ and WT mice were treated with AAV-IL-27 or AAV-Ctrl vectors. Two weeks later, the expression of Sca-1 in spleen was examined by flow cytometry and MFI of Sca-1 was quantified. Three to five mice were used in each group for the experiments. Data are expressed as means of individual determinations ± SE and represent two independent experiments using both male and female mice. Statistical analysis was performed using the unpaired Student's *t*-test. ***$P < 1 \times 10^{-3}$.

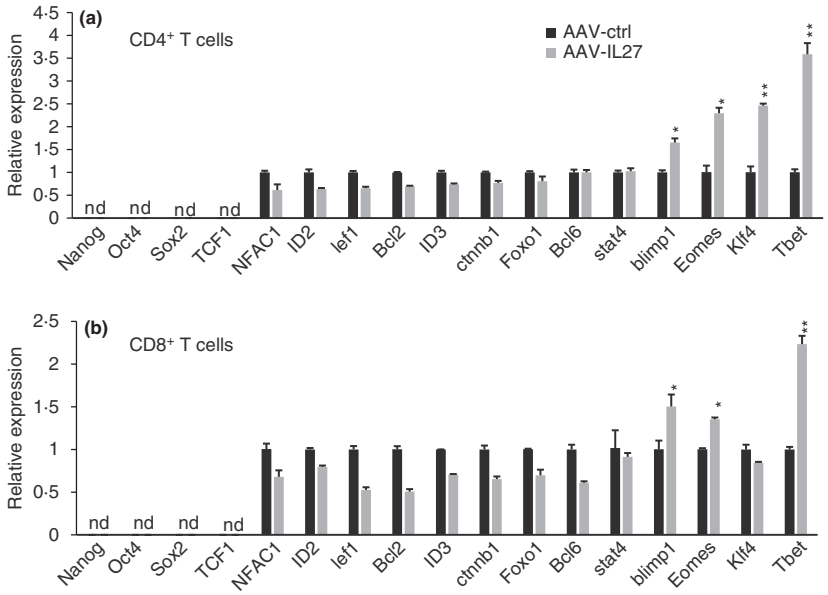

Figure 6. Expression of transcription factors and stemness genes in T cells from Adeno-associated virus–interleukin-27 (AAV-IL-27) and AAV-ctrl-treated mice. C57BL/6 mice were treated with AAV-ctrl or AAV-IL-27 viral vectors intramuscularly at a dose of 2×10^{11} DNAse resistant particle (DRP)/mouse. Two weeks later $CD4^+$ and $CD8^+$ T cells were sorted from the spleens by FACS. Total RNA was prepared from purified T cells and quantitative PCR was used to quantify transcription factors associated with T-cell differentiation and stemness in $CD4^+$ (a) and $CD8^+$ T cells (b). Samples were pooled from three mice in each group. Data are expressed as means of individual determinations ± SE of three replicates, and represent two independent experiments. Statistical analysis was performed using the unpaired Student's *t*-test. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$. nd, not detectable.

stem cell self-renewal and the development of committed progenitor cells.[25] Sca-1 was also shown to play a role in c-kit expression in haematopoietic stem cells[42] and in haematopoietic commitment to granulocyte development.[43] However, our current results suggest that IL-27-induced Sca-1 expression does not appear to be associated with stemness of mature T cells, as the classic transcription factors associated with cell stemness, including *Nanog*, *Oct4* and *Sox2*, were undetectable in Sca-1^+ T cells from AAV-IL-27-treated mice (Fig. 6), whereas *Klf4* expression was only found to be elevated in $CD4^+$ but not $CD8^+$ T cells. Induction of *Ctnnb1* (β-catenin) has also been shown to be associated with T-cell stemness.[28] However, we found that *Ctnnb1* was slightly reduced in T cells from AAV-IL-27-treated mice (Fig. 6). Hence, IL-27-induced Sca-1^+ T cells do not show many features of conventional stem cells. These results suggest that Sca-1 expression in haematopoietic stem cells and mature T lymphocytes play different roles.

Although IL-27-induced Sca-1^+ T cells do not express many stem cell-associated genes, they have elevated expression of memory T-cell-associated molecules such as Eomes, which have been shown to promote T-cell memory.[44,45] In this study, we have found that IL-27 induces Sca-1 expression in all three populations of T cells, i.e. $CD62L^+$ $CD44^-$ naive, $CD62L^+$ $CD44^+$ central memory and $CD62L^-$ $CD44^+$ effector memory T cells. Although the significance for induction of Sca-1 in central memory and effector memory T cells remains to be studied, Gattinoni *et al.* [27,28] have identified a new subset of $CD62L^+$ $CD44^-$ Sca-1^+ T_{SCM}. They have shown that T_{SCM} cells can be generated *in vitro* by programming naive T cells in the presence of small molecules such as glycogen synthase-3β inhibitors[27,28] and cytokines such as IL-15[29] and IL-21.[30] Our results provide the first evidence that IL-27 signalling induces the expansion of $CD62L^+$ $CD44^-$ Sca-1^+ T_{SCM} cells *in vivo*. These findings, taken together, explain why IL-27 promotes T-cell memory.[18,21,22] However, these results do not suggest that Sca-1 itself is important for T-cell memory, as Sca-1-deficient mice have normal T-cell memory responses.[46]

Taken together, we have found that IL-27 signalling can directly induce Sca-1/Ly6A/E expression in naive and memory populations of T cells. However, IL-27-induced Sca-1^+ T cells do not express the classic transcription factors for stemness. IL-27 induces the expansion of a memory pool of T cells including T_{SCM} cells. Given that IL-27 may potentially be used as a therapeutic for cancer[47] and autoimmune diseases,[48] identification of Sca-1/Ly6A/E as an IL-27-responsive biomarker in T cells *in vivo* may potentially be useful for determining therapeutic response in pre-clinical settings.

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authors declare no financial or commercial conflict of interest.

Author contributions

ZL, LW, JZ, XZ and JZ performed experiments; JQL, JZ and JPD produced AAV viruses; SV and ARS provided Stat1 knockout mice and helped in designing the experiments; JZ, MSL and XFB designed experiments and generated funding support for this study. XFB wrote the manuscript.

Disclosures

The authors have no conflicts of interest to disclose.

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