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IL-27 improves adoptive CD8$^+$ T cells' antitumor activity via enhancing cell survival and memory T cell differentiation

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Abstract

IL-27 is an anti-inflammatory cytokine that triggers enhanced antitumor immunity, particularly cytotoxic T lymphocyte responses. In the present study, we sought to develop IL-27 into a therapeutic adjutant for adoptive T cell therapy using our well-established models. We have found that IL-27 directly improved the survival status and cytotoxicity of adoptive OT-1 CD8$^+$ T cells in vitro and in vivo. Meanwhile, IL-27 treatment programs memory T cell differentiation in CD8$^+$ T cells, characterized by upregulation of genes associated with T cell memory differentiation (T-bet, Eomes, Blimp1, and Ly6C). Additionally, we engineered the adoptive OT-1 CD8$^+$ T cells to deliver IL-27. In mice, the established tumors treated with OT-1 CD8$^+$ T-IL-27 were completely rejected, which demonstrated that IL-27 delivered via tumor antigen–specific T cells enhances adoptive T cells' cancer immunity. To our knowledge, this is the first application of CD8$^+$ T cells as a vehicle to deliver IL-27 to treat tumors. Thus, this study demonstrates IL-27 is a feasible approach for enhancing CD8$^+$ T cells' antitumor immunity and can be used as a therapeutic adjutant for T cell adoptive transfer to treat cancer.

KEYWORDS
antitumor, enhanced T cell survival, IL-27, memory T cell differentiation, T cell adoptive transfer

Abbreviations: 7AAD, 7-amino-actinomycin D; AAV, adeno-associated virus; CCK-8, cell-counting kit-8; CFSE, 5,6-carboxyfluorescein diacetate, succinimidyl ester; ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon-γ; IL-27, interleukin-27; MHC I, major histocompatibility complex I; $OVA_{257-264}$, ovalbumin residues 257-264; qRT-PCR, quantitative real-time polymerase chain reaction; RV, retrovirus; TCR, T cell receptor; TIL, tumor-infiltrating lymphocyte; TNF-α, tumor necrosis factor-α.

Miao Ding and Yi Fei contributed equally to the article.

1 | INTRODUCTION

Adoptive T cell therapy utilizing either tumor-infiltrating lymphocyte (TIL)-derived T cells or T cells genetically engineered to express tumor-recognizing receptors has emerged as a powerful curative therapy for cancers. The infusion of manipulated T cells in tumors has been associated with a favorable prognosis in various cancer types.[1-5] Many adoptive T cell therapy–based therapies have recently entered late-phase clinical testing.[6-9] The ultimate goal of adoptive T cell therapy is to generate a robust immune-mediated antitumor response via the infusion of ex vivo–manipulated T cells. However, there are several barriers that need to be overcome before this type of therapy becomes a widely accepted standard treatment for different cancers. Among the barriers, the poor persistence of adoptive T cells is a critical challenge.

More recently, several exciting cytokines have been characterized as considerable and promising tools in the treatment of cancer, opening up a novel avenue in the development of cancer immunotherapy. IL-27, a member of the IL-12 cytokine family, with important roles in both innate and adoptive immunity,[10-12] is one of these exciting antitumor cytokines. Accumulating evidence by recent studies has revealed that IL-27 possesses potent antitumor activity against a variety of tumors, such as melanoma,[13,14] B acute lymphoblastic leukemia,[15] acute myeloid leukemia,[16] large B cell lymphoma,[17] and multiple myeloma.[18] It also exerts indirect antitumor effects driven by its immune-stimulatory activity in melanoma,[19] colon carcinoma,[20,21] neuroblastoma,[22,23] lung cancer,[24] and head and neck squamous cell carcinoma[25] tumor models. The route of IL-27 administration ranged from a transplanted mouse tumor genetically engineered to secret IL-27 to a human therapeutic model by injection of IL-27 protein into immunodeficient mice after transplantation of human tumor as preclinical tumor models. IL-27 is considered to be a cytokine with low toxicity and efficient antitumor effect.[26]

Although the role of IL-27 in tumor immunity has been appreciated for more than a decade, the critical effect for IL-27 in $CD8^+$ T cells and how to develop IL-27 into a therapeutic adjutant for adoptive T cell therapy has not been well studied. In the present study, we sought to investigate this by using our well-established models. Because TILs are not available for most cancer types, we tested if using genetically modified T cells with IL-27 for cancer therapy is an effective approach. We first used an in vitro culture system to generate antigen-specific OT-1 $CD8^+$ T cells in the presence or absence of IL-27 and compared the phenotype and cytotoxicity of the cells. OT-1 $CD8^+$ T cells were separated from gene-modified OT-1 mice that have specificity for ovalbumin peptide residues 257-264 ($OVA_{257-264}$) presented by the MHC I molecule.[27] We also generated tumor-reactive OT-1 $CD8^+$ T cells for adoptive transfer into an animal model bearing tumor. The results showed that AAV-IL-27 significantly enhances the survival of adoptive $CD8^+$ T cells and induces a memory phenotype in $CD8^+$ T cells, characterized by upregulation of genes associated with T cell memory differentiation (T-bet, Eomes, Blimp1, and Ly6C). Additionally, we engineered adoptive OT-1 $CD8^+$ T cells to deliver IL-27. In mice, the established tumors treated with OT-1 $CD8^+$ T-IL-27 were completely rejected, which indicates that IL-27 delivered enhances the antitumor activity of adoptive OT-1 $CD8^+$ T cells and has the potential to be used for the immunotherapy of cancer.

2 | MATERIALS AND METHODS

2.1 | Mice

C57BL/6 mice were purchased from Shanghai Lingchang Biotech Company and were maintained in the animal facilities of Shanghai Children's Medical Center. CD45.1 C57BL/6 mice and OT-1 C57BL/6 transgenic mice, whose T cell receptor (TCR) was designed to recognize $OVA_{257-264}$ peptide, were purchased from Shanghai Model

TABLE 1 Primers used for amplifying related genes

Primer	Sequence (5′–3′)
CD44 Forward	TCGATTTGAATGTAACCTGCCG
CD44 Reverse	CAGTCCGGGAGATACTGTAGC
CD122 Forward	TGGAGCCTGTCCCTCTACG
CD122 Reverse	TCCACATGCAAGAGACATTGG
Ly6c Forward	GCAGTGCTACGAGTGCTATGG
Ly6c Reverse	ACTGACGGGTCTTTAGTTTCCTT
Blimp1 Forward	TTCTCTTGGAAAAACGTGTGGG
Blimp1 Reverse	GGAGCCGGAGCTAGACTTG
Eomes Forward	GCGCATGTTTCCTTTCTTGAG
Eomes Reverse	GGTCGGCCAGAACCACTTC
T-bet Forward	AGCAAGGACGGCGAATGTT
T-bet Reverse	GGGTGGACATATAAGCGGTTC
β-actin Forward	GGCTGTATTCCCCTCCATCG
β-actin Reverse	CCAGTTGGTAACAATGCCATGT
perforin Forward	AGCACAAGTTCGTGCCAGG
perforin Reverse	GCGTCTCTCATTAGGGAGTTTTT
IFN-γ Forward	ATGAACGCTACACACTGCATC
IFN-γ Reverse	CCATCCTTTTGCCAGTTCCTC
TNF-α Forward	CCCTCACACTCAGATCATCTTCT
TNF-α Reverse	GCTACGACGTGGGCTACAG
Granzyme G Forward	TCTGGTACACCTATTCCAAGACC
Granzyme G Reverse	CTCCTGGAGTGATTGTCCTGT
Granzyme B Forward	CCACTCTCGACCCTACATGG
Granzyme B Reverse	GGCCCCCAAAGTGACATTTATT
Granzyme C Forward	GCAGAGGAGATAATCGGAGGC
Granzyme C Reverse	GCACGAATTTGTCTCGAACCA

Organisms. The CD45.1 mice were bred with OT-1 mice to generate CD45.1 OT-1 mice expressing both OT-1 TCR and CD45.1 on CD8$^+$ T cells. The initial tumor implantation was conducted in animals at the age of 6-8 weeks. Tumor heights and widths were measured with a caliper every 2-3 days to calculate tumor volume (width^2 × height × 0.5).

2.2 | Flow cytometry

Antibodies were purchased from Biolegend or BD Biosciences. T-Select H-2Kb OVA Tetramer-SIINFEKL-APC was purchased from MBL to identify OVA-specific T cells. Stained cells were prepared according to the manufacturer's indications and analyzed on a FACSCalibur flow cytometer.

2.3 | Cytolysis assay

CCK-8 assay (Beyotime Biotechnology) was used to measure the cytolysis rate elicited by OT-1 CD8$^+$ T cells against target cells. B16.OVA or B16F10 (negative control) cells (2 × 10^4) were cocultured with OT-1 CD8$^+$ T cells for 24 hours. The supernatant was removed and incubated with CCK-8 reaction mixture. Cytolysis rate was calculated on the basis of the following equation: cytotoxicity (%) = (effector and target cell mixture-blank)/(target cell control-blank) ×100.

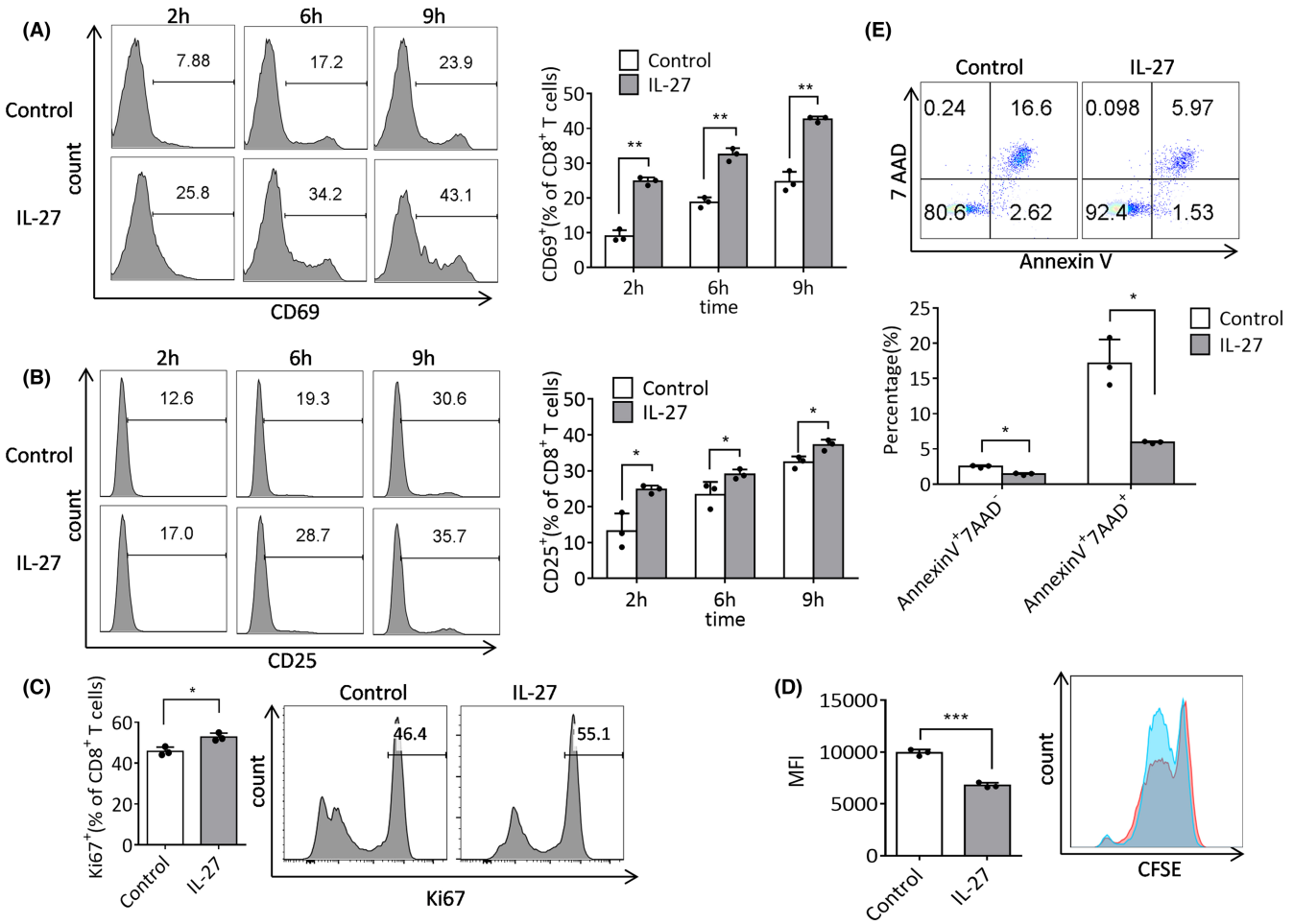

FIGURE 1 IL-27 enhances the activation and proliferation ability of T cells in vitro. A, B, Cultured OT-1 CD8$^+$ T cells were stimulated with CD3/CD28 beads in the presence or absence of 50 ng/ml of IL-27, and CD69 and CD25 expressions were analyzed at the indicated time. C, OT-1 CD8$^+$ T cells were stimulated with CD3/CD28 beads in the presence or absence of 50 ng/ml of IL-27 for 24 h. Ki67 expression was analyzed by flow cytometry. D, Stimulated OT-1 CD8$^+$ T cells were labeled with CFSE (5 nM) and cultured in the presence (blue) or absence (red) of 50 ng/ml IL-27 for 72 h. Labeled cells were gated on CD8$^+$ T cells and analyzed by flow cytometry to compare the intrinsic proliferative capacity. E, Stimulated OT-1 CD8$^+$ T cells were cultured in the presence or absence of 50 ng/ml IL-27 for 96 h, stained for Annexin V and 7AAD, and analyzed by flow cytometry. All these data are shown as mean ± SD of three samples in each group and represent three experiments performed with similar results

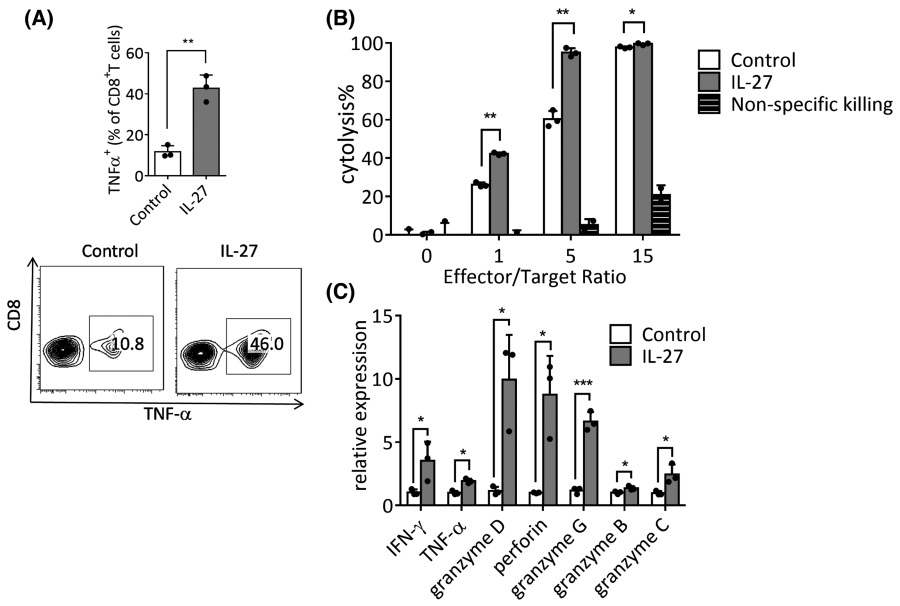

FIGURE 2 IL-27 enhances the antigen-specific cytotoxicity of activated OT-1 $CD8^+$ T cells. OT-1 $CD8^+$ T cells were cultured in the presence or absence of 50 ng/ml IL-27 for 5 d. A, OT-1 $CD8^+$ T cells producing TNF-α were analyzed by flow cytometry. B, Cytotoxicity assay using B16.OVA cells as targets and B16 cells as nonspecific killing controls. The effector functions of OT-1 $CD8^+$ T cells were quantified by CCK-8–based cytolysis assay. C, mRNAs involved in the effector functions of $CD8^+$ T cells were analyzed by qRT-PCR. Data are shown as mean ± SD of three samples in each group and represent two to three experiments with similar results

2.4 | OT-1 $CD8^+$ T cells generation

The OT-1 $CD8^+$ T cells were harvested from the spleens of CD45.1 OT-1 mice by CD8 MicroBeads (MACS) and cultured in 50 U/ml IL-2 (Shanghai Huaxin High Biotechnology INC) in complete media with or without 50 ng/ml rmIL27 (Sino Biological). The purified $CD8^+$ T cells were activated by Dynabeads Mouse T-Activator CD3/CD28 (Invitrogen) and split when confluent. These activated $CD8^+$ T cells were used for subsequent in vitro and in vivo tumor killing studies.

2.5 | Production of AAVs and retroviruses (RVs) with IL-27

AAV–IL-27 and AAV-ctrl were produced as we previously described[1] or produced by Genomedi Technology Corp. Retroviruses with IL-27 (RV-IL-27) were produced by compacting MigR1-IL-27 with a pCL-Eco help vector (Addgene) in 293T cells. The generated viruses were aliquoted and stored at −80°C before use.

2.6 | ELISA

ELISA kits for the detection of IL-27 levels in mouse blood serum or culture medium were purchased from eBiosciences. Standard procedures were followed to detect releases of cytokines in supernatants in a variety of settings.

2.7 | Real-time PCR

Quantitative real-time PCR was performed using previously determined conditions.[28] The primers used are listed in Table 1.

2.8 | Statistics

Statistical analyses were performed using GraphPad Prism software. Differences between different groups were analyzed by Student's *t* test. Kaplan-Meier analysis and log-rank test were used to evaluate the differences in animal survival. Ns indicates no significant difference. Data were considered statistically significant as follows: $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

3 | RESULTS

3.1 | IL-27 enhances $CD8^+$ T cells activation, proliferation, and antigen-specific cytotoxicity in vitro

Our initial aim was to evaluate the direct impact of IL-27 on $CD8^+$ T cells. We cultured the $CD8^+$ T cells separated from mice's spleens in the absence or presence of IL-27 in vitro. After culturing for 2, 6, and 9 hours, we analyzed the earliest T cell activation marker (CD69) expression.[29,30] CD69 expression with IL-27 treatment was generally higher than that without IL-27 (Figure 1A). CD25, a subunit of IL-2R (IL-2Ra), is also involved in the IL-27–related T cell activation and proliferation[31,32] (Figure 1B). In Figure 1C,D, IL-27 remarkably increased Ki67 expression in $CD8^+$ T cells and enhanced their proliferation ability. Furthermore, Annexin V/7AAD double-staining assay revealed IL-27 treatment can slow down $CD8^+$ T cells' apoptosis (Figure 1E). We found that IL-27 amplified TNF-α expression (Figure 2A) in cultured $CD8^+$ T cells. To investigate the cytotoxicity of $CD8^+$ T cells, we performed an in vitro T cell–killing assay using OT-1 mouse–derived $CD8^+$ T cells. In Figure 2B, the OT-1 $CD8^+$ T cells exposed to IL-27 were significantly efficient at killing target B16.OVA cells. We also found effector function genes (IFN-γ, TNF-α, granzyme B, C, D, G, and perforin, Table 1) were highly increased

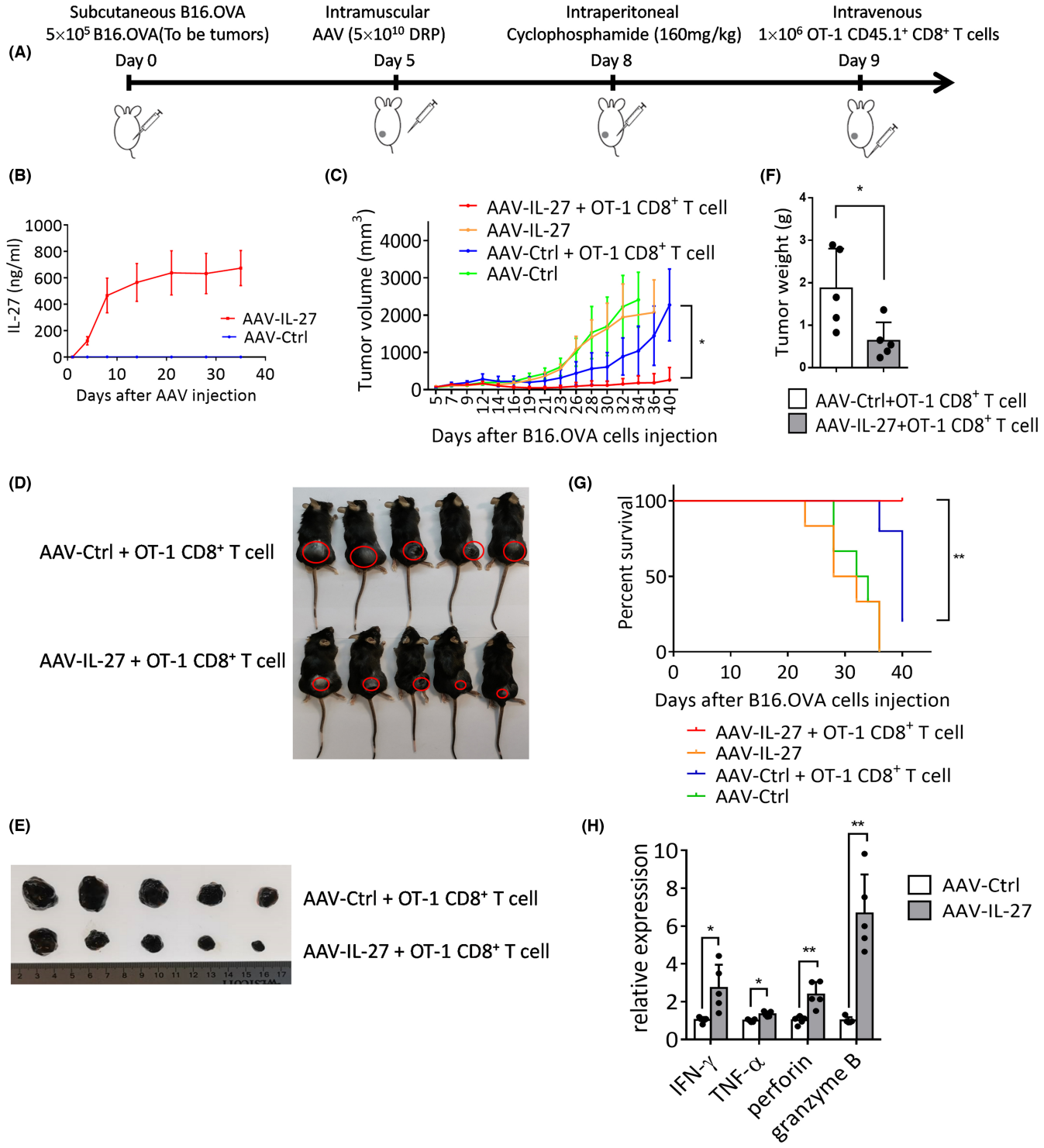

FIGURE 3 AAV-IL-27 treatment enhances the antitumor activity of adoptive OT-1 CD8⁺ T cells. (A), Schematic diagram of the combination therapy of AAV-IL-27 and adoptive OT-1 CD8⁺ T cells. Mice were injected with B16.OVA tumor cells subcutaneously. Five days later, tumor-bearing mice were treated with adeno-associated virus (AAV) followed by intravenous injection of OT-1 CD8⁺ T cells. Mice were intraperitoneally administered cyclophosphamide 24 h before OT-1 CD8⁺ cells treatment. (B), A single dose of AAV-IL-27 treatment resulted in sustained IL-27 production in mice. C57BL/6 mice injected with AAV-IL-27 were bled over time, and the concentrations of IL-27 in serum were detected by ELISA. (C), Tumor volumes of tumor-bearing mice with AAV or OT-1 CD8⁺ T cells treatment. Tumor volume changes (F), mice survival (G), and tumor weight (D, E) were examined. H, Gene expression of CD8⁺ T cells from mice treated with AAVs. Data represent mean ± SD of five samples in each group per time point and two experiments with similar results

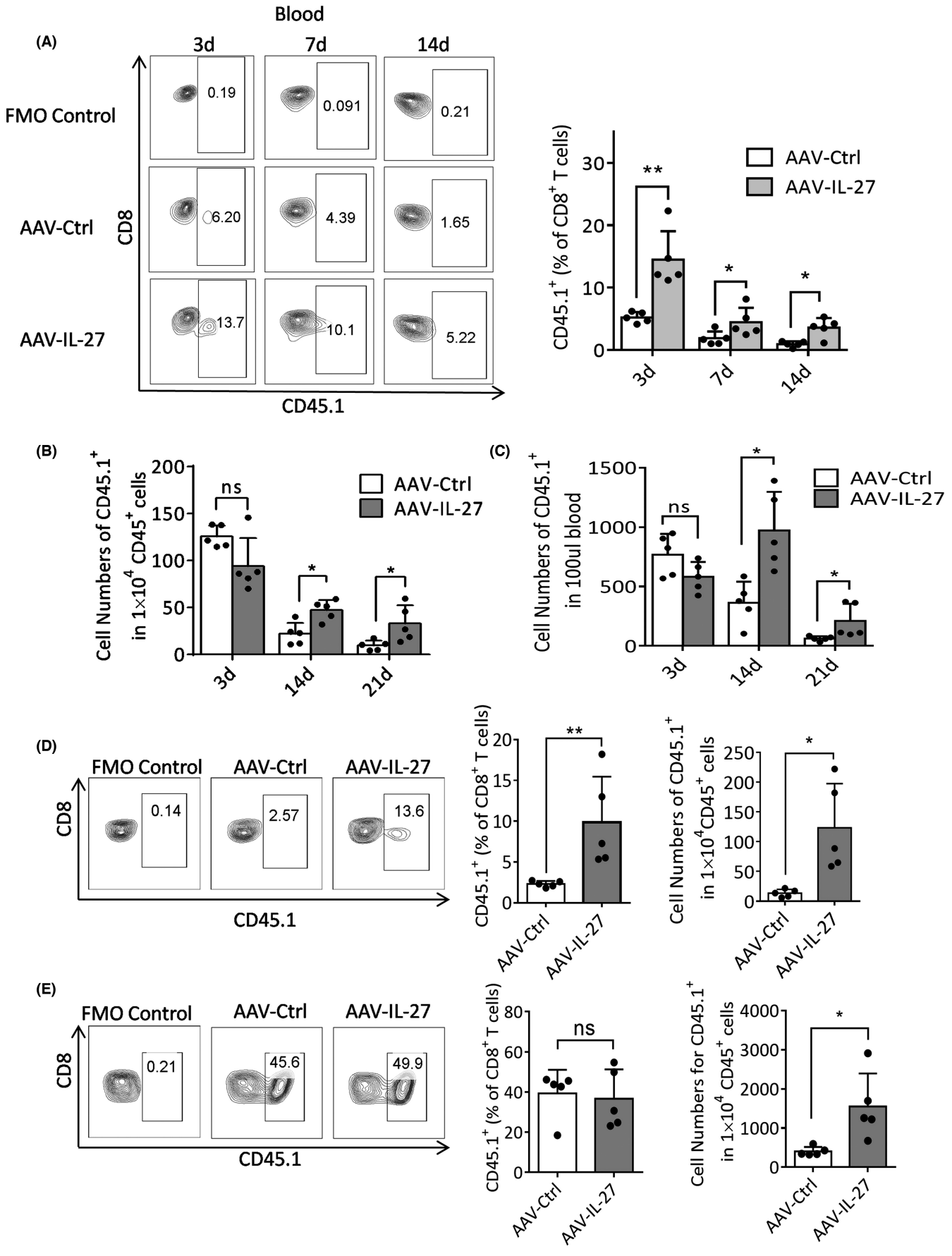

FIGURE 4 Legend on next page

FIGURE 4 IL-27 treatment improves the survival capacity of adoptive T cells. A-C, Tumor-bearing mice were treated with adeno-associated virus (AAV) and adoptive OT-1 CD45.1$^+$CD8$^+$ T cells. Mice were bled over time, and the percentages (A) and numbers (B, C) of CD45.1$^+$ T cells in blood were examined at the indicated time. Mice were sacrificed 28 d later. The percentages and numbers of surviving CD45.1$^+$ T cells in spleens (D) and tumor-infiltrating lymphocytes (TILs) (E), were examined. Data represent mean ± SD of five samples in each group per time point

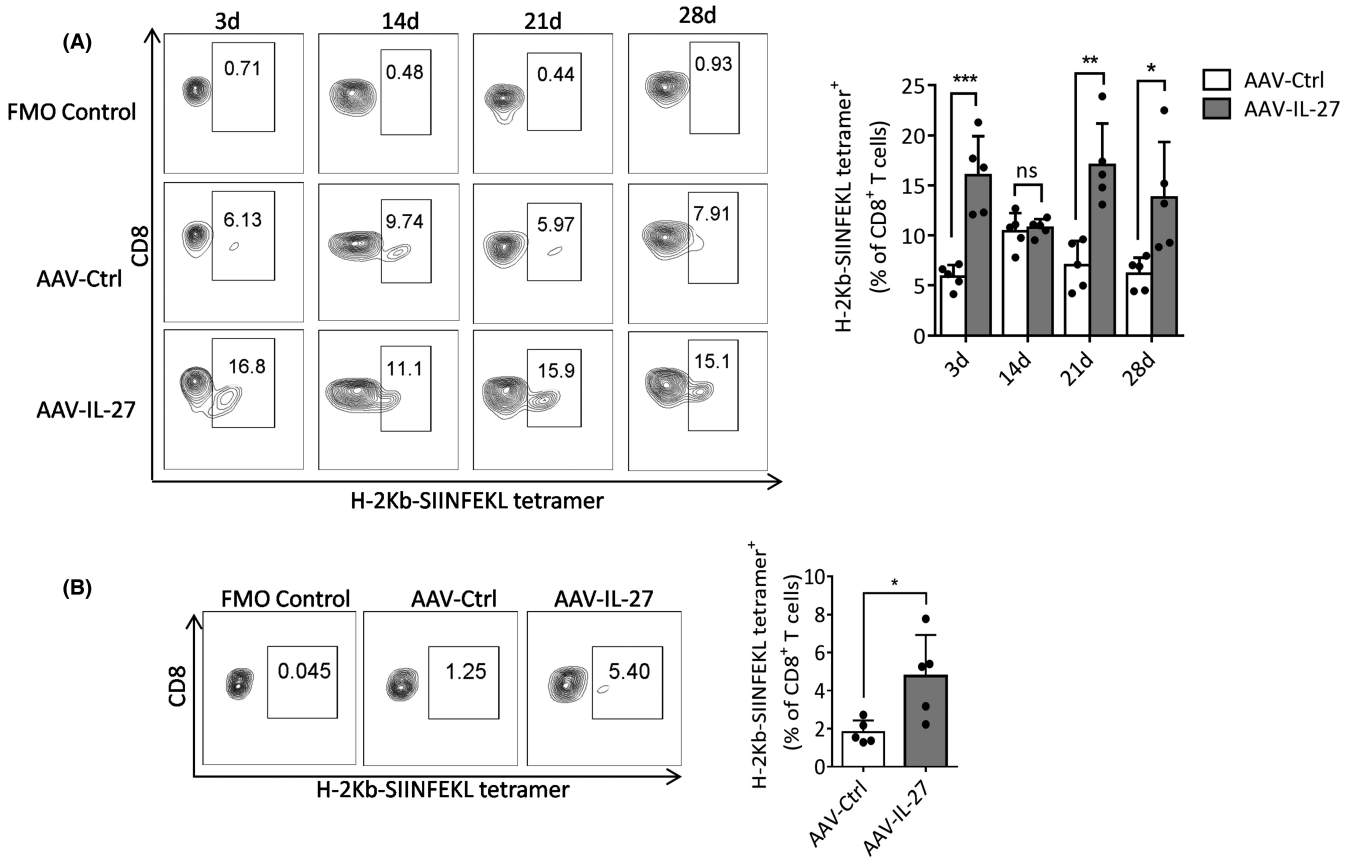

FIGURE 5 IL-27 treatment induced high frequency of $OVA_{257-264}$–specific CD8$^+$ T cells. A, B, Tumor-bearing mice were treated with adeno-associated virus (AAV) and adoptive OT-1 CD45.1$^+$CD8$^+$ T cells. Mice were bled over time, and the percentages of OVA-specific CD8$^+$ T cells in blood were examined at the indicated time. B, Mice were sacrificed 28 d later, and the percentages of OVA-specific CD8$^+$ T cells in spleens were examined. Data represent mean ± SD of five samples in each group per time point

in IL-27–treated OT-1 CD8$^+$ T cells (Figure 2C). Thus, our results suggest that IL-27 treatment stimulates CD8$^+$ T cells' activation and proliferation and boosts their antigen-specific cytotoxicity.

3.2 | AAV-IL-27 treatment enhances the antitumor activity of the adoptive T cells

Our above data indicates that IL-27 may be used as an adjuvant for adoptive T cell therapy. Our previous study used anAAV-IL-27 to treat mice and resulted in sustained IL-27 production in the blood of mice.[10,33] We established the B16.OVA xenograft model in C57BL/6 mice, and treatments were carried out as described in Figure 3A. Figure 3B shows a sustained IL-27 production in mice with AAV-IL-27 treatment. We observed that mice treated with OT-1 CD8$^+$ T cells and AAV-IL-27 showed significant tumor inhibition effect compared with those treated with OT-1 CD8$^+$ T cells and AAV-ctrl virus (Figure 3C-G). Meanwhile, the effector function genes (IFN-γ, TNF-α, granzyme B, and perforin) were significantly increased in IL-27–treated T cells (Figure 3H). Thus, AAV-IL-27 administration showed significant synergy with adoptive T cell therapy.

3.3 | IL-27 treatment improves the survival capacity of adoptive T cells and induces high frequency of OVA-specific T cells

We used OT-1 CD45.1$^+$CD8$^+$ cells as adoptive cells and examined their survival capacity in treated mice. An improved survival percentage of adoptive cells was observed in AAV-IL-27–treated mice from day 3 to day 14 (Figure 4A). We also analyzed the number of persistent adoptive T cells in CD45$^+$ cells and blood, and the survival improvements were also significant (Figure 4B,C). In Figure 4D,E, AAV-IL-27 treatment upregulated adoptive T cells' survival capacity

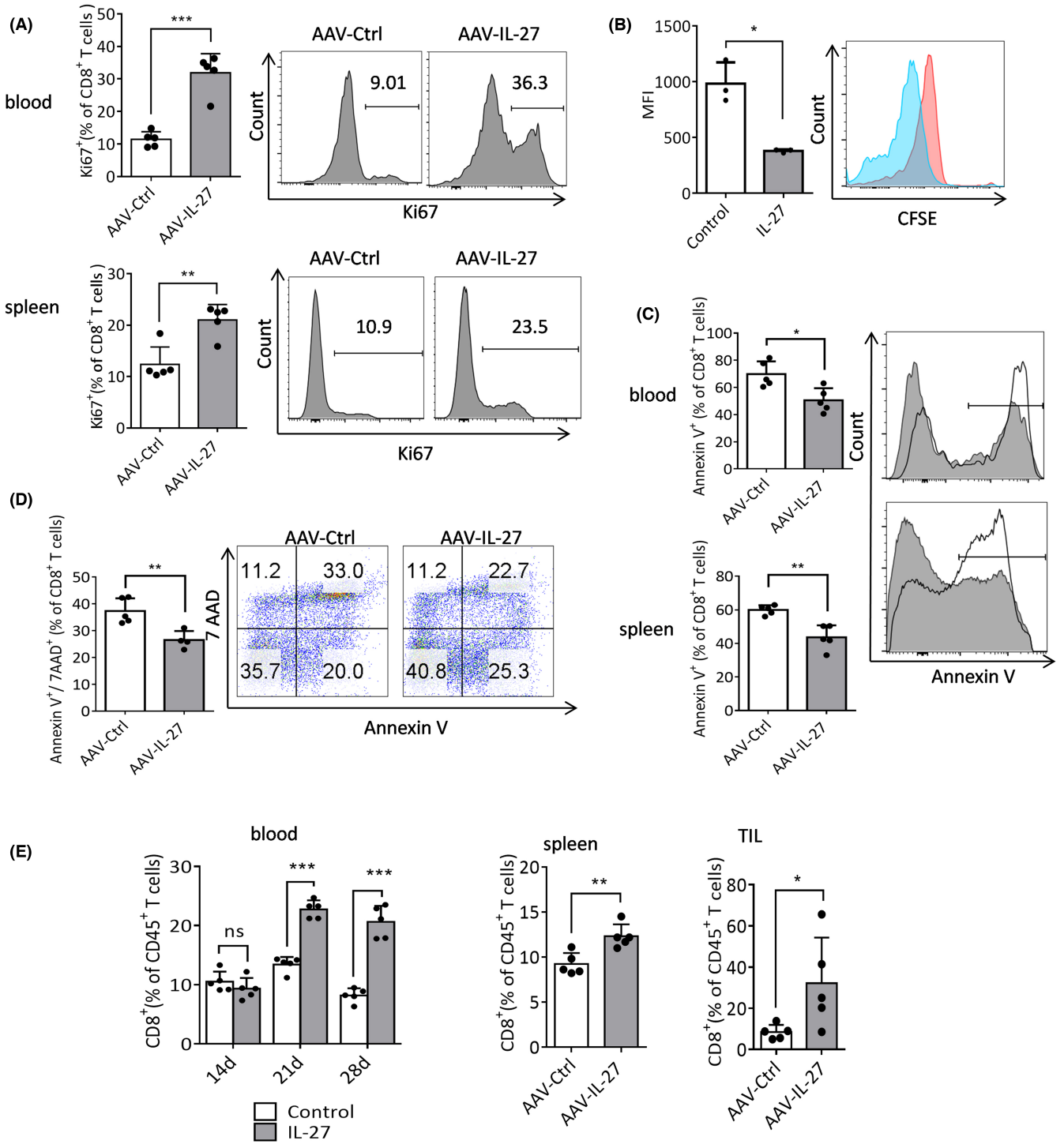

FIGURE 6 IL-27 treatment increases CD8$^+$ T cell proliferation and inhibits apoptosis in vivo. (A), Mice were treated with adeno-associated virus (AA)V, bled at 21 d, and sacrificed at 28 d after treatment. Ki67 expression of CD8$^+$ T cells in blood or spleens were analyzed by flow cytometry. (B), Cultured CD8$^+$ T cells were labeled with CFSE and transferred into the AAV-treated mice for 72 h. Labeled cells in blood were analyzed by flow cytometry. Blue line indicates AAV-IL-27 treatment, and red line indicates AAV-ctrl treatment. Apoptosis percentage (C, D) and total percentage (E) of CD8$^+$ T cells in blood, spleens, and tumor-infiltrating lymphocytes (TILs) from AAV-treated mice were analyzed by flow cytometry. Data are shown as mean ± SD of five samples in each group and represent two experiments with similar results

in spleens and TILs. The larger number of CD45.1$^+$ T cells also indicated the improvement of the localization of adoptive T cells with IL-27. We concluded IL-27 improved the survival capacity of adoptive cells, which is crucial for improved antitumor responses. We also found the percentage of OVA-specific CD8$^+$ T cells in tumor-bearing mice with AAV-IL-27 was higher than with AAV-Ctrl (Figure 5), and IL-27 treatment potentiated antigen-specific CD8$^+$ T cell generation in vivo.

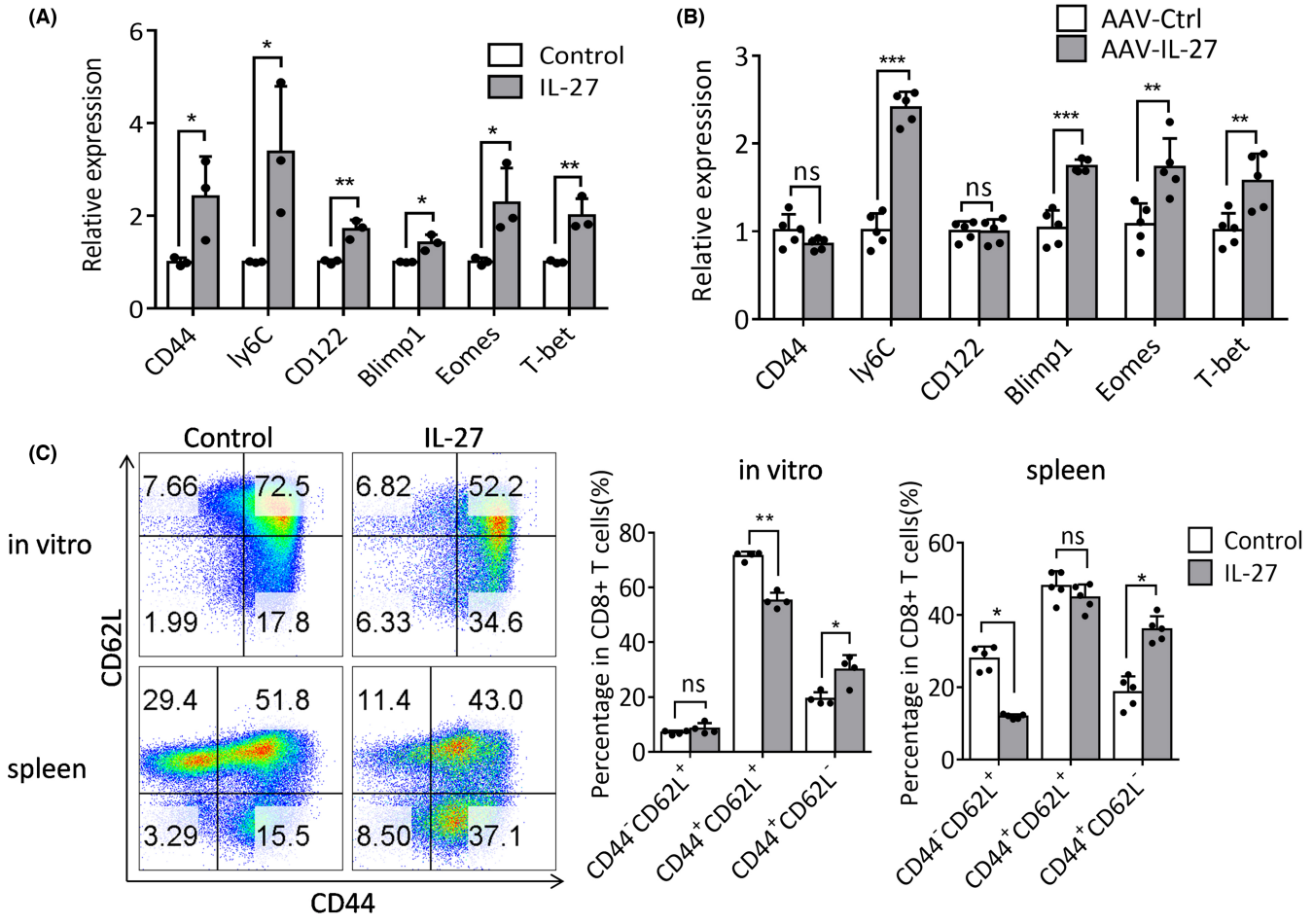

FIGURE 7 IL-27 treatment programs CD8$^+$ memory T cell differentiation. A, mRNA levels of genes involved in memory T cell differentiation in cultured CD8$^+$ T cells in the presence or absence of 50 ng/ml IL-27. B, mRNA levels of genes involved in memory T cell differentiation in CD8$^+$ T cells separated from mice treated with adeno-associated virus (AAV). C, The upper panel shows the cultured CD8$^+$ T cells in the presence or absence of 50 ng/ml IL-27 for 6 d. The lower panel shows CD8$^+$ T cells separated from mice treated with AAV for 21 d. Data are shown as mean ± SD of three or five samples in each group and represent two experiments with similar results

3.4 | IL-27 treatment enhances CD8$^+$ T cell proliferation and inhibits apoptosis in vivo

Ki67 is strictly associated with cell proliferation.[34] We analyzed Ki67 expression in CD8$^+$ T cells from treated mice. In Figure 6A, CD8$^+$ T cells from mice with AAV-IL-27 shows higher proliferation ability than controls. Figure 6B shows CFSE-labeled CD8$^+$ T cells proliferate rapidly in AAV-IL-27–treated mice. IL-27 treatment resulted in substantially reduced cell apoptosis in CD8$^+$ T cells (Figure 6C,D). Last, we found AAV-IL-27 treatment significantly increased the percentages of CD8$^+$ T cells in blood, spleens, and TILs (Figure 6E), which was consistent with enhancing CD8$^+$ T cell proliferation and inhibiting apoptosis effect.

3.5 | IL-27 treatment programs memory CD8$^+$ T cell differentiation

To further characterize the effects of IL-27 for CD8$^+$ T cells, we examined various genes associated with CD8$^+$ T cell differentiation.[35-38] CD122, CD44, and Ly6C have been verified to be highly expressed in memory T cells. Blimp1, Eomes, and T-bet have been shown to promote memory T cell differentiation. Figure 7A,B shows IL-27 significantly upregulated the mRNA of Ly6C, Blimp1, Eomes, and T-bet in CD8$^+$ T cells in vitro and in vivo (Table 1). We failed to detect the upregulated expression of CD44 and CD122 in vivo, which may be due to the low activation status of T cells in spleens. Memory T cells can be separated into two populations: CD62L$^+$CD44$^+$ (central memory) and CD62L$^-$CD44$^+$ (effector memory) T cells.[39,40] Figure 7C shows that the effector memory T cells were significantly enriched after being treated with IL-27 both in vitro and in vivo.

3.6 | IL-27 delivered via tumor-specific T cells is a feasible approach for cancer immunotherapy

We have previously demonstrated that AAV-IL-27 enhanced adoptive T cells' antitumor activity. We had generated a retroviral vector with IL-27 (RV-IL-27, Figure 8A) to modify OT-1 CD8$^+$

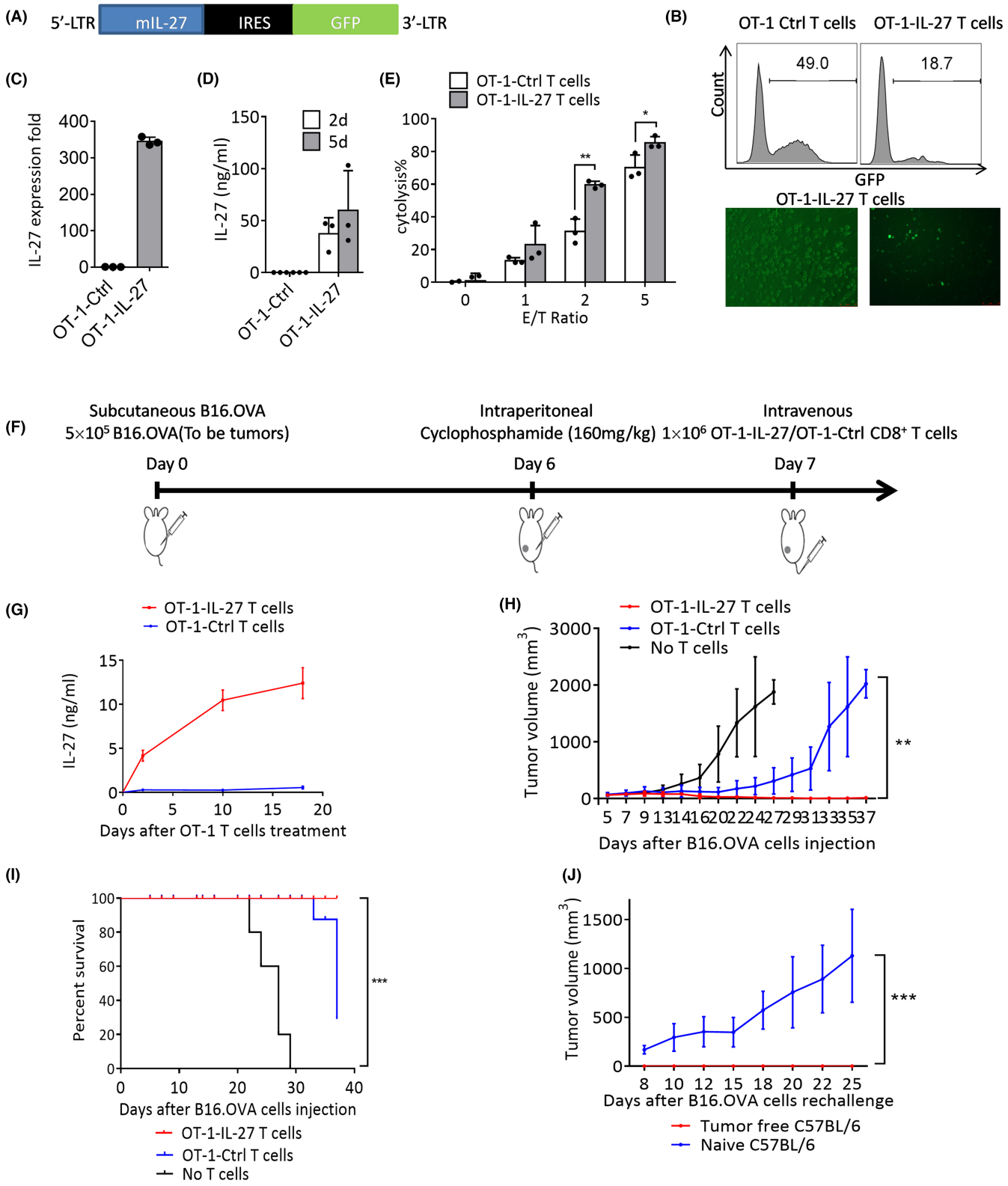

FIGURE 8 IL-27 delivered via OT-1 CD8⁺ T cells enhances adoptive T cells' antitumor activity. (A), Schematic of retroviral vector expressing mouse IL-27 and GFP. (B), Flow cytometry analysis of RV-IL-27-GFP– and RV-GFP–infected OT-1 CD8⁺ T cells (upper panel). Cultured cells were photographed under a fluorescence microscope (lower panel). IL-27 mRNA expression (C) in RV-IL-27-GFP T cells and IL-27 protein (D) in the cell culture supernatants were quantified by qPCR or ELISA. (E), Cytolysis assay of OT-1-IL-27 CD8⁺ T cells. Effector cells and target cells were cocultured for 24 h, and target cell cytolysis was quantified by CCK-8 assays. (F), Schematic diagram for adoptive OT-1-IL-27/OT-1-Ctrl CD8⁺ T cells. Mice were subcutaneously injected with B16.OVA tumor cells. Six days later, mice were intraperitoneally administered cyclophosphamide, followed by intravenous injection of OT-1-IL-27/OT-1-Ctrl CD8⁺ T cells. C57BL/6 mice injected with OT-1-IL-27 were bled over time, and the concentrations of IL-27 in serum were detected by ELISA (G). Tumor volume changes (H) and mice survival (I) were examined and shown. J, B16.OVA cells were injected into mice whose tumors were rejected after OT-1-IL-27 therapy and naive C57BL/6 mice. Tumor growths were compared. Data are shown as mean ± SD of four to five samples in each group

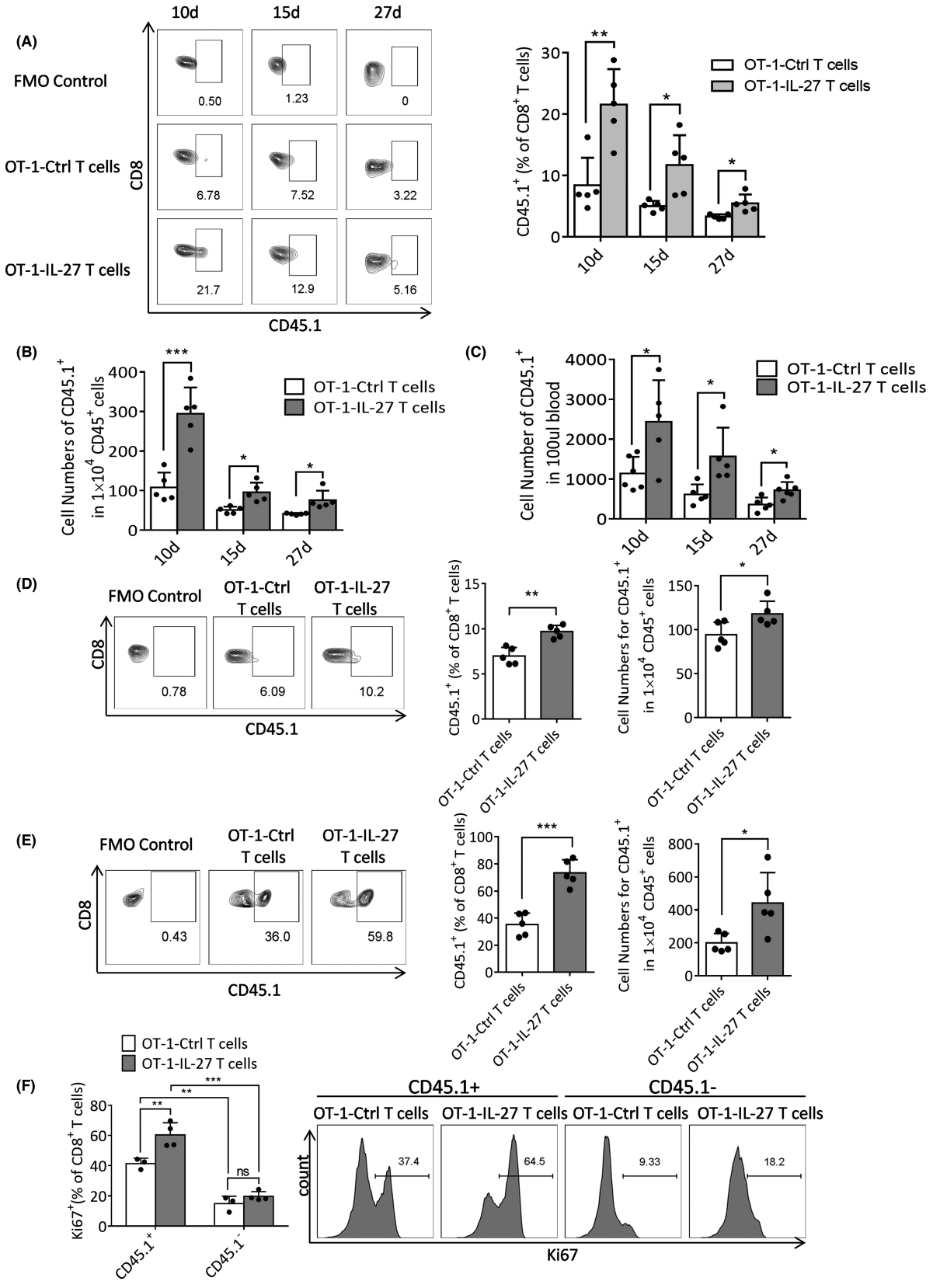

FIGURE 9 IL-27 delivered via OT-1 $CD8^+$ T cells improves the survival capacity of adoptive T cells. A-C, Tumor-bearing mice were treated with adoptive OT-1-Ctrl or OT-1-IL-27 $CD45.1^+CD8^+$ T cells. Mice were bled over time, and the percentages (A) and numbers (B, C) of $CD45.1^+$ T cells in blood were examined at the indicated time. Mice were sacrificed 28 d later. Percentages and numbers of surviving $CD45.1^+$ T cells in spleens (D) and tumor-infiltrating lymphocyte (TILs) (E) were examined. (F), Ki67 expression of $CD45.1^+CD8^+$ and $CD45.1^-CD8^+$T cells in TILs were analyzed. Data represent mean ± SD of three to five samples in each group per time point

T cells and cultured the cells for about 4 days (Figure 8B,C,D). As Figure 8E shows, OT-1-IL-27 cells exhibited superb cytotoxicity against B16.OVA cells. Additionally, we treated mice bearing tumor with OT-1-IL-27 or OT-1-Ctrl T cells as shown in Figure 8F. Figure 8G shows a sustained IL-27 production in mice with OT-1-IL-27 treatment. Tumor growth inhibition and prolonged survival was observed in OT-1-IL-27–treated mice (Figure 8H,I). A defining characteristic of memory T cells is rapid responsiveness to antigen and providing a more effective protection from reinfection.[41] So we reinjected B16.OVA cells into mice whose tumors were inhibited and found that these mice were resistant to B16.OVA cell rechallenging (Figure 8J). An improved survival percentage of CD45.1$^+$ T cells was observed in OT-1-IL-27–treated mice in blood, spleens, and TILs (Figure 9A,D,E). We analyzed the number of persistent CD45.1$^+$ T cells in CD45$^+$ cells from blood, spleens, and TILs. The survival improvement of adoptive T cells was also significant (Figure 9B,C). We also found OT-1-IL-27 treatment remarkably increased Ki67 expression in CD45.1$^+$CD8$^+$ TILs (Figure 9F). The above study demonstrated that IL-27 delivered via tumor-specific T cells has the potential to be used for the immunotherapy of cancer.

4 | DISCUSSION

In this article, we have found that IL-27 directly improves the survival status and cytotoxicity of adoptive CD8$^+$ T cells to inhibit tumor growth. Furthermore, IL-27 treatment programs effector memory T cell (CD62L$^-$CD44$^+$) differentiation, characterized by the upregulation of genes associated with effector functions and memory differentiation. We also demonstrated that IL-27 delivered via tumor-specific T cells enhances adoptive T cell cancer immunity and has the potential to be used for the immunotherapy of cancer.

Cytotoxic antigen–specific CD8$^+$ T cells are an important component of successful antitumor immune responses; therefore, predictably, most cancer therapies aim to expand tumor antigen–specific CD8$^+$ T cells. In our study, IL-27 was a cytokine which can potentiate tumor-specific CD8$^+$ T cells' expanding (Figure 5). This outcome may be due to several mechanisms. First, IL-27 treatment enhances CD8$^+$ T cell proliferation and inhibits apoptosis in vivo. Second, priming and expanding tumor-specific CD8$^+$ T cell requires the interaction between T cells and antigen-presenting cells, where antigen-derived peptides in complexes with MHC class I molecules are presented to TCRs. Previous studies have reported IL-27 plays a key role in activation and proliferation of antigen-presenting cells,[42,48,49,50] which indicates the enhanced presenting capacity of antigen-presenting cells with IL-27 treatment. Although the mechanism is not clear yet, it predicts a new avenue to clarify the antitumor activity of IL-27. Third, IL-27–mediated depletion of Tregs (Figure S1) can bypass the obstacle of immune inhibition and lymphoinhibition prior to T cell transfer, which makes room for T cell homeostatic proliferation.[51] Thus, our results suggest that IL-27 can potentiate tumor-specific CD8$^+$ T cell expansion and is a potential adjutant combined with T cell adoptive transfer therapy.

We have found IL-27 induces a memory T cell phenotype (CD62L$^-$CD44$^+$) with a unique effect, characterized by upregulation of genes associated with effector functions (IFN-γ, TNF-α, granzyme B, and perforin) and T cell memory differentiation (T-bet, Eomes, Blimp1, and Ly6C). The memory T cells have a robust proliferative potential, long-term survival capacity, and the ability to mediate superior tumor regression upon adoptive transfer into tumor-bearing mice.[35,52] These are consistent with the improved survival capacity, enhanced proliferation ability, and antitumor functions of the adoptive CD8$^+$ T cells with IL-27 treatment.

IL-27 is a member of the IL-12 cytokine family that consists of an EBV-induced gene 3 (EBI3, and a p35-related subunit (p28).[42] Accumulating evidence from previous studies has indicated that both endogenous[43-46] IL-27 and exogenous[22,47,48] IL-27 enhance tumor immunity and inhibit tumor growth. Our studies have also revealed that IL-27 plays a pivotal role in CD8$^+$ T cell proliferation and cytotoxicity and programs CD8$^+$ T cells into a memory cell phenotype which enhances the antitumor effect of adoptive T cells in mice. In Figure 8, although only about 20% of OT-1 CD8$^+$ T cells delivered IL-27 gene, nearly all the tumors of mice receiving OT-1-IL-27 CD8$^+$ T cells shrunk and disappeared. The IL-27 level in mice's blood with OT-1-IL-27 T cells transferred (Figure 8G) was lower compared with AAV-IL-27–treated mice; however, IL-27 improved the status of transferred CD8$^+$ T cells (Figure 9) and the tumor microenvironment (Figure S2), which leads to the tumor inhibition in OT-1 CD8$^+$ T-IL-27–transferred mice. Moreover, we found mice that rejected B16.OVA tumors were completely resistant to rechallenging (Figure 8J), which was evidence for the immune cell memory differentiation with IL-27 treatment. The unique functions of IL-27 demonstrate that it could be an attractive candidate to enhance tumor immunity and an agent applicable in adoptive T cell therapy against cancers.

Improvements to T cell culture systems that promote long-term engraftment and function of adoptive T cells will likely result in superior clinical benefit to more individuals. Porter et al. observed a strong correlation between T cell persistence and improved clinical responses, suggesting that efforts to enhance persistence of engineered T cells will result in improved clinical responses.[53] Our results have implications for the design of human adoptive immunotherapy trials. In our currently used protocols, the cells generated for adoptive T cell transfer acquire memory T cell attributes before transfer. Our data suggest that adoptive T cell transfer of these populations may be appropriate. Their transfer might increase the proliferation and persistence of cells upon adoptive transfer in vivo. Our findings may have significant implications for the generation of more appropriate antitumor T cells for adoptive cell transfer in cancer patients in human clinical trials.

Taken together, we have found that IL-27 directly improved the survival status and cytotoxicity of adoptive CD8$^+$ T cells in vivo.

Furthermore, IL-27 treatment programs effector memory T cell ($CD62L^{-}CD44^{+}$) differentiation, characterized by the upregulation of the expression levels of genes associated with effector functions (IFN-γ, TNF-α, granzyme B, and perforin) and T cell memory differentiation (T-bet, Eomes, Blimp1, and Ly6C). Finally, we demonstrated the feasibility of engineering tumor-specific T cells to deliver IL-27 to tumors and develop practical approaches for engineering T cells to secrete IL-27 for cancer immunotherapy. Thus, the proposed studies demonstrate IL-27 is a feasible approach for enhancing $CD8^{+}$ T cells' antitumor immunity and can be used as a therapeutic adjutant of T cell adoptive transfer for the treatment of cancer.

DISCLOSURE

The authors have no conflict of interest.

ETHICAL APPROVAL

The research protocol was approved by the Institutional Reviewer Board. The animal studies were reviewed and approved by the Institutional Ethics Committee for Animal Research of Shanghai Childrens' Medical Center.

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SUPPORTING INFORMATION

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