

In vitro effects of *Staphylococcus aureus* enterotoxin C3 on T cell activation, proliferation and cytokine production

YIXIN XIE¹, MIN WANG¹, ZHIHUI DONG¹, HUAN SONG¹, LIANPING LI¹, MIN YANG¹, PENG LING LI¹, JINGJING TIAN¹, KAN ZHANG¹, XIAOMENG XIA², TINGTING ZHANG¹ and AIGUO TANG¹

Departments of ¹Laboratory Medicine, and ²Obstetrics and Gynecology,
The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, P.R. China

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Abstract. The present study aimed to investigate the effects of *Staphylococcus aureus* enterotoxin C3 (SEC3), including recombinant (r)SEC3 protein and lentivirus-mediated SEC3, on the activation, proliferation and cytokine production of human T cells. HeLa cells were infected with SEC3 lentiviral vector (LV-SEC3) and viability was determined using the Cell Counting Kit-8 (CCK-8) assay. Subsequently, infected cells or rSEC3 protein were co-cultured with human peripheral blood mononuclear cells (PBMCs) for 10 days, after which the culture supernatant and T cells were incubated with untreated HeLa cells, which were subjected to a CCK-8 assay to determine cytotoxicity. In addition, IL-6 and IFN- γ expression was detected by chemiluminescence and enzyme-linked immunospot analyses, respectively. Subpopulations of activated T cells were sorted by flow cytometry. The results demonstrated that, following infection with LV-SEC3 or negative control lentiviral vector (LV-NC), >80% of HeLa cells presented green fluorescent protein-positive signals. All five groups of co-cultured T cells exhibited proliferation. Co-culture of PBMCs with rSEC3 protein or LV-SEC-infected cells resulted in elevated IL-6 and IFN- γ secretion. In addition, rSEC3-activated and monocultured T cells were predominantly cluster of differentiation (CD)4⁺ (62.7 and 59.6%, respectively) whereas phytohemagglutinin-stimulated T cells were predominantly CD8⁺ (57.8%). Compared with the LV-NC group, T cells and culture supernatants from the LV-SEC3 group significantly attenuated proliferation of HeLa cells. These results suggest that rSEC3 protein, and LV-SEC3-infected HeLa cells, are able to potentially activate T cells, increasing cytokine production and amplify the antitumor immune response.

Introduction

Cervical cancer affects women worldwide, with >500,000 new cases and 275,000 cases of mortality reported annually, according to GLOBOCAN in 2013 (1). Chemotherapy, radiotherapy and surgery are the three most common therapeutic strategies used to treat cervical cancer, and along with improved screening programs have markedly increased patient survival and quality of life. However, improved treatment paradigms with reduced toxicity and risk of recurrence are urgently required. Cancer gene therapy and immunotherapy have garnered attention among clinical studies. By targeting the underlying genetic mechanisms, gene therapy is considered a promising alternative for the conventional treatment of cancer, as evidenced by a significant number of clinical trials (2-4). Cancer immunotherapy, including cancer vaccines, adoptive T cell therapy, immune checkpoint blockade and immune-modulating agents, has exhibited promising clinical effects as a novel therapeutic approach. The US Food and Drug Administration has approved the use of sipuleucel-T to treat prostate cancer and ipilimumab to treat malignant melanoma, and numerous phase I and II clinical trials of cancer immunotherapy are in development, and the preliminary results are promising (5).

T cells are well known for their various potent antitumor effects through the release of cytotoxic effector molecules, including perforin or cytokines, such as interleukin (IL)-6, IL-8, interferon (IFN)- γ and tumor necrosis factor (TNF)- α . However, tumor-specific T cells are often suppressed or are present in low numbers; therefore, they are unable to efficiently attack tumor cells. Through binding to the T cell receptor β chain, superantigens, such as *Staphylococcus aureus* enterotoxins (SEs) are able to stimulate a large fraction of T cells [cluster of differentiation (CD) 4⁺ and CD8⁺], and consequently help to boost the antitumor immune response (6). In the last decade, numerous studies have demonstrated the association between superantigens and cancer immunotherapy, including using SEA to increase the antitumor ability of oncolytic adenovirus in bladder cancer (7), employing retrovirus-mediated toxic shock syndrome toxin-1 to promote cytotoxicity against colorectal cancer LoVo cells (8), and fusing superantigens to the Fab moiety of a tumor-reactive monoclonal antibody to achieve therapeutic goals (9). Furthermore, coupling of SEA with the CD80 transmembrane region driven by specific

Correspondence to: Professor Min Wang, Department of Laboratory Medicine, The Second Xiangya Hospital, Central South University, 139 Middle Renmin Road, Changsha, Hunan 410011, P.R. China
E-mail: wangmin0000@aliyun.com

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tumor antigen enhancer/promoter (10), or anchoring SEA with the hydrophobic transmembrane domain on tumor-derived exosomes, efficiently induced tumor-specific T cells (11), which meets the demands of personalized medicine and precision medicine. Therefore, superantigens may exhibit potential in cancer vaccination and treatment, with precise tumor targeting and decreased systemic toxic side effects.

SEC is a type of SE, which has already been clinically used as a supplementary medicine for tumor treatment in China, due to its ability to stimulate proliferation of T cells (12). There are three SEC subtypes (C1-C3), which are classified according to minor epitope differences. Compared with SEC2, there has been relatively less research conducted on the antitumor effects of SEC3 (12,13). Therefore, it may be hypothesized that SEC3 exhibits the same, or even stronger, antitumor effects and may be used as a powerful therapeutic agent. According to previous unpublished results from our group, lentivirus-mediated SEC3 expression may induce cytotoxic effects on HeLa cells, and affect proliferation, migration and invasion. In the present study, HeLa cells were infected with a previously constructed SEC3 lentiviral vector (LV-SEC3), and the effects of LV-SEC3-infected cells and purified recombinant (r)SEC3 protein on T cell proliferation, activation and cytokine secretion were determined.

Materials and methods

Ethical approval. The present study was approved by the ethics committee of The Second Xiangya Hospital, Central South University (Changsha, China). Written informed consent was obtained from the patient.

Cell culture. HeLa cervical cancer cells were purchased from Shanghai Genechem Co., Ltd. (Shanghai, China). The cells were plated in a 25 cm² flask and were maintained in complete medium, which comprised 89% high-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd., Hangzhou, China) and 1% penicillin (100 U/ml)/streptomycin (100 µg/ml) (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) at 37°C in an atmosphere containing 5% CO₂.

Extraction of human peripheral blood mononuclear cells (PBMCs). Venous blood samples were acquired from a 22-year-old healthy female volunteer recruited in March 2016 from The Central South University (Changsha, China) in a lithium heparin tube and human PBMCs were separated by density gradient centrifugation using lymphocyte separation medium (Tianjin MD Pacific Technology Co., Ltd., Tianjin, China). Briefly, 5 ml RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.) was diluted 1:1 with the blood sample, which was carefully layered onto 5 ml lymphocyte separation medium. The mixture was then centrifuged at 1,000 x g for 22 min at 20°C, and the PBMCs were collected and washed two times with RPMI-1640 (700 x g for 7 min and 400 x g for 7 min, respectively). Subsequently, the sediment containing PBMCs was gently resuspended in complete medium for further use.

Cell transduction with lentiviral vectors. HeLa cells at 80% confluence were trypsinized using a 0.25% trypsin-0.02% EDTA solution (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China), were seeded in 6-well plates (10⁵ cells/well) with complete medium and cultured at 37°C in an atmosphere containing 5% CO₂. After 24 h of attaching and growing (30-50% cell confluence), the cells were infected with LV-SEC3 (Shanghai GeneChem Co., Ltd, Shanghai, China) or an empty negative control lentiviral vector (LV-NC), without the SEC3 gene (Shanghai GeneChem Co., Ltd.), at multiplicity of infection of 5, with the medium containing 5 g/ml polybrene and enhanced infection solution (Shanghai GeneChem Co., Ltd.). A total of 16 h post-infection, the medium was discarded and replaced with high-glucose DMEM. The growth status of infected cells was observed every day for a total of 5 days, and images were captured under an inverted light microscope (Zeiss AG, Oberkochen, Germany) and a fluorescent microscope (Leica Microsystems GmbH, Wetzlar, Germany).

Cell viability assay and co-culture with PBMCs. After 5 days of infection, LV-SEC3- and LV-NC-infected cells were harvested, counted and were sub-cultured in a 96-well plate (2,000 cells/well) for viability assay and a 6-well plate (10⁵ cells/ml) for co-culture in complete medium at 37°C with 5% CO₂. Cell viability was measured after 3 days using a standard Cell Counting kit-8 assay (CCK-8; Sangon Biotech Co., Ltd., Shanghai, China) according to the manufacturer's protocol. Briefly, 10 µl CCK-8 solution was added to each well (100 µl cell suspension), and absorbance at 450 nm was measured using a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) following a 2 h incubation. Each group included 5 parallel wells.

Following counting with a blood cell counter (XS500i; Sysmex Corporation, Kobe, Japan), the PBMCs were diluted in complete medium and were added to the attached infected HeLa cells at a final ratio of 10 volumes PBMCs:1 volume LV-infected HeLa cells for co-culture, according to a previous study (14). The experiment was divided into five groups: i) PBMCs co-cultured with LV-SEC3-infected HeLa cells; ii) PBMCs co-cultured with LV-NC-infected HeLa cells; iii) PBMCs treated with purified rSEC3 protein stored in our laboratory, 1 µg/ml/well; iv), PBMCs treated with phytohemagglutinin (PHA; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany; 100 µg/ml/well), as a positive control; and v) PBMC monoculture, as a negative control. The total volume was 2 ml per well, and the density of PBMCs was 10⁶/ml. In addition, 50 U/ml IL-2 (Jiangsu Kingsley Pharmaceutical Co., Ltd., Yixing, China) was added to maintain T cell activity after an overnight culture. At days 4 and 7, 1 ml fresh complete medium was mixed into each well. T cells were stained with Wright-Giemsa dye (Beijing Dingguo Changsheng Biotechnology Co., Ltd., Beijing, China) every day to observe morphological alterations.

rSEC3 was obtained from a prokaryotic recombinant plasmid SEC3-pET-32a, which has been described in our previous study (15). Briefly, the total bacterial genomic DNA was extracted from *Staphylococcus aureus* cells using a DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's protocol. SEC3 was detected

by PCR with primers synthesized using Primer 5.0 (forward, 5'-CCGAATTCATGGAGAGCCAACCAGACCCT-3' and reverse, 5'-CCGCTCGAGTTATCCATTCTTTGTTGT-3') in a 50 μ l reaction system containing 5 μ l 10X Taq Buffer (Mg^{2+} plus), 4 μ l dNTP (10 mmol/l), 1 μ l of each primer, 4 μ l template DNA (2 mmol/l), 1 μ l Taq DNA polymerase (5 U/ μ l) and 34 μ l ddH₂O. Thermocycling conditions were as follows: At 95°C for 4 min, followed by 35 cycles at 94°C for 1 min, at 55°C for 1 min, at 72°C for 1 min, with a final extension at 72°C for 10 min. PCR-generated amplicons were separated on a 2% agarose gel at 100 V for 30 min. The 720 bp PCR product was recovered using a DNA gel recovery and purification kit (Tiagen Biotech Co., Ltd.), and mixed with the pUCm-T vector (Sangon Biotech Co., Ltd) overnight at 16°C, then transformed to *Escherichia coli* DH5 α competent cells and inoculated into Luria Bertani (LB) medium (Ameresco, Inc., Framingham, MA, USA) without ampicillin (AMP). Following gentle agitation at 37°C for 45 min, the culture was incubated in LB agar plates, which containing 1 mmol/l isopropyl β -D-1-thiogalactopyranoside (Ameresco, Inc.) and 100 μ g/ml AMP (Sigma-Aldrich; Merck KGaA) overnight at 37°C. Positive colonies were screened and identified by PCR, and culture was continued in AMP-containing LB liquid medium at 37°C. Following overnight agitation, the plasmids were extracted using a plasmid extraction kit (Tiagen Biotech Co., Ltd.) and digested with restriction enzymes *Eco*RI and *Xho*I (Fermentas; Thermo Fischer Scientific, Inc.). Positive bacteria were sequenced by Sangon Biotech Co., Ltd. The correct recombinant plasmid SEC3-pUCm-T and the pET-32a (+) (Sangon Biotech Co., Ltd.) expression vector was digested with *Eco*RI and *Xho*I, mixed for connection at 16°C overnight, and transduced into *Escherichia coli* DH5 α competent cells to obtain SEC3-pET-32a-positive bacteria as aforementioned. SEC3-pET-32a plasmids were extracted and transduced into *Escherichia coli* BL21 (DE3) competent cells (Sangon Biotech Co., Ltd.) overnight at 37°C in LB medium containing 100 μ g/ml AMP. Isopropyl β -D-1-thiogalactopyranoside was added at a concentration of 1 mmol/l and cells were cultured for 4 h, centrifuged at 3,000 \times g for 5 min at 4°C, and collected cells were suspended in buffer A (50 mmol/l Tris-HCl, pH 8.0; Roche Diagnostics, Basel, Switzerland). Bacterial cells were repeatedly freeze-thawed and the sediments containing rSEC3 were centrifuged at 3,000 \times g for 5 min at 4°C, collected and dissolved in a buffer A containing 8 mol/l urea (Borer Chemie AG, Zuchwil, Switzerland).

Detection of T cell proliferation, classification and IL-6 secretion. After co-culturing for 10 days, the activated T cells were separated from the supernatant following centrifugation for 5 min at 300 \times g at 20°C. T cells were resuspended in RPMI-1640, counted following Trypan Blue staining, then diluted to the appropriate cell number for future experiments. T lymphocyte subtypes were sorted by flow cytometry using a FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's protocol. Furthermore, the supernatant of all five groups was tested for IL-6 using a cobas4000 chemiluminescence analyzer (Roche Diagnostics) with an IL-6 diagnostic kit (cat. no. 12150202; Roche Diagnostics), according to the manufacturer's protocol.

Enzyme-linked immunospot (ELISPOT) assay for interferon (IFN)- γ . The T-SPOT[®].TB kit (Oxford Immunotec, Inc., Commerce, CA, USA) was used for IFN- γ ELISPOT. According to the manufacturer's protocol, each well of a 96-well plate was coated with a mouse IFN- γ monoclonal antibody, after which 250,000 \pm 50,000 viable T cells per well were transferred onto plate for all five groups; fresh extracted T cells were applied as a negative control. Following a 16-20 h incubation at 37°C and 5% CO₂, the contents were discarded and each well was washed 3-4 times with PBS with force. Subsequently, 50 μ l working strength Conjugate Reagent solution was pipetted into each well and the plate was incubated at 2-8°C for 1 h. The conjugate was discarded and 4 PBS washes were performed, followed by the addition of 50 μ l substrate solution to each well and further incubation at room temperature for 7 min. After thoroughly washing the plate with distilled water to terminate the detection reaction, the plate was thoroughly dried and images were captured using the T-SPOT[®].Tutor magnifying imager (Oxford Immunotec, Inc.).

Cytotoxic assay of CTLs. The ability of activated PBMCs [effector cells, cytotoxic T cells (CTLs)] to mediate cytotoxicity to HeLa cells was determined by culturing uninfected HeLa cells with T cells following activation by purified rSEC3 protein or LV-infected cells. Briefly, the HeLa cells (2,000 cells/well) were initially treated with 20 μ g/ml Mitomycin C (Meilun Biotech Co., Ltd., Dalian, China) for 60 min at 37°C; after being maintained in complete medium overnight, the medium was replaced with activated T cells at a ratio of 10:1. A total of 3 days later, cell suspensions were discarded and substituted with complete medium. HeLa cells cultured without stimulation were considered the control group. Cytotoxicity was detected using the CCK-8 assay, as aforementioned. With the exception of the addition of activated T cells, the cytotoxic effects of the supernatant (100 μ l/well) from all five groups were measured in the same manner.

Statistical analysis. Data are presented as the mean \pm standard deviation of 3 independent experiments. Analyses were conducted using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA). Statistical analysis was performed using independent samples Student's t-test or one-way analysis of variance followed by a least significant difference post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Cellular morphology and viability. As presented in Fig. 1, >80% of HeLa cells presented green fluorescent protein-positive signals following infection with LV-SEC3 or LV-NC, indicating the high efficiency of infection. The images were photographed 72 h postinfection at 100 \times magnification. The LV-NC-infected cells were obviously fusiform; however, the LV-SEC3-infected cells were relatively round and refractive, indicating the cytopathic effects of LV-SEC3. In addition, the LV-NC-infected HeLa cells grew faster compared with the LV-SEC3-infected cells, as determined by cell viability assay. Based on the results of the CCK-8 assay, 72 h after seeding the infected cells into a 96-well plate, proliferation of the

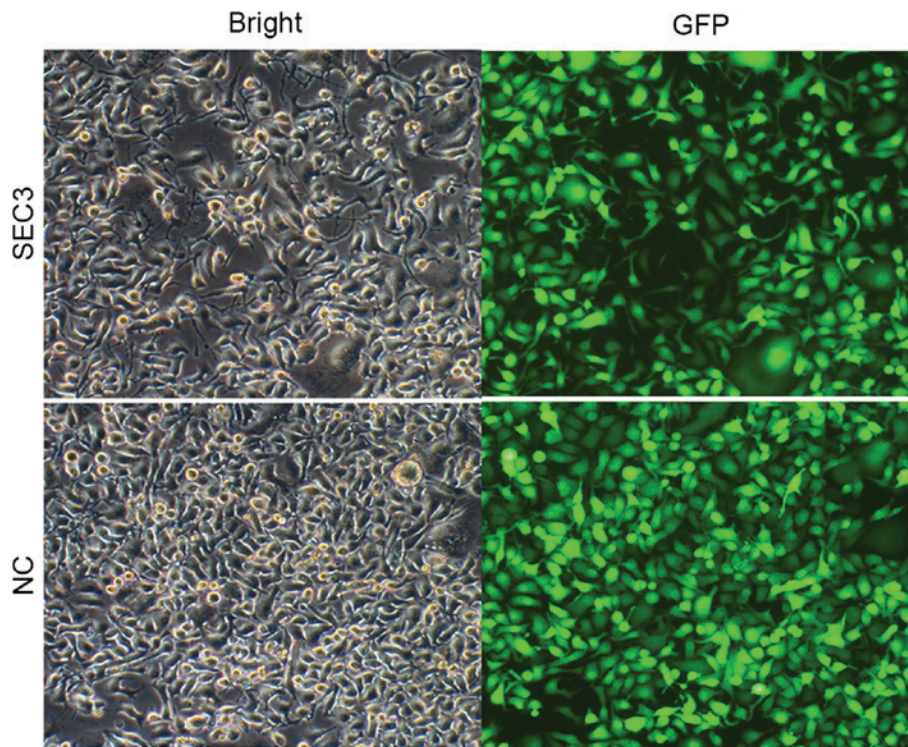


Figure 1. Infection of HeLa cells with LV-SEC3 and LV-NC (multiplicity of infection, 5; magnification, x100). Bright and fluorescent microscopy images of LV-SEC3- and LV-NC-infected HeLa cells are presented. GFP, green fluorescent protein; LV, lentiviral vector; NC, negative control; SEC3, *Staphylococcus aureus* enterotoxin C3.

LV-SEC3 cells was significantly reduced compared with the negative controls (29.10% reduction; $P < 0.001$; data not shown).

PBMC proliferation. During co-culture, the PBMCs were stained with Wright-Giemsa dye and images were captured under a light microscope on days 1, 5 and 10 before they were collected. As presented in Fig. 2, T cells appeared normal on day 1, but appeared slightly activated on day 5, becoming larger and irregularly shaped, this was particularly noticeable for T cells stimulated with rSEC3 and PHA. On day 10, the T cells appeared to be clustering and proliferating, this was also detected in the T cell monoculture, probably due to the addition of IL-2. After 10 days of co-culture, the remaining T cells were considered to be CTLs; the CTL densities of the five groups were as follows: 650,000 (PBMCs co-cultured with LV-SEC3-infected HeLa cells), 975,000 (LV-NC-infected HeLa cells), 3,070,000 (purified rSEC3 protein), 5,240,000 (PHA) and 1,070,000/ml (PBMC monoculture). Notably, the number of cells from the LV-SEC3 and LV-NC groups were fewer than the original number of added T cells (1,000,000/ml), and were therefore unable to undergo flow cytometry due to the low cell number. As shown in Fig. 3, rSEC3-activated and monocultured T cells were predominantly CD4⁺ (62.7 and 59.6%, respectively), whereas PHA-stimulated T cells were predominantly CD8⁺ (57.8%), suggesting greater cell cytotoxicity.

Increased cytokine secretion by CTLs. The concentrations of IL-6 within the cell supernatants are presented in Fig. 4. The lowest concentration of IL-6 was detected in the T cell monoculture. Compared with the PHA group, CTLs stimulated by purified rSEC3 protein secreted increased levels of IL-6,

whereas the levels of IL-6 secreted from the LV-SEC3 group were markedly greater than those from the LV-NC group. With regards to IFN- γ secretion, every spot observed was contributed to an active IFN- γ -secreting T cell. As shown in Fig. 5, CTLs co-cultured with LV-SEC3-infected HeLa cells, rSEC3 protein and PHA were able to release large amounts of IFN- γ compared with the T cell monoculture and the T cells co-cultured with LV-NC-infected HeLa cells.

Cytotoxicity of activated PBMCs against HeLa cells. The cytotoxicity of CTLs against HeLa cells was measured using the CCK-8 assay. As presented in Fig. 6, compared with the LV-NC group, the CTLs and supernatant from the LV-SEC3 group significantly attenuated proliferation of HeLa cells ($P < 0.05$ and $P < 0.01$, respectively), indicating that LV-SEC3-infected cells exhibit a stronger ability to activate T cells and attack tumor cells. With the exception of the supernatant from the LV-NC group, the LV-NC and T cell monoculture groups exhibited weak antitumor activity with no significant difference compared with the controls. With regards to rSEC3, its T cell-activating ability was markedly weaker than PHA, and whereas its supernatant exhibited cytotoxic effects, the specific CTLs from this group failed to induce toxicity, with no significant difference compared with the control group.

Discussion

Cervical cancer is the fourth most common cause in women worldwide, and is particularly prevalent in developing countries (1). To ease the burden, novel therapeutic strategies urgently need to be developed, including immunotherapy

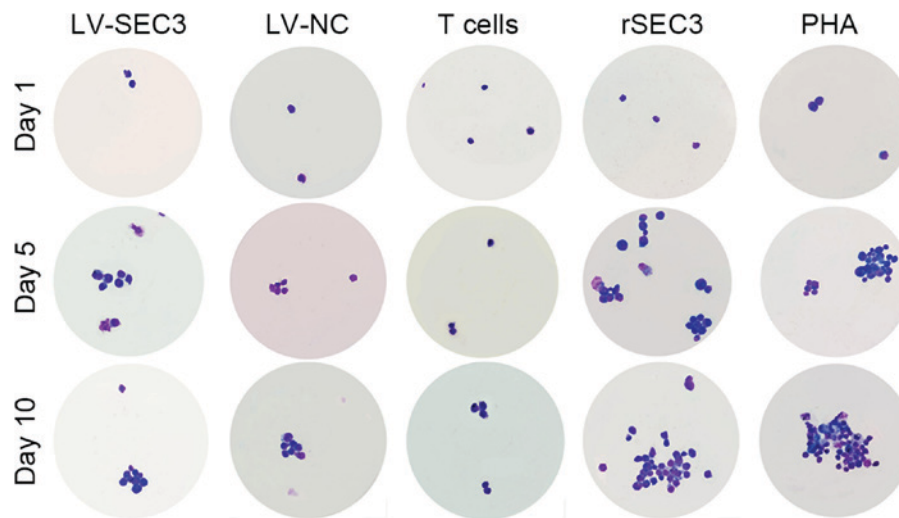


Figure 2. Morphology of activated T cells (Wright-Giemsa staining; magnification, 10x). LV, lentiviral vector; NC, negative control; PHA, phytohemagglutinin; r, recombinant; SEC3, *Staphylococcus aureus* enterotoxin C3.

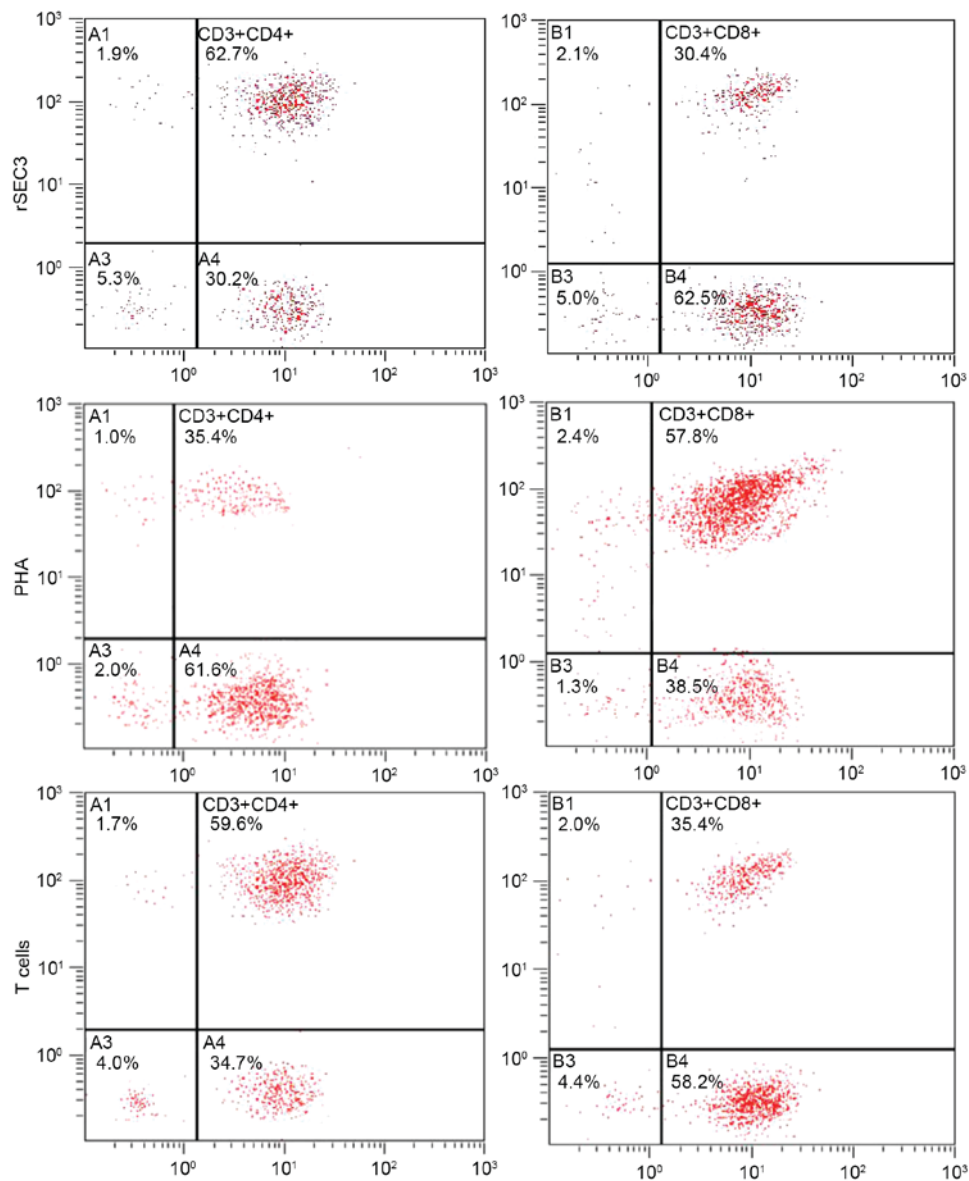


Figure 3. Proportion of cytotoxic T lymphocyte subsets in each group, as determined by flow cytometry. CD, cluster of differentiation; PHA, phytohemagglutinin; r, recombinant; SEC3, *Staphylococcus aureus* enterotoxin C3.

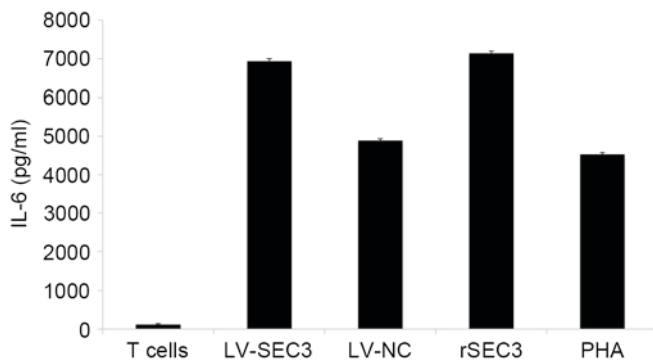


Figure 4. IL-6 concentration among the supernatants secreted by cytotoxic T lymphocytes. IL-6, interleukin-6; LV, lentiviral vector; NC, negative control; PHA, phytohemagglutinin; r, recombinant; SEC3, *Staphylococcus aureus* enterotoxin C3.

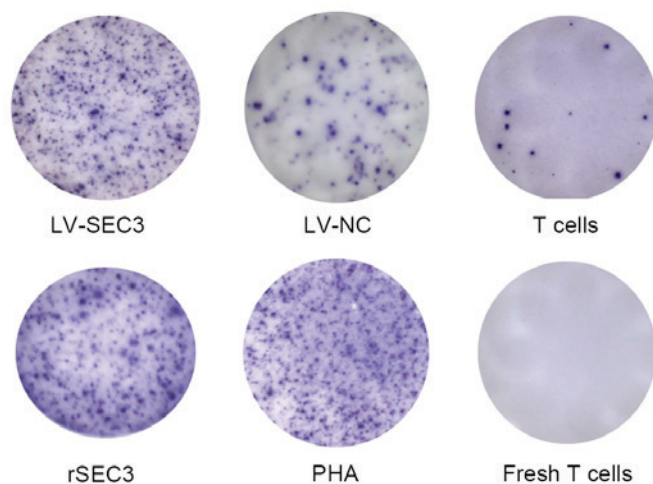


Figure 5. IFN- γ secretion into the supernatants of cytotoxic T lymphocytes from each group. IFN- γ , interferon- γ ; LV, lentiviral vector; NC, negative control; PHA, phytohemagglutinin; r, recombinant; SEC3, *Staphylococcus aureus* enterotoxin C3.

with superantigens. Similar to other types of SE, SEC is a powerful T cell activator, a well-known immune-modulator, and an efficient cytokine inducer via its ability to cross-link major histocompatibility complex II molecules and the T cell receptor. With its enhanced ability to activate T lymphocytes compared with common antigens, SEC has been tested for its anti-tumor efficiency in numerous studies (16,17). During previous unpublished experiments from our group, an LV-SEC3 was constructed, and its effects were determined on the proliferation, migration and invasion of infected HeLa cells *in vitro*. However, the antitumor effects of the vector not only depend on its cytotoxicity but also on stimulation of the immune system. Therefore, prior to an *in vivo* experiment using animals, the present study aimed to conduct *in vitro* experiments to investigate the effects of LV-SEC3 on proliferation and cytokine secretion of T cells, which may shed light on the subsequent research.

LV-SEC3 induced positive effects on the growth of HeLa cells, as determined using a cell viability test (previous unpublished data). To the best of our knowledge, until now, the molecular alterations of LV-SEC3-infected HeLa cells,

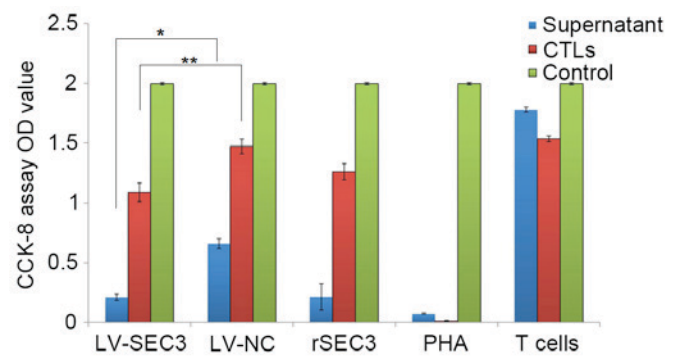


Figure 6. Cytotoxicity of CTLs and T cell supernatants to HeLa cells, as determined by CCK-8 assay. Blue bars refer to HeLa cells co-cultured with previously activated T cell supernatants; red bars refer to HeLa cells co-cultured with previously activated T cells. Data are presented as the mean \pm standard deviation of triplicate samples. * $P < 0.05$; ** $P < 0.01$. CCK-8, Cell Counting Kit-8; CTLs, cytotoxic T lymphocytes; LV, lentiviral vector; NC, negative control; OD, optical density; PHA, phytohemagglutinin; r, recombinant; SEC3, *Staphylococcus aureus* enterotoxin C3.

including surface molecule expression or secretions, were unknown. Through co-culture with extracted human PBMCs and subsequent IL-6, IFN- γ and cytotoxicity analyses, it was revealed that the LV-SEC3-infected cells were able to activate T cells and potentially induce antitumor effects. Out of the numerous cytokines, IL-6 and IFN- γ were chosen as markers of activity. IL-6, which is also known as IFN- β 2 or hybridoma/plasmacytoma growth factor, is a multifunctional cytokine with a central role in numerous physiological inflammatory and immunological processes (18). In addition, IL-6 has been reported to be involved in the pathogenesis of several human malignancies, including cervical cancer (19). Similar to IL-6, IFN- γ is also a proinflammatory cytokine, which is often referred to as a 'master regulator' of antitumor immunity (20). According to the present results, the LV-SEC3 group produced increased levels of IL-6 and IFN- γ compared with the LV-NC group. In addition, the cytotoxicity experiment exhibited a consistent result, indicating the marked antitumor efficiency of LV-SEC3. It is therefore hypothesized that LV-SEC3 may exert an underlying influence on HeLa cells, including the release of specific molecules, which activate T cells and initiate antitumor effects. However, while T cells were activated, the cell number after co-culture was relatively less; this is probably due to the long duration of co-culturing or the induction of T cell apoptosis by HeLa cells (21). The control T cell monoculture group exhibited a slight ability to secrete IFN- γ and induce HeLa cytotoxicity; however, this may be due to the addition of IL-2, which aimed to keep the T cells alive but is also essential for T cell activation (22).

With regards to the rSEC3 protein, its ability to activate T cells, for T cell proliferation and cytokine production, was demonstrated by CCK-8, IL-6 analysis and IFN- γ ELISPOT. T cells are normally composed of CD4⁺ and CD8⁺ subsets. CD4⁺ T cells are also known as T helper cells, which mainly release cytokines such as IL-2 and IFN- γ ; however, the CD8⁺ subset directly induces cell death (23). Compared with PHA-stimulated T cells, T cells stimulated with rSEC3 exhibited a weaker cytotoxic efficacy against HeLa cells; this may be because there were fewer CD8⁺ T cells in this group. Flow

cytometry demonstrated that the ratio of CD8⁺/CD4⁺ cells in the PHA group was 1.6, whereas for the rSEC3 group it was only 0.5. This finding is consistent with previous studies (24,25).

In conclusion, the present study revealed a novel insight into superantigen-mediated cancer immunotherapy. The *in vitro* experiments indicated that HeLa cells infected with LV-SEC3 exerted positive effects on the activation and cytokine production of human T cells. However, the mechanisms underlying the immunotherapy effects of LV-SEC3 on T cells remain to be fully elucidated. Further tests, particularly *in vivo* experiments, are required to investigate the potential mechanisms and signal pathways associated with the immune response to tumor cells treated with LV-SEC3.

Acknowledgements

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