PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2014, 41(12): 1245~1254
www.pibb.ac.cn

Tumor Repression Mediated by IL-15 Expanded CD8 T Cell Correlated With Sustained Tumor-specific CD8 T Cells in Spleen*

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Abstract Adoptive cell transfer (ACT) of tumor infiltrating or genetically engineered T cells is an effective immunotherapy in patients with metastatic cancer, but without additional therapies such as chemotherapy or radiotherapy, the effect was diminished and the tumor regression was also transient. To develop more effective adoptive T cell transfer therapy, central memory T cell (TCM) was expanded *in vitro* using IL-15 followed by transferring them into mice with B16-OVA melanoma. We found that TCM conferred a prolonged suppression of tumor growth whereas effector T cell (TEFF) cultured by IL-2 showed shorter suppression for tumor growth, which was correlated with sustained high number of tumor-specific CD8 T cells in spleen from IL-15 group. Interestingly, the expression of MHC-Ⅰ in the tumor from IL-15 group was upregulated which suggested that the cross presentation in IL-15 group was efficient. These data collectively suggested that IL-15 treated CD8 T cells elicit more effective anti-tumor response than IL-2 treated CD8 T cells owing to sustained tumor-specific CD8 T cells in spleen. Furthermore, expression of MHC-Ⅰ was upregulated in tumor in response to IL-15 mediated adoptive T cell transfer, suggesting that antigen presenting cells (APCs) were involved in adoptive T cell transfer. Our research may have implications for developing more effective tumor immunotherapy.

Key words adoptive transfer, CD8 T cell, IL-15, TCM, tumor microenvironment
DOI: 10.3724/SP.J.1206.2014.00094

Understanding the molecular and cellular bases of T-cell-mediated antitumour responses is one of the most important questions in tumor immunology [1-3]. Currently, IL-2 is added to the culture medium to make T-cell proliferation, differentiation and survival in most protocols. Either TILs from human tumor tissue cultured by IL-2 or activated tumor antigen specific CD8 T cells from murine expanded by IL-2 display high IFN-γ expression and elicit strong cytotoxic function to certain targets[4-5].

Except for the aquirement of T cell effector function, the capability of persistence *in vivo* is another vital determinant for ACT efficacy. Mescher, *et al*.[6] have reported in EG7 model that IL-2- expanded OT-Ⅰ cells were eliminated soon after adoptive transter even with continuous IL-2 administration. A pilot clinical trial in 25 metastasis melanoma patients demonstrated that there was a significant correlation between tumor regression and the degree of persistence in peripheral blood of adoptively transferred T cell clones[7]. Thus, it is a big challenge to find a procedure which will raise long-lived tumor specific T cells *in vivo*. T cells are subdivided into several subsets including traditional TCM, TEM (effecter memory T cell), and TEFF based on their phenotypic markers, functional attributes, migratory properties as well as renewal abilities[2, 8]. Transfer of polyclonal tumor-reactive TEFF could cause an objective response rate reaching 50%, there are reasons to believe that transfer of memory properties cells with a potential recall response and the ability to undergo self-renewal may be superior for anti-tumor therapy[9].

*This work was supported by a grant from The National Natural Science Foundation of China(31300728).
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Received: April 14, 2014 Accepted: May 13, 2014

In company with these thoughts, TCM was found to have superior therapeutic effects when compared with TEFF cell on a per cell basis *in vivo*[10].

It has been reported that IL-15 has substantial potential as an immunotherapeutic molecular for increasing immune response. Specifically, IL-15 is transpresented to NK and T cell by its receptor alpha to cause NK cell expansion and $CD44^{+}CD8$ T cell proliferation *in vivo*[11]. Manjunath *et al*. firstly showed that P14 TCR-tg mice CD8 T cells,which was specific for gp33 epitope, acquired a central memory phenotype with low effector cell functions in the presence of exogenous IL-15 in LCMV model [12]. Further researches revealed that although IL-2 and IL-15 were equivalent mitogens for antigen-primed CD8 T cells, these two cytokines were strikingly different in the ability to regulate cellular amino acid uptake and protein synthesis[13].

However, some studies have suggested tumor cells induce local immune-tolerance during progression to evade immune surveillance [14]. To overcome the immune-tolerance in tumor, we performed adoptive transfer of OT-Ⅰ CD8 T cells expanded with IL-2 or IL-15 into mice with 6～7 mm diameter B16-OVA melanoma. We found that both CD8 T cells expanded with IL-2 and IL-15 have striking efficacy in repressing tumor growth, but the tumors treated with IL-15-expanded CD8 T cells were sustainedly repressed while the tumors treated with IL-2-expanded CD8 T cells re-grow later. The consistent suppression of tumor growth in IL-15 group was mediated by higher percentage of tumor-specific T cells in spleen than that in IL-2 group. Moreover, the increased tumor repression was also correlated with higher MHC-Ⅰ expression in tumor of IL-15 group suggesting efficient antigen presenting activity in IL-15 group.

1 Materials and methods

1.1 Animals

6 to 8-week-old female C57BL/6J (B6) mice were used in all animal experiments. Housing and animal experimental procedures were approved by the Institutional Care and Use Committee of Institute. OT-Ⅰ mice was a gift from Prof. Lieping Chen from Yale University. The TCR transgenes were derived from the CD8 OVA-specific T cell clone 149.42, which expresses the Vα2 and Vβ5 variable regions of the T cell Receptor [15]. The generation of the OVA-specific TCR transgenic CD8 T cells (OT-I) has been previously described[16].

1.2 Cell lines and reagents

B16-OVA cell line (a gift from Prof. Lieping Chen) was cultured in 1640 medium supplemented with 10% heat-inactivated fetal bovine serum(Hyclone), 2 mmol/L L-glutamine (Hyclone), 0.1 mmol/L MEM nonessential amino acids(Hyclone), 100 U/L penicillin, and 100 μg/L streptomycin.

1.3 Generation of OT-$\text{Ⅰ}_{\text{IL-2}}$ and OT-$\text{Ⅰ}_{\text{IL-15}}$ and adoptive transfer

Spleen and LN from 10～16 weeks old OT-Ⅰ mice were grinded and suspended into single cells which were then put into a 12-well plates with 0.1 μg/L OVA257 in the density of 3.0×10^{6}/ml. 48 h later, cells were washed and added with recombinant 10 μg/L IL-2 (eBioscience) or recombinant 20 μg/L IL-15 (eBioscience). 9 days post *in vitro* culture, cells with 95% purity CD8 T were collected and adoptively transferred into tumor-bearing mice.

1.4 Animal model

6～8 weeks old C57BL/6J mice were subcutaneously injected with 1×10^{6} B16-OVA melanoma. The diameter of tumor was measured in two dimensions using a caliper. Tumor diameter was calculated as follow: tumor diameter = (length + width) / 2.

1.5 RNA extraction and RT-PCR

Total RNA from day 30 tumor was isolated using Trizol Reagent (Invitrogen) and reverse-transcribed to cDNA with the M-MLV transcriptase (NEB). Analysis of H-2kb and OVA mRNA expression were carried out on ABI 7500 real-time PCR system (Applied Biosystems) and the amplifications were done using the SYBR Green PCR Master Mix. All RT-PCR reactions were performed in triplicate. Expression data were normalized to the geometric mean of housekeeping gene GAPDH to control the variability in expression levels and were analyzed using the $2^{-\Delta\Delta CT}$ method The primers were shown in Table 1.

Table 1 Sequences of primers for the amplification of PCR

Primers	Sense	Anti-sense
GAPDH	5′ GGCAAATTCAACGGCACAGT 3′	5′ AGAATGGTGATGGGCTTCCC 3′
OVA	5′ AGTGGCATCAATGGCTTCT 3′	5′ GTTGATTATACTCTCAAGCTGCTCA 3′
$H\text{-}2K^{b}$	5′ GGTGGTGTTAAGATGTGGATGA 3′	5′ GCTCCAGAGACAAGTCAGAGGT 3′

1.6 Isolation of TILs

Tumor tissues were dissected and digested in complete RPMI1640 medium supplemented with 2% FBS, 2 mmol/L L-glutamine, 50 μmol/L 2-ME, 100 U/ml penicillin and 100 mg/L streptomycin, 1 g/L collagenase, and 0.2 g/L hyaluronidase (Sigma) at 37℃ for 30 min; 0.5 g/L DNase Ⅰ (Sigma) was then added and the suspension was left at 37℃ for another 15 min. The tumor suspension was next filtered through a 70 μm nylon mesh and live lymphocytes were collected by 75% /100% Ficoll gradient centrifugation. The TILs were washed for 3 times and processed to FACS analysis.

1.7 Flow cytometric analysis

Single cell suspensions were firstly incubated with anti-CD16/32 (anti-Fcγ Ⅲ / Ⅱ receptor, clone 2.4G2) for 10 min, then stained with conjugated Abs followed by washing twice and suspended in 300 μl FACS buffer (PBS with 2% fetal bovineserum). Analysis by flow cytometry was performed on a FACS Caliber flow cytometer (Becton-Dickinson, Mountainview, CA, USA). All incubations were performed on ice in the dark using 5×10^5 cells per sample. Data were analyzed using FlowJo software (TreeStar). Anti mouse CD45 FITC (30-F11), CD3 FITC (145-2C11), CD3 PE (145-2C11), CD8 APC (53-6.7), FITC CD62L (MEL-14), PE CD25 (PC61) APC CD44 (IM7) and PE CD69 (H1.2F3), were purchased from BioLegend or eBioscience. H-2Kb-OVA Tetramer-PE was purchased from Beckman Coulter.

1.8 Statistical analysis

All statistical analysis were performed using Prism 5 (GraphPad Software, San Diego, CA, USA). Mean values were compared using an unpaired Student's two-tailed *t* test. Differences were considered significant at $P < 0.05$.

2 Results

2.1 Adoptive transfer of IL-2-expanded OT-Ⅰ cells resulted in temporary regression of B16-OVA

B16-OVA is a derivative of the H-2^b melanoma B16, which was transfected with cDNA encoding full-length chicken OVA and displays the immunodominant epitope of chicken OVA on its surface with the amino acid sequence SIINFEKL [17]. Subcutaneous B16-OVA tumors grew progressively in C57BL/6 mice (Figure 1). The CD8 T cells of OT-Ⅰ TCR-transgenic mice specifically recognize the SIINFEKL peptide in the context of kb class Ⅰ MHC[17] and were used to generate OT-Ⅰ effector cells by stimulating with OVA257 peptide. To determine how many T cells was suitable for adoptive transfer to eliminate B16-OVA melanoma, spleen and lymph node cells from OT-Ⅰ mice were activated using 0.1 μg/L OVA257 for 48 h *in vitro*, and 3.0×10^6, 1.0×10^7 and 3.0×10^7 activated OT-Ⅰ CD8 T cells were transferred individually to mice at day 9 post tumor inoculation. Three doses of OT-Ⅰ cells were all effective in inhibiting tumor growth without significant dose-dependent manner. But all tumors in three groups began to re-grow 5 days post treatment. This result indicated that adoptive transfer of IL-2-expanded OT-Ⅰ cells alone cannot repress tumor constantly in our model.

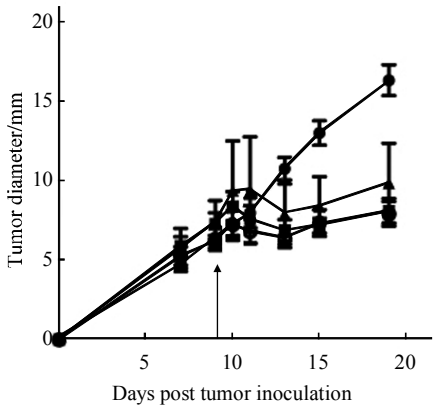

Fig. 1 Adoptive transfer of activated OT-Ⅰ cells resulted in temporary regression of B16-OVA

8×10^5 B16-OVA cells were subcutaneously injected into the right flank of C57BL/6. LN and spleen from OT-Ⅰ mice were stimulated with 0.1 μg/L OVA257 for 48h. Afterwards, 3.0×10^7, 1.0×10^7 and 3.0×10^6 activated OT-Ⅰ cells were injected through tail vein at 9 days post tumor inoculation. The average diameter of tumor was measured every 2～3 days (3～5 mice/group). Data are shown as a repesentative of three experiments. ●—●: Control; ▲—▲: OT-Ⅰ (3×10^6); ■—■: OT-Ⅰ (1×10^7); ○—○: OT-Ⅰ (3×10^7).

2.2 Administration of OT-Ⅰ cultured with IL-15 repressed tumor growth persistently

To explore more effective therapeutic strategies, OT-Ⅰ CD8 T cells were activated with 0.1 μg/L OVA257 followed by culturing in the presence of IL-2 or IL-15 respectively (Figure 2a). The FSC *vs*. SSC of transferred cells in flow cytometry was shown in Figure 2b. As shown in Figure 2c, the culture with IL-15 gradually up-regulated CD62L and down-

regulated CD25 expression on activated OT-Ⅰ cells, while the culture with IL-2 down-regulated both of CD25 and CD62L. After 9-day culture *in vitro*, the IL-15-expanded OT-Ⅰ aquired the central memory phenotypes with high expression of CD62L and low expression of activation marker such as CD25, CD69 and CD44, compared with IL-2-expanded OT-Ⅰ (Figure 2d).

1.0×10^7 OT-Ⅰ cells expanded by IL-2 or IL-15 respectively were transferred into mice 12 days after

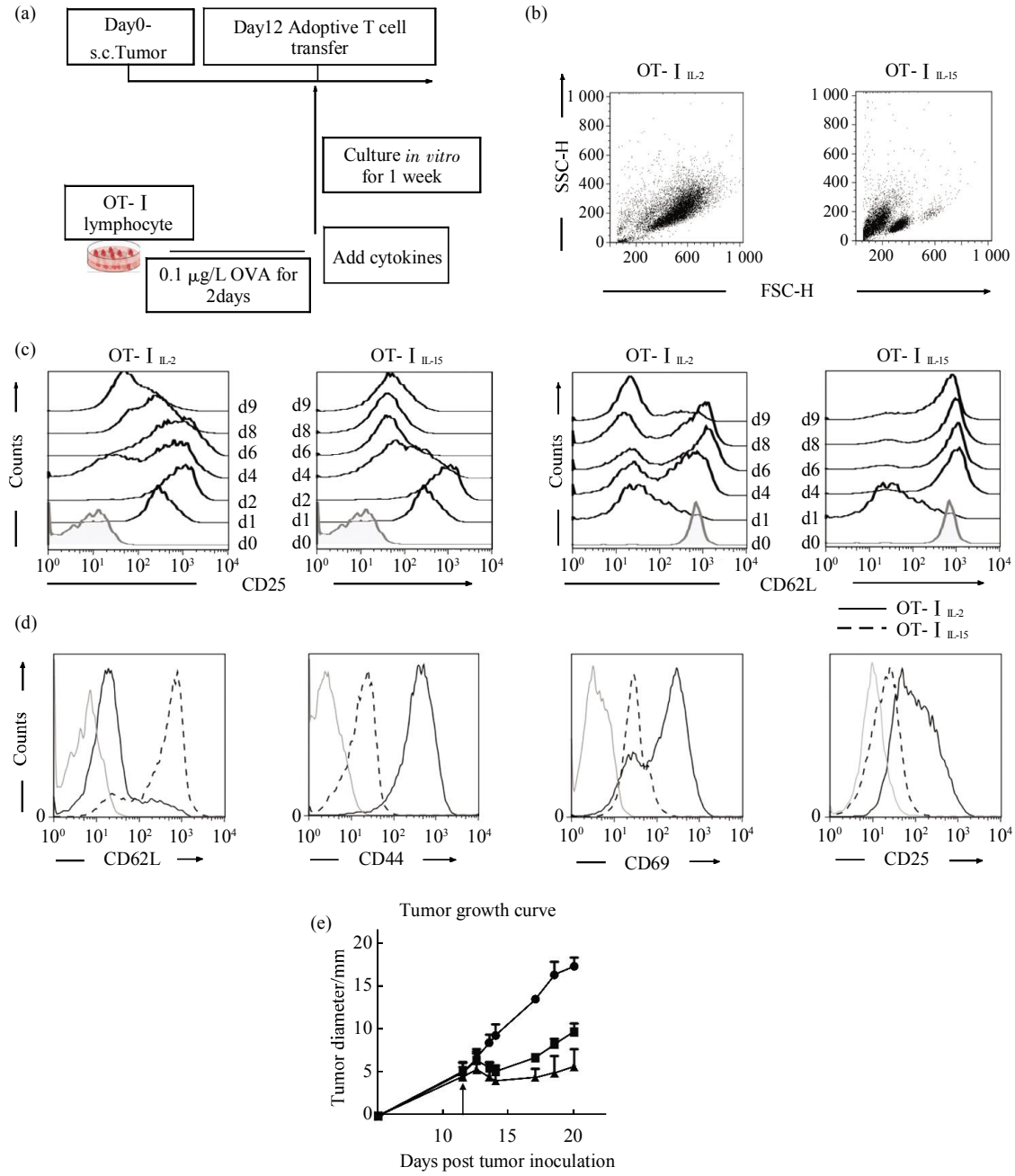

Fig. 2 Administration of OT-Ⅰ cultured by IL-15 persistently repressed tumor growth

8×10^5 B16-OVA cells were subcutaneously injected into the right flank of C57BL/6 mice. OT-Ⅰ was activated with 0.1 μg/L OVA257 in the presence of IL-2 (20 μg/L) or IL-15 (10 μg/L) *in vitro*. The flow chart was shown in (a) and the FSC *vs*. SSC of the transferred cell was exhibited in (b). Expression level of CD25 and CD62L was shown in (c) and the shadow indicated FACS staining of naïve CD8 T cell. Before adoptive transfer (9 days post *in vitro* culture), OT-Ⅰ CD8 T cells were stained with FITC-CD62L, PE-CD25, APC-CD44 and PE-CD69. IL-15-treated T cells were shown as dashed line and that IL-2-treated group were shown as solid line in (d).The isotype controls were illustrated in gray line. CD8 T cells were gated in all FACS analysis. Experiments were performed three times and a representative data is shown here. 1.0×10^7 OT-Ⅰ cells were transferred into mice 12 days after tumor inoculation. The growth curve of tumor was shown in (e) (3～5 mice/group). Data is shown as a representative of two experiments. ●—●: Control; ■—■: OT-Ⅰ$_{IL-2}$; ▲—▲: OT-Ⅰ$_{IL-15}$.

tumor inoculation, and the tumor diameter was measured every 2～3 days using a caliper. As shown in Figure 2e, both IL-2 and IL-15-expanded OT-Ⅰ CD8 T cells exhibited strong anti-tumor effect, but the tumor in IL-2 group began to re-grow 5 days after adoptive transfer, whereas IL-15 group display a nearly horizontal growth curve. The result suggested that OT-Ⅰ CD8 T cells differentiated with IL-15 *in vitro* acquired more efficient anti-tumor response than OT-Ⅰ cultured with IL-2.

2.3 The spleen from mice treated with IL-expended OT-Ⅰ cells exhibited more tumor specific CD8 T cells than that with IL-2-expanded OT-Ⅰ

Next, we investigated the frequency of OVA-specific T cells in spleen of tumor-bearing mice at day 30 after tumor injection. As illustrated in Figure 3a, lymphocytes in spleen were gated in which $CD3^+CD8^+$ T cells were gated. Finally, $Tetramer^+$ T cells were gated from $CD3^+CD8^+$ T cells. As shown in

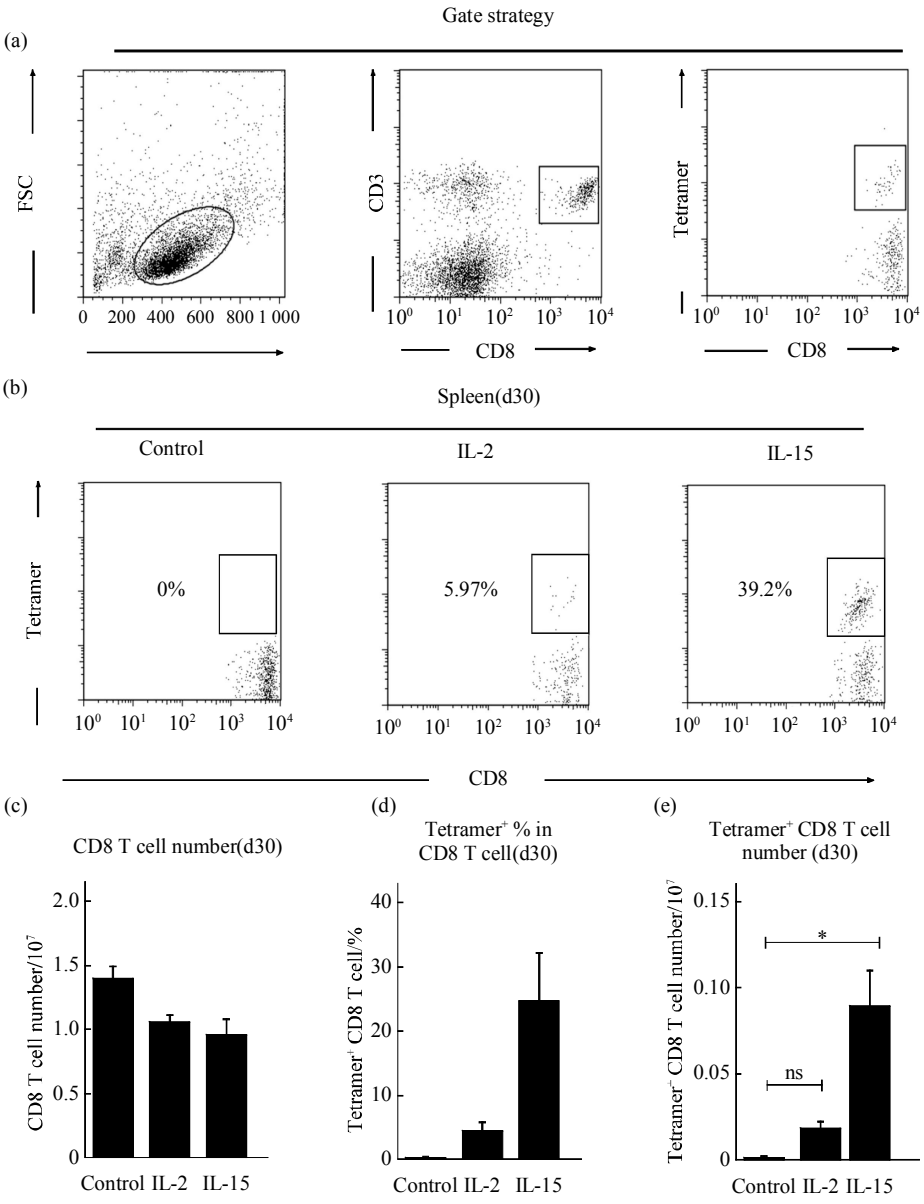

Fig. 3 The higher frequency and number of OVA-specific T cell in speen of tumor-bearing mice treated with IL-15-expanded OT-Ⅰ cells

Mice were transferred with OT-Ⅰ at day 12 and sacrificed at 30 days post tumor inoculation. OT-Ⅰ was prepared as Figure 2. Gating strategy was illustrated in (a). FACS analysis of CD8 T cell and OVA-tetramer T cell from spleen was show in (b). The absolute number of CD8 T cells (c), the percentage (d) and absolute number (e) of OVA-specific T cells in spleen were shown. Data are representives of two groups.(*$P < 0.05$, t-test).

Figure 3b and 3d, the percentage of tetramer$^+$ CD8 T cell in IL-15 group was 6 fold more than that in IL-2 group ((24.67±7.51)% *vs*. (4.38±1.60)%). Both were higher than that in control group ((0.23±0.23)%) in which the OVA-tetramer$^+$ CD8 T cell was rare. Although the absolute number of total CD8 T cells in IL-15 group were fewer than either IL-2 or control groups (Figure 3c), the number of OVA-specific CD8 T cells in IL-15 group was more than that in other two groups ((1.0±0.2)×10^6 *vs*. (0.02±0.004)×10^6 from IL-15 and IL-2 respectively, Figure 3e). Further, we questioned why the transferred T cells cannot eliminate the tumor completely. The composition of tumor-infiltrated lymphocytes were consequently analyzed at day 19 after tumor inoculation in tumor-bearing mice (Figure 4a). The results showed that (32.04±3.02)% of CD45$^+$ cells were CD11b$^+$F4/80$^+$ macrophage. However, only (12.45±0.74)% of CD45$^+$ cells were CD8 T cells (Figure 4b). This result may indicate that the tumor microenvironment is not proper for CD8 T cell-mediated cytotoxic effect. It didn't exhibit higher CD8 T cell percentage in OT-Ⅰ$_{IL\text{-}15}$ group ((13.49±4.92)% in OT-Ⅰ$_{IL\text{-}15}$ group, (19.5±1.9)% and (7.44±1.52) in OT-Ⅰ$_{IL\text{-}2}$ and PBS group respectively, Figure 4d). The representative figures of the three groups were shown in Figure 4c. The percentage and phenotype of macrophage post transfer need to be further investigated. These results collectively suggested that the growth equilibrium of tumor was mediated by both tumor-specific T cells in spleen and tumor microenvironment.

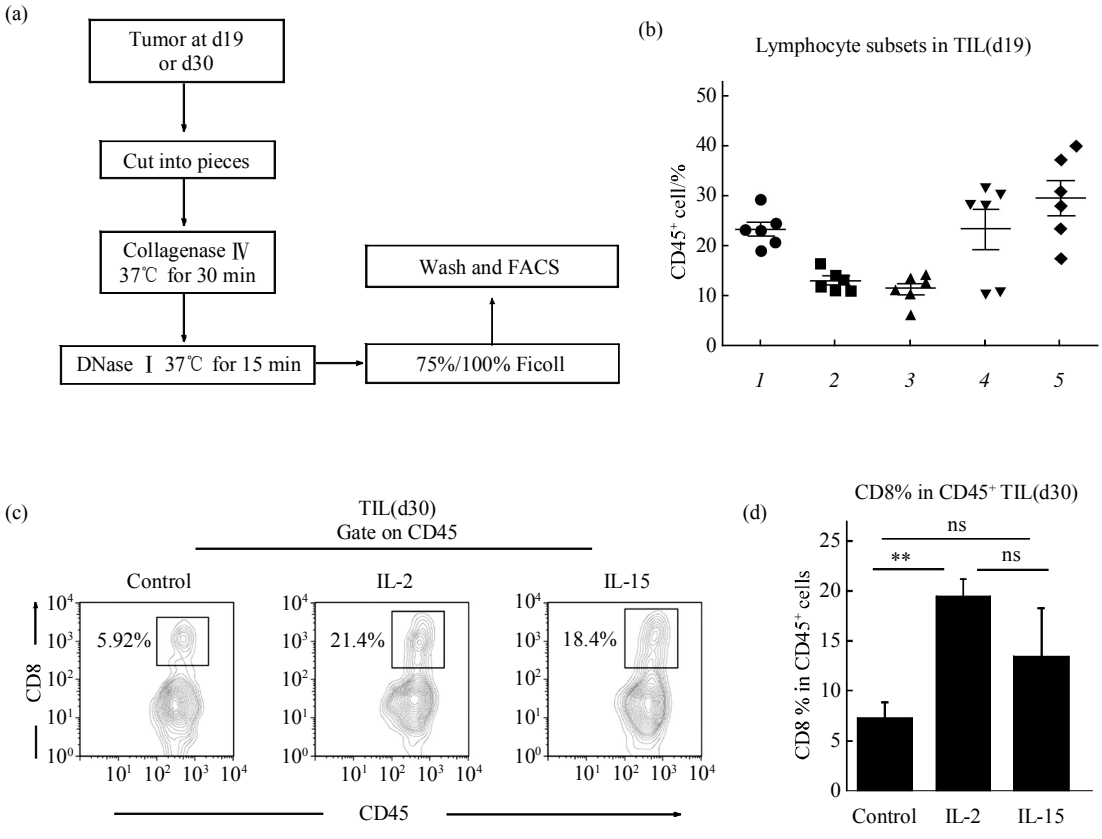

Fig. 4 The frequency of CD8 T cells in tumor-infiltrated lymphocytes (CD45$^+$cells) has no difference between IL-2- and IL-15-expanded groups

The flow chart of TILs separation was shown in (a). The composition of lymphocytes in tumors were shown in (b) through gating CD45$^+$ cells in TILs at day 19 after tumor inoculation. *1*: CD3 T cell; *2*: CD8 T cell; *3*: NK; *4*: B220; *5*: CD11b$^+$F480$^+$. Tumor-bearing mice were treated as in Figure 2a and sacrificed at day 30. The frequency of CD8 T cells in CD45$^+$ TILs were exhibited in (c) and (d). Each data points represents the mean SEM. Data shown is a representive of two experiments. Unpaired Student *t* test was performed. (**P < 0.01, *t*-test).

2.4 The treatment of IL-15-expanded OT-Ⅰ cells increased expression of MHC-Ⅰ in tumor

Modulation of MHC molecule expression has been reported as a tumor evade strategy [18–19]. We detected the mRNA level of MHC-Ⅰ and OVA in the tumor tissue after adoptive T cell transfer. As shown in Figure 5a, 2 of 3 mice in IL-15 group had high MHC-Ⅰ expression in tumor tissue whereas the expression of MHC-Ⅰ was not up-regulated in IL-2 and control groups. OVA mRNA level in tumor tissue had no significant difference among three groups (Figure 5b). The increased expression of MHC-Ⅰ in tumor treated with IL-15-expanded OT-Ⅰ suggested that the cross presentation was active in tumor tissue.

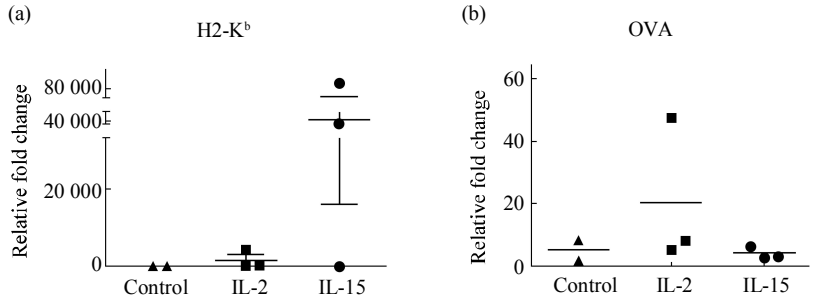

Fig. 5 Tumor tissue expressed higher MHC-Ⅰ after treatment with IL-15-expanded OT-Ⅰ cells

Tumor-bearing mice were adoptively transferred with 1.0×10^7 IL-15- or IL-2 expanded OT-Ⅰ cell at day 12 after tumor inoculation and sacrificed at day 30. Tumors were grinded in TRIZOL followed by RNA extraction and cDNA synthesis. RT-PCR was further performed. The relative fold changes of expression of MHC-Ⅰ (a) and OVA (b) were calculated using C_t value of target gene compared with GAPDH. The data was a representative of the two experiments.

3 Discussion

In this study, we found that activated T cells alone or more effector-prone T cell induced by IL-2 couldn't repress the tumor growth persistently, but T cell with central memory properties expanded by IL-15 *in vitro* could constantly repress the tumor growth. Further studies revealed that IL-15 group elicited more tumor-antigen specific T cells in spleen and higher expression of MHC-Ⅰ in tumor. Remarkably, analysis of TIL demonstrated substantial involvement of macrophage in tumor which may explain why adoptive transfer of the differentiated OT-Ⅰ cells cannot completely eliminate tumor.

Since in clinical ACT trials, the absolute number of infused CD8 T cells has retrospectively been correlated with the likelihood of obtaining an objective response. We set out to determine the number of adoptive transfer T cell in ACT without any lymph depletion procedure. Firstly we found that three doses of 3.0×10^7, 1.0×10^7 and 3.0×10^6 has no significant dose dependent efficacy in B16-OVA tumor model. It has been reported that during chemotherapy or radiotherapy, dying tumor cells release danger signal molecular such as HMGB1 (High-mobility group protein-1) and calreticulin. These danger signal molecules license DCs for antigen uptake and TLR4-dependent antigen processing, which are required for the priming of antigen-specific CD8 T cells[20].

The experiments done by Restifo [21] showed that infusion of the maximum possible number of T cells should be a goal of future T cell transfer protocol. However, it is a huge cost and full of risks to get a proper TIL cell culture. TILs are generated from multiple, independently grown tumor debris, which results in fewer individual TIL cultures from only one patient. While the establishment of TIL culture requires 21 to 36 days. What's more important is that the longer TILs are expanded *ex vivo*, the more senescent they become. Interestingly, some reports suggested that IFN-γ secretion should not been considered as the only decisive factor for TILs availability, as short culture duration and telomere length were repeatedly reported to have a significant positive correlation to clinical response [22–23]. An *in vitro* investigation comparing short-term cultured TILs directly to older IFN-γ-selected TILs showed that

younger TILs have longer telomeres and high levels of the co-stimulatory molecules CD27 and CD28, which can lead to persistence *in vivo* [24]. Interestingly, the "younger" T cells cultured *in vitro* exhibit central memory phenotype after transferred into the recipients[24].

Thus, we evaluated the impact of T-cell differentiation status on treatment outcome while holding the number of cells infused constant. We found that IL-2 expanded OT-Ⅰ infusion exhibited striking tumor growth inhibition in B16-OVA melanoma model. However, the tumor began to re-grow 5 days after treatment. Although infusion of IL-15-expanded OT-Ⅰ couldn't eliminate the tumor completely, the extended repression of tumor growth was remarkable. This was consistent with the result in a melanoma pulmonary metastasis model, in which T cell adoptive transfer was performed only two days after intravenously injection of tumor cells [4]. And strikingly, IL-15 group strongly inhibited the tumor growth in lung. They found that both IL-15- and IL-2-expanded CD8 T cells rapidly located in lung after transfer. But, only IL-15-expanded T cell existed in lung, liver and spleen 48 h after transfer and lasted for 1 week. Thus, we investigated the percentage and number of OVA-specific OT-Ⅰ in tumors. Our results showed that the percentage of tetramer$^+$ CD8 T cells from IL-15 group was 3-fold higher than that of IL-2 group. However, IL-2 group didn't show significant increased tetramer$^+$ CD8 T cells in tumors compared with control group which may be contributed by the shored lived properties of IL-expanded OT-Ⅰ cells.

MHC-Ⅰ plays critical role in cross presentation. However, tumor down-regulate the expression of MHC-Ⅰ to evade the antitumor immunity. For example, overexpression of c-Myc oncogene in melanoma cells was correlated with selective HLA-B locus down-regulation[25]. The gene products of HLA-C are often expressed poorly or not at all[26]. The loss of MHC-Ⅰ was also reported in some recurrence tumor models [27]. In addition, IFN-γ can also induce the expression of MHC-Ⅰ in tumor. We found the upregulation of MHC-Ⅰ in the tumor from the mice treated with IL-15-expanded OT-Ⅰ cells, which may be attributed by tumor cells or tumor infiltrated cells. The upregulation may significantly contribute to the repression of tumor growth from OT-$Ⅰ_{IL\text{-}15}$ group.

Additionally, we also found a high frequency of macrophage in tumor. It has been reported that macrophages in tumor play important role in providing pro-angiogenic factors as well as inhibiting CD8 T cell-mediated anti-tumor immunity. This inspired us to further investigate the role of macrophage in adoptive transfer.

In conclusion, our study demonstrated that OT-Ⅰ with central memory properties posed superior function in treating large B16 melanoma as well as manifested the potential of tumor microenvironment regulation during ACT therapy which may supply a guideline for developing more effective therapies.

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IL-15 扩增的 OT-Ⅰ细胞持续抑制小鼠黑色素瘤生长 *

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摘要 过继免疫治疗(adoptive cell transfer，ACT)是肿瘤治疗中一种有效的免疫治疗手段，但是在没有化疗或者放疗等辅助 治疗手段时，过继免疫治疗缓解肿瘤生长的效果非常短暂．为了探索一种更为有效的过继免疫治疗手段，我们使用白介素 15(IL-15)体外扩增 OT-Ⅰ CD8 T 细胞，使其分化成为中央记忆性 T 细胞 (central memory T cells，TCM)，并将其过继转移至携带 B16-OVA 肿瘤的小鼠中．我们发现，与 IL-2 体外扩增的 CD8 T 细胞(effector T cells，TEFF)相比，TCM 对肿瘤的生长具有长时间的缓解作用，而 IL-2 分化的 TEFFs 治疗肿瘤在短暂的缓解后反弹性生长．进一步的研究发现，TCM 治疗的小鼠脾脏内肿瘤抗原特异性的 T 细胞数量和比例明显高于 TEFF 组，并且 RT-PCR 分析表明 TCM 治疗的小鼠肿瘤内细胞高表达 MHC Ⅰ类分子．这些现象提示了抗原提呈对过继细胞转移治疗的效果具有重要作用．我们的研究对于发展更为有效的肿瘤免疫治疗具有提示意义．

关键词 过继免疫治疗，CD8 T 细胞，IL-15，中央记忆型 T 细胞，肿瘤微环境

学科分类号 Q2，Q5 **DOI**: 10.3724/SP.J.1206.2014.00094

* 国家自然科学基金资助项目(31300728).

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收稿日期：2014-04-14，接受日期：2014-05-13