IL-15 receptor alpha reduces the toxicity of IL-15 during CAR-T immunotherapy

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Abstract

Background:

Chimeric antigen receptor (CAR)-T cell therapy is a powerful adoptive immunotherapy against both B cell malignancies and some types of solid tumors. Interleukin (IL) -15 is an important immune stimulator that may offer ideal long-term persistent CAR-T cells. However, higher base line or peak serum IL-15 levels also relate to severe toxicity, such as cytokine release syndrome (CRS), graft-versus-host disease (GVHD) and neurotoxicity.

Methods:

We successfully constructed CD19 specific armored CAR-T cells overexpressing IL-I5 and IL-15 receptor alpha (IL-15Ra). In vitro cell differentiation and viability were monitored by flow cytometry, and an in vivo xenograft mouse models was used to evaluate the anti-tumor efficiency and toxicity of CAR-T cells.

Results:

CAR-T cells overexpressing just IL-15 demonstrated enhanced viability, retarded exhaustion in vitro and superior tumor inhibitory effects in vivo. However, these tumor-cured mice had lower survival rate, because of, to some extent, toxicity. Nevertheless, CAR-IL-15 T cells transduced with IL-15Ra had reduced CD132 expression and released less cytokines (IFNγ, IL-2 and IL-15) in vitro, and improved mouse survival compared to CAR-IL-15 T cells.

Conclusion:

These results indicated that the importance of IL-15 in enhancing T cells persistence and IL-15Ra in reducing the adverse events of IL-15, with superior tumor retardation during CAR-T therapy, which paves the way for the rapid exploitation of IL-15 in adoptive cell therapy.

Introduction

Due to the great ability to expand and stimulate T cells, IL-2 functions as the first cytokine to be used in clinical cancer trials. However, the administration of IL-2 leads to the exhaustion of T cells limiting its application, especially in adoptive T cell therapy. Recently, IL-15 drew much attention because of its similarity to IL-2 in cytokine receptor biology. Studies have reported that the administration of recombinant IL-15 or over-expression of IL-15 protects mice from various infections [1,2], and because of the ability to prolong T cells' longevity, the use of IL-15 clinically was further strengthened.

Belonged to the common γ-chain family cytokines, both IL-15 and IL-2 are involved in regulation of T cell homeostasis and differentiation. They share many biological activities, presumably because they share components of the IL-2 receptor complex [3]. The shared activities include growth and migration of activated T and NK cells, and induction of B cell differentiation and proliferation [4]. IL-15 and IL-2 each

utilize a heterotrimeric receptor complex, which consists of the IL-2Rβ and γc subunits and a specific, unique α subunits [3]. IL-15 receptor alpha (IL-15Ra) is an IL-15 specific receptor with high affinity. Following secretion by immune cells, IL-15 binds to IL-15Ra expressed on effector cells and this IL-15-IL-15Ra fusion protein is then bound to the IL-2Rβγ (IL-15Rβγ) complex expressed on nearby effector cells, such as NK, B and T cells, which leads to the recruitment and activation of JAK1 and JAK3 [5]. Activated JAK1 and JAK3 further prompt the transcription of IL-15-regulated genes in effector cells [6] via STAT3 and STAT5. Beyond that, IL-15 alone can also bind to the IL-15Rβγ complex in the absence of IL-15Ra, resulting in the activation of PI3K and MAPK pathways [7]. Thus, IL-15 and IL-15-IL-15Ra complex exhibit different contributions to T cell-mediated immune responses.

Chimeric antigen receptor (CAR) is synthetic molecules that was constructed by an extracellular tumor antigen-binding domain, hinge, transmembrane, and intracellular signaling domains [8]. T cells expressing CAR (CAR-T cells) can directly recognize tumor-associated antigens through single-chain variable fragment (scfv) of the extracellular domain. After recognition, CAR-T cells are activated to release multiple cytokines, such as granzyme, perforin, interferon-γ (IFN-γ), and then induce the apoptosis of tumor cell [9,10]. The development of CAR-T cells includes four stages. The first-generation CAR, which did not contain a co-stimulatory signal domain, was developed to treat cancers but with poorly persistent in vivo. The second generation CAR was incorporated with co-stimulatory signal domains from CD28 or 4-1BB into the CAR intracellular structure to solve the problem. Since the two domains determine different functional properties of CAR-T cells, the third generation of CARs containing CD28 and 4-1BB were established [11]. However, despite the significant breakthrough of CAR-T therapy in terms of its clinical curative effects, there are still several factors limit the anti-tumor efficient of CAR-T cells. For example, the exhaustion of CAR-T cells was universal [12], which leads to poor anti-tumor activity and relapse. Thus, the fourth generation CAR, which is also called armored CAR and based on the third-generation CAR combined with CRISPR/Cas9 technology, cytokine, antibody and so on [13,14] was constructed to overcome these deficiencies of CAR-T cells.

There is ample pre-clinical and clinical evidence demonstrating CAR-T cells are predisposed to exhaustion and poor persistence that limits efficacy of the immunotherapy [11]. The application of IL-15, to some extent, has been reported to solve this problem. Even so, other adverse events are occurred such as toxicity. CAR-T cells can cause toxicity by several mechanisms. If the tumor-associated antigen to which the CAR is targeted is expressed on normal tissues, those tissues may be damaged, as is the case that normal B cells were damaged and depleted by CD19 CAR-T cells [15]. In addition, the most prominent and well-described toxicity of CAR-T cells is CRS, which is caused by the cytokines released by infused T cells with symptoms including hypotension and fever. Neurologic toxicities may also occur caused by CAR T-cell therapy [16,17]. It is worth noting that the application of IL-15, which has been reported to induce better anti-tumor response, was always accompanied with more serious toxicity [18,19].

In this study, to reduce the adverse effect and amplify the advantage of IL-15 during CAR-T therapy, we generated CD19 specific armored CARs connecting IL-15 and IL-15Ra. We found IL-15Ra together with IL-

15 could block the toxicity but had no effect on the persistence and anti-tumor activity of CAR-T cells induced by IL-15. This provides clue for the rapid application of IL-15 to treat cancer patients during adoptive cell therapy.

Materials And Methods

Cell lines

The human NALM-6, and the retrovirus packaging cell line PG13 were purchased from the American Type Culture Collection (ATCC). The NALM-6 cell expressing GFP was generated by retroviral infection. NALM-6 cell was maintained in RPMI-1640 (Lonza) and containing 10% fetal bovine serum (Biosera) and 10,000 IU/mL penicillin/10,000 μg/mL streptomycin (EallBio Life Sciences). All cells were cultured in 5% CO_2, 95% air at 37°C in a humidifed incubator.

Generation of CD19 specific CAR-T cells

CD19 Specific CAR coding sequence was synthesized by GeneArt (Invitrogen) and then subcloned into the SFG retroviral vector. The cDNAs of IL-15 (HG10360-M) and IL-15Ra (HG18366-G) were purchased from Sino Biological. All cloning of the CARs were verified by sequencing. PG13 cells were used to produce retroviral particles after plasmid transient transfection. Human peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated by Lymphoprep (MP Biomedicals) gradient centrifugation. After stimulated with anti-CD3/CD28 beads, T cells from PBMCs were then infected with retrovirus. After 7 days, the CAR-T cells were subjected to CAR expression detection and then expanded in X-VIVOTM15 serum-free medium containing 5% GemCellTM Human Serum AB with IL-2 (138 U/ml). This research was approved by the Beijing Shijitan Hospital Institutional Review Board and informed consent was obtained from all the healthy donors.

Flow cytometry

Flow cytometry was performed on a FACSCanto Plus instrument (BD Biosciences) and FlowJo v.10 (FlowJo, LLC) was used for data analysis. All antibodies were purchased from BD Biosciences. CAR-T cells were detected after staining with APC-cy7-labeled mouse anti-human CD3 antibody, FITC-labeled mouse anti-human CD8 antibody, Alexa Fluor 700-labeled mouse anti-human CD8 antibody, BV421 labeled mouse anti-human CD4 antibody, V450-labeled mouse anti-human CD107a, BV605-labeled mouse anti-human CD45RO, PE-cy7-labeled mouse anti-human CCR7, Alexa Fluor 700-labeled mouse anti-human CD27, PE-cy5-labeled mouse anti-human CD95, and Alexa Fluor 647-labeled goat anti-mouse IgG (Fab specific) F(ab')2 antibody (Jackson ImmunoResearch).

Cytotoxicity assay

CAR-T cells were co-cultured with or without NALM6-GL at different effector to target (E:T) ratios in a 24-well plate. After 24 h, cells were collected and tumor cells were detected by the surface marker using flow cytometry (BD FacsCanto II Plus).

Proliferation assay

CD19-CAR-T cells, CD19-CAR-IL-15 T cells and CD19-CAR-IL-15-IL-15Ra T cells were cultured with IL-2. The cell number at the day 0, day 7, day 14 and day 21 were counted by Viable through Trypan blue exclusion using the Vi-CELL Cell Viability Analyzer.

Analysis of cytokine production

The CAR-T cells were co-cultured with NALM-6 at an E:T ratio of 2:1 for 24 h. The supernatants were collected and subjected to IFN-γ, IL-15 and IL-2 detection via ELISA kits (DY285B, D1500, DY202, R&D systems) according to the manufacturer's instructions.

PCR

IL-15 and IL-15Ra expression in antigen-stimulated CAR-T cells was confirmed by PCR. Total RNAs were extracted from CAR-T cells using TRIzol Reagent (Invitrogen) following the manufacturer's instructions. cDNA was synthesized using the High Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific). The expression of IL-15 gene was amplified using primers 5'- ATGGATGCAATGAAGAGAGGG-3' (sense) and 5'- CGACGTGTTCATGAACATCTGGA-3' (antisense); IL-15Ra was amplified using primers 5'- ATGGCCCCGAGGCGGGCGCGAGG-3' (sense) and 5'- TAGGTGGTGCGAGCAGT-3' (antisense); GAPDH was amplified using primers 5'- tgaccacagtccatgccatc-3' (sense) and 5'-gtgagcttcccgttcagctc-3' (antisense) functions as the control.

Xenograft mouse model with NALM-6 cells injection

Six to eight week-old NOD-SCID mice were purchased from Charles River Laboratories. 1×10^6 NALM-6-GFP cells were injected into NOD-SCID mice intravenously to construct the xenograft mouse model. One days after tumor cell injection, 1×10^7 CAR-T cells were injected into tail vein once a day for three days. Tumor development was monitored using IVIS (IVIS, Xenogen, Alameda, CA, USA). All experiments including mice were approved by the Beijing Shijitan Hospital Institutional Review Board.

Statistical analyses

Graphs and statistical analyses were performed using Graphpad Prism 8.0.2. The data were analyzed using a student *t* test with *p* values < 0.05 considered as significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; n.s., not significant. Overall survival of mice with NALM-6 xenografts was measured using the Kaplan-Meier method, with Cox proportional hazard regression analysis for group comparison. All experiment was repeated at least three times.

Results

1. Armored CD19 specific CAR-T cells are developed.

The IL-15 gene and IL-15Ra gene connected to the CD19-CAR gene was constructed (Fig. 1A) and these co-expressing retroviral vector were transduced into T cells. Fig. 1B shows the transfection efficiency, and the CD4/CD8 ratios were similar between the three groups (Fig. S1A). Next, the successful expression of IL-15 and IL-15Ra were confirmed using PCR. Total RNAs of CAR-T cells were extracted. It was showed that CD19-CAR-IL-15 T cells overexpress IL-15 and CD19-CAR-IL-15-IL-15Ra T cells overexpress IL-15 as well as IL-15Ra (Fig. 1C).

2. IL-15 armored CAR-T cells exhibit higher proliferative and less-differentiated phenotype in vitro.

Direct cell counting showed that IL-15 and IL-15Ra overexpressed CAR-T cells exhibited higher proliferation capacity (Fig. 2A). In addition, as IL-2 is the growth factor for T cells, the concentrations of IL-2 in the supernatant were measured and it was showed that CD19-CAR-IL-15 and CD19-CAR-IL-15-IL15Ra T cells released more IL-2 compared with CD19-CAR T cells (Fig. 2B). The differentiated phenotype of CAR-T cells was determined. After stimulated with NALM-6 cells for 7 days, result showed that only 1.67% of $CD8^+$ CD19-CAR T cells were Tscm, whereas CD19-CAR-IL-15 and CD19-CAR-IL-15-IL-15Ra T cells had more Tscm cells (9.23% and 4.84% respectively) (Fig. 2C). In addition, it is reported that less-differentiated T cells produce less IFNγ upon antigen stimulation. Thus, CD19-CAR, CD19-CAR-IL-15 and CD19-CAR-IL-15-IL-15Ra T cells were stimulated with NALM-6 cells for 24 h and the concentration of IFNγ was measured via ELISA. As showed in Fig. 2D, IL-15 and IL-15Ra exhibited T cells less IFNγ production, implying the less-differentiated phenotype for CD19-CAR-IL-15 and CD19-CAR-IL-15-IL-15Ra T cells. Subsequently, as IL-15 amplifies T cell proliferation, the percentages of apoptotic cell and cell viability were analyzed. We found that IL-15 and IL-15Ra depressed CAR-T cells apoptosis and enhanced cell viability (Fig. 2E).

3. IL-15Ra reduces the IL-15-induced toxicity in vitro.

To study the influence of IL-15Ra on IL-15 in cell culture condition, the supernatant of armored CAR-T cells were collected to detect the concentration of IL-15 using ELISA. It was showed that CD19-CAR-IL-15 T cells had the highest IL-15 release, whereas CD19-CAR-IL-15-IL-15Ra T cells performed the same as CD19-CAR-T cells. As Fig. 1C has already confirmed that CAR-IL-15-IL-15Ra T cells can express IL-15 and IL-15Ra successfully, this result demonstrates IL-15Ra combine with IL-15 to reduce the concentration of IL-15 in the medium, which had the potential to reduce toxicity. In addition, another IL-15 receptor, CD132, whose high expression is related with GVHD [20], was detected. Fig. 3B shows CAR-IL-15-IL-15Ra T cells have the lowest CD132 expression (60.8% compared with 93.2% and 65.5%). The anti-tumor activity of armored CAR-T cells were investigated. CD19-CAR T cells and CAR-IL-15-IL-15Ra T cells had the same anti-tumor capacity and CD19-CAR-IL-15 T cells had the lowest.

4. IL-15 armored CAR-T cells co-expressed with IL-15Ra exhibit enhanced anti-tumor activity and reduced toxicity in vivo.

To further examine the anti-tumor activity of armored T cells in vivo, NALM-6-GFP cells were injected intravenously into NOD-SCID mice to generate a xenograft mouse model. After one day, T cells were

injected intravenously with non-transduced T cells (NT) as the negative control, and the mice were monitored for more than three months (Fig. 4A). As shown in Figs. 4B and 4C, compared with control mice with a rapid progression of tumor development, mice treated with CD19-CAR-IL-15 T cells and CD19-CAR-IL-15-IL-15Ra T cells had no tumor relapse, implying the enhanced anti-tumor activity induced by IL-15. However, despite no tumor relapse, the survival rate of CD19-CAR-IL-15 T cells treated group was lowest compared with CD19-CAR and CD19-CAR-IL-15-IL-15Ra T cells treated groups that all mice were died within 70 days (Figs. 4B and 4D), implying the serious toxicity of IL-15. Compared with CD19-CAR group that only 20% mice lived for more than 90 days, the survival rate of CD19-CAR-IL-15-IL-15Ra was 40% (Figs. 4B and 4D). On day 50, bloods from every mouse were collected and the sera were used to detect the concentration of human IL-15. Fig. 4E demonstrates CD19-CAR-IL-15 T treated mice had the most human IL-15 in the blood, which indicates the co-expression of IL-15Ra blocked the toxicity of IL-15 in the serum and prolonged the survival of tumor-treated mice.

Discussion

In the present study, we evaluated the effects of IL-15 and IL-15-IL-15Ra complex on CAR-T therapy. Our results demonstrated that IL-15 armored CAR-T cells resulted in the highest percentage of Tscm cells and cell viability in cell culture condition. On the aspect of IL-15 combined with IL-15Ra, it had higher percentage of Tscm despite lower than IL-15. IL-15-IL-15Ra armored CAR-T cells performed lowest toxicity with less cytokine release (IFNγ) and CD132 expression. In the xenograft mouse model, IL-15 armored CAR-T cells inhibit tumor relapse totally but with severe toxicity that all mice died within 70 days, whereas IL-15-IL-15Ra armored CAR-T cells inhibit tumor relapse totally with lower toxicity that 40% mice lived for more than 90 days, demonstrating the inhibiting effect of IL-15Ra on toxicity induced by IL-15.

CD19 specific CAR-T therapy has demonstrated significant anti-tumor effects for B cell malignancies and some types of solid tumors [21]. However, various studies showed that CAR-T products generated by different methods or from different laboratories exhibit inconsistent anti-tumor efficiency. Several factors limit the anti-tumor efficient of CAR-T cells, such as antibody affinity, off-target toxicity, impressive tumor microenvironment as well as terminal differentiation [22]. CAR-T cell persistence is the crucial problem and considerable progression has been made to obtain cell products that result in enhanced expansion or persistence and anti-tumor response. Studies have shown that anti-tumor benefits for using IL-15 as well as IL-7 in culture [23] or IL-15 genetically engineered into T cells with CAR synchronously [24,25]. Furthermore, high serum IL-15 in patients with DLBCL and B-ALL were associated with the better outcome after CD19 CAR-T therapy [26,27], demonstrating the combined adjuvant IL-15 improved the anti-tumor efficacy of CAR-T therapy. Indeed, in this study we developed the armored CAR for leukemia treatment. Our in vitro cell cultivation result indicated that this armored CAR-targeting CD19 increased the cell viability, inhibited apoptosis and maintained CAR-T cells with a less-differentiated phenotype, demonstrating the enhanced longevity of T cells [28,29].

Despite several clinical studies reported that elevated IL-15 expression correlates with improved patient survival, the administration of IL-15 has caused considerable toxicity including hypotension, fever, thrombocytopenia, and so on in cancer patients, which may prevent further the safety approval of IL-15 [18,30]. In line with these studies, using the NALM-6 tumor bearing mouse, we found IL-15 armored CD19-CAR-T cells inhibit tumor relapse totally but the toxicity was serious and all mice were die within 70 days, implying the severe adverse effect of IL-15. IL-15-IL-15Ra complex has been shown to significantly stimulate $CD8^+$ T cells, especially memory $CD8^+$ T cells, to enhance cytotoxicity against multiple tumors, such as myeloma [31], breast cancer [32], colorectal carcinoma [33], etc., and prolonged survival of tumor-bearing mice, established a long-term immune memory against tumor re-challenge [34,35]. A fusion protein of IL-15-IL-15Ra and anti-FAP caused superior targeted anti-tumor killing ability, which provided rationale for the development of antibody-IL-15-IL-15Ra fusion proteins for cancer immunotherapy in the future [36]. Thus, in the present study, after using IL-15Ra as a part of CAR cassette, we found overexpression of IL-15Ra together with IL-15 enhanced cell viability, reduced apoptotic cell and retain T cell differentiation with more Tscm compared with conventional CAR-T cells, and most importantly inhibit tumor relapse totally for tumor-bearing mice with lower toxicity compared with CD19-CAR-IL-15 T cells, providing a new choice for improving CAR construct.

It is demonstrated IL-15 cis presentation by IL-15Ra expressed on the $CD8^+$ T cells was also able to enhance the proliferation and viability of these $CD8^+$ T lymphocytes in vivo [37] and IL-15Ra significantly increased the stability of IL-15 in serum [38]. In one study, peripheral T cells stably expressing second generation CD19-CAR and IL-15-IL-15 Ra retarded leukemia development and sustained resistance after tumor clearance with long-lived T-memory stem cells [24]. Even so, it did not indicate the toxicity effect of IL-15. In our study, we discovered IL-15 combined with IL-15Ra reduced the adverse events significantly during CAR-T therapy and prolonged survival rate of tumor-bearing mice, which indicates a novel function of IL-15Ra. There are two reasons that may explain. Firstly, the CD19 specific CAR we used was the third generation of CARs having CD28 and 4-1BB into the CAR intracellular structure, which may influence the intracellular signal transduction [39]. Secondly, unlike previous studies, we constructed CD19-specific armored CARs connecting CAR, IL-15 and IL-15Ra together. Therefore, the co-expression of CD19-CAR and IL-15-IL-15Ra fusion protein within one cells may influence the function of IL-15Ra. Anyway, the mechanism of reduced toxicity induced by IL-15Ra need to do further research.

CD132, as a common γ chain, is the subunit for the IL receptors including IL-2, IL-4, IL-7, IL-9 and IL-15. Because levels of these cytokines were shown to be increased in the serum of patients developing acute and chronic GVHD and inhibition of CD132 could have a profound effect on GVHD [20], the CD132 expression on the armored CAR-T cells was examined and the expression level of CD132 was lowest for CAR-T cells together with IL-15 and IL-15Ra compared with CD19-CAR-T and CD19-CAR-IL-15 T. In addition, studies showed that inflammatory bowel disease was associated with increased soluble CD132 [40], and increased expression of IL-7Rα together with CD132 was positively related with psoriasis-like skin

inflammation [41]. Therefore, for the IL-15-IL-15Ra complex armored CAR-T cells, it can be predicted the low-toxicity towards patients after application.

Toxicities caused by CAR-T cells are varied and not fully understood, and the administration of immunosuppressive agents to decrease toxicity is an evolving practice [42,43]. IL-15 stimulates the proliferation and activation of various immune cells, especially $CD8^+$ T cells, leading to increased cytotoxicity and production of cytokines, but also amply the adverse effects of CAR-T cells [44]. Herein, we found IL-15 combined IL-15Ra reduced the expression of CD132 compared with conventional CAR-T and IL-15 armored CAR-T cells, leading to the highest survival rate of tumor-bearing mouse, providing a novel application of IL-15Ra. Despite the observation, the precise relationship between IL-15Ra and CD132 is unclear.

In summary, sustaining the Tscm population during ex vivo expansion prior to adoptive T-cell therapy is challenging. Here, we demonstrate that the combination of IL-15 for CAR-T cell generation preserves Tscm phenotype and results in enhanced self-renewing capacity but with higher toxicity in vivo. We next successfully constructed the CD19 specific CAR combined with IL-15-IL-15Ra fusion protein and then examined its cell viability in vitro and immunotoxicity in xenograft mouse models, which provide a candidate tool for clinical leukemia treatment.

Declarations

Ethics approval and consent to participate

This research was approved by the Beijing Shijitan Hospital Institutional Review Board.

Consent for publication

Not applicable.

Conflict of interest statement

The authors declare no competing interests.

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Author contribution

Conception and design, Xiaosong Zhong; **Data acquisition**, Ying Zhang, Qinghui Zhuang, Fang Wang, Can Zhang, Chang Xu, Shuai Tong, Zhen Yu, Zhijing An, Aiqin Gu and Yi Hu; **Data analysis**, Ying Zhang, Qinghui Zhuang and Fang Wang; **Funding acquisition,** Xiaosong Zhong; **Software**, Ying Zhang and Yi Hu;

Writing, Ying Zhang; Review & editing, Xiaosong Zhong and Yi Hu. **Final approval**, Qinghui Zhuang, Fang Wang, Can Zhang, Chang Xu, Shuai Tong, Zhen Yu, Zhijing An, Aiqin Gu, Yi Hu and Xiaosong Zhong.

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Availability of data and material

The original data of the study are available from the corresponding authors upon reasonable request.

References

1. Yajima T, Nishimura H, Ishimitsu R, Watase T, Busch HD, Pamer GE, Kuwano H, Yoshikai Y. Overexpression of IL-15 in vivo increases antigen-driven memory CD8 + T cells following a microbe exposure. J Immunol. 2002;168:1198–203. doi:10.4049/jimmunol.168.3.1198.
2. Thompson AL, Staats HF. (2011). Cytokines: the future of intranasal vaccine adjuvants. Clin. Dev. Immunol. *2011*, 289597. doi:10.1155/2011/289597.
3. Kennedy MK, Park LS. Characterization of interleukin-15 (IL-15) and the IL-15 receptor complex. J Clin Immunol. 1996;16:134–43. doi:10.1007/bf01540911.
4. Giri JD, Ahdieh M, Eisenman J, Shanebeck K, Grabstein K, Kumaki S, Namen A, Park LS, Cosman D, Anderson D. Utilization of the beta and gamma chains of the IL-2 receptor by the novel cytokine IL-15. EMBO J. 1994;13:2822–30.
5. Lodolce JP, Burkett PR, Koka RM, Boone DL, Ma A. Regulation of lymphoid homeostasis by interleukin-15. Cytokine Growth Factor Rev. 2002;13:429–39. doi:10.1016/s1359-6101(02)00029-1.
6. Sato N, Patel HJ, Waldmann TA, Tagaya Y. (2007). The IL-15/IL-15Ralpha on cell surfaces enables sustained IL-15 activity and contributes to the long survival of CD8 memory T cells. Proc. Natl. Acad. Sci. U. S. A. *104*, 588–593. doi:10.1073/pnas.0610115104.
7. Jakobisiak M, Golab J, Lasek W. Interleukin 15 as a promising candidate for tumor immunotherapy. Cytokine Growth Factor Rev. 2011;22:99–108. doi:10.1016/j.cytogfr.2011.04.001.
8. Fesnak AD, June CH, Levine BL. Engineered T cells: the promise and challenges of cancer immunotherapy. Nat Rev Cancer. 2016;16:566–81. doi:10.1038/nrc.2016.97.
9. Martinez-Lostao L, Anel A, Pardo J. How Do Cytotoxic Lymphocytes Kill Cancer Cells? Clin. Cancer Res. 2015;21:5047–56. doi:10.1158/1078-0432.CCR-15-0685.
10. Eshhar Z, Waks T, Gross G, Schindler DG. (1993). Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc. Natl. Acad. Sci. U. S. A. *90*, 720–724. doi:10.1073/pnas.90.2.720.

11. Weber EW, Maus MV, Mackall CL. The Emerging Landscape of Immune Cell Therapies. Cell. 2020;181:46–62. doi:10.1016/j.cell.2020.03.001.
12. Poorebrahim M, Melief J, Coaña YP, Wickström ST, Cid-Arregui A, Kiessling R. Counteracting CAR T cell dysfunction. Oncogene. 2021;40:421–35. doi:10.1038/s41388-020-01501-x.
13. Li P, Yang LC, Li T, Bin SF, Sun BH, Huang YT, Yang KY, Shan DM, Gu HH, Li H. The Third Generation Anti-HER2 Chimeric Antigen Receptor Mouse T Cells Alone or Together With Anti-PD1 Antibody Inhibits the Growth of Mouse Breast Tumor Cells Expressing HER2 in vitro and in Immune Competent Mice. Front Oncol. 2020;10:1143. doi:10.3389/fonc.2020.01143.
14. Huang R, Li X, He Y, Zhu W, Gao L, Liu Y, Gao L, Wen Q, Zhong FJ, Zhang C, et al. Recent advances in CAR-T cell engineering. J Hematol Oncol. 2020;13:86. doi:10.1186/s13045-020-00910-5.
15. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, Teachey DT, Chew A, Hauck B, Wright JF, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013;368:1509–18. doi:10.1056/NEJMoa1215134.
16. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood. 2016;127:3321–30. doi:10.1182/blood-2016-04-703751.
17. Kochenderfer JN, Dudley ME, Kassim SH, Somerville RPT, Carpenter RO, Stetler-Stevenson M, Yang JC, Phan GQ, Hughes MS, Sherry RM, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol. 2015;33:540–9. doi:10.1200/jco.2014.56.2025.
18. Gust J, Ponce R, Liles WC, Garden GA, Turtle CJ. Cytokines in CAR T Cell-Associated Neurotoxicity. Front Immunol. 2020;11:577027. doi:10.3389/fimmu.2020.577027.
19. Shi SX, Li YJ, Shi K, Wood K, Ducruet AF, Liu Q. IL (Interleukin)-15 Bridges Astrocyte-Microglia Crosstalk and Exacerbates Brain Injury Following Intracerebral Hemorrhage. Stroke. 2020;51:967–74. doi:10.1161/strokeaha.119.028638.
20. Hechinger AK, Smith BAH, Flynn R, Hanke K, McDonald-Hyman C, Taylor PA, Pfeifer D, Hackanson B, Leonhardt F, Prinz G, et al. Therapeutic activity of multiple common γ-chain cytokine inhibition in acute and chronic GVHD. Blood. 2015;125:570–80. doi:10.1182/blood-2014-06-581793.
21. Han D, Xu Z, Zhuang Y, Ye Z, Qian Q. Current Progress in CAR-T Cell Therapy for Hematological Malignancies. J Cancer. 2021;12:326–34. doi:10.7150/jca.48976.
22. Wang E, Cesano A, Butterfield LH. Improving the therapeutic index in adoptive cell therapy: key factors that impact efficacy. J Immunother Cancer. 2020;8:e001619. doi:10.1136/jitc-2020-001619.
23. Xu Y, Zhang M, Ramos CA, Durett A, Liu E, Dakhova O, Liu H, Creighton CJ, Gee AP, Heslop HE, et al. Closely related T-memory stem cells correlate with in vivo expansion of CAR.CD19-T cells and are preserved by IL-7 and IL-15. Blood. 2014;123:3750–9. doi:10.1182/blood-2014-01-552174.
24. Hurton LV, Singh H, Najjar AM, Switzer KC, Mi T, Maiti S, Olivares S, Rabinovich B, Huls H, Forget M, et al. (2016). Tethered IL-15 augments antitumor activity and promotes a stem-cell memory subset in tumor-specific T cells. Proc. Natl. Acad. Sci. U. S. A. *113*, E7788-e7797. doi:10.1073/pnas.1610544113.

25. Krenciute G, Prinzing BL, Yi Z, Wu MF, Liu H, Dotti G, Balyasnikova IV, Gottschalk S. Transgenic Expression of IL15 Improves Antiglioma Activity of IL13Rα2-CAR T Cells but Results in Antigen Loss Variants. Cancer Immunol Res. 2017;5:571–81. doi:10.1158/2326-6066.cir-16-0376.
26. Rossi J, Paczkowski P, Shen Y, Morse K, Flynn B, Kaiser A, Ng C, Gallatin K, Cain T, Fan R, et al. Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. Blood. 2018;132:804–14. doi:10.1182/blood-2018-01-828343.
27. Kochenderfer JN, Somerville RPT, Lu T, Shi V, Bot A, Rossi J, Xue A, Goff SL, Yang JC, Sherry RM, et al. Lymphoma Remissions Caused by Anti-CD19 Chimeric Antigen Receptor T Cells Are Associated With High Serum Interleukin-15 Levels. J Clin Oncol. 2017;35:1803–13. doi:10.1200/jco.2016.71.3024.
28. Gattinoni L, Lugli E, Ji Y, Pos Z, Paulos CM, Quigley MF, Almeida JR, Gostick E, Yu Z, Carpenito C, et al. A human memory T cell subset with stem cell-like properties. Nat Med. 2011;17:1290–7. doi:10.1038/nm.2446.
29. Lanitis E, Rota G, Kosti P, Ronet C, Spill A, Seijo B, Romero P, Dangaj D, Coukos G, Irving M. Optimized gene engineering of murine CAR-T cells reveals the beneficial effects of IL-15 coexpression. J Exp Med. 2021;218:e20192203. doi:10.1084/jem.20192203.
30. Conlon KC, Lugli E, Welles HC, Rosenberg SA, Fojo AT, Morris JC, Fleisher AT, Dubois SP, Perera LP, Stewart DM, et al. Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer. J Clin Oncol. 2015;33:74–82. doi:10.1200/jco.2014.57.3329.
31. Wong HC, Jeng EK, Rhode PR. The IL-15-based superagonist ALT-803 promotes the antigen-independent conversion of memory CD8(+) T cells into innate-like effector cells with antitumor activity. Oncoimmunology. 2013;2:e26442. doi:10.4161/onci.26442.
32. Guo S, Smeltz RB, Nanajian A, Heller R. IL-15/IL-15Rα Heterodimeric Complex as Cancer Immunotherapy in Murine Breast Cancer Models. Front Immunol. 2020;11:614667. doi:10.3389/fimmu.2020.614667.
33. Bessard A, Solé V, Bouchaud G, Quéméner A, Jacques Y. High antitumor activity of RLI, an interleukin-15 (IL-15)-IL-15 receptor alpha fusion protein, in metastatic melanoma and colorectal cancer. Mol Cancer Ther. 2009;8:2736–45. doi:10.1158/1535-7163.mct-09-0275.
34. Mathios D, Park C, Marcus WD, Alter S, Rhode PR, Jeng EK, Wong HC, Pardoll DM, Lim M. Therapeutic administration of IL-15 superagonist complex ALT-803 leads to long-term survival and durable antitumor immune response in a murine glioblastoma model. Int J Cancer. 2016;138:187–94. doi:10.1002/ijc.29686.
35. Dubois S, Patel HJ, Zhang M, Waldmann TA, Müller JR. Preassociation of IL-15 with IL-15R alpha-IgG1-Fc enhances its activity on proliferation of NK and CD8+/CD44high T cells and its antitumor action. J Immunol. 2008;180:2099–106. doi:10.4049/jimmunol.180.4.2099.
36. Rubinstein MP, Kovar M, Purton JF, Cho JH, Boyman O, Surh CD, Sprent J. (2006). Converting IL-15 to a superagonist by binding to soluble IL-15R{alpha}. Proc. Natl. Acad. Sci. U. S. A. *103*, 9166–9171.

doi:10.1073/pnas.0600240103.

37. Rowley J, Monie A, Hung CF, Wu TC. Expression of IL-15RA or an IL-15/IL-15RA fusion on CD8 + T cells modifies adoptively transferred T-cell function in cis. Eur J Immunol. 2009;39:491–506. doi:10.1002/eji.200838594.
38. Ochoa MC, Fioravanti J, Rodriguez I, Hervas-Stubbs S, Azpilikueta A, Mazzolini G, Gúrpide A, Prieto J, Pardo J, Berraondo P, et al. Antitumor immunotherapeutic and toxic properties of an HDL-conjugated chimeric IL-15 fusion protein. Cancer Res. 2013;73:139–49. doi:10.1158/0008-5472.can-12-2660.
39. Cappell KM, Kochenderfer JN. A comparison of chimeric antigen receptors containing CD28 versus 4-1BB costimulatory domains. Nat Rev Clin Oncol. 2021;18:715–27. doi:10.1038/s41571-021-00530-z.
40. Nielsen OH, Kirman I, Johnson K, Giedlin M, Ciardelli T. The circulating common gamma chain (CD132) in inflammatory bowel disease. Am J Gastroenterol. 1998;93:323–8. doi:10.1111/j.1572-0241.1998.00323.x.
41. Vranova M, Friess MC, Jahromi NH, Collado-Diaz V, Vallone A, Hagedorn O, Jadhav M, Willrodt A, Polomska A, Lerouxet J, et al. Opposing roles of endothelial and leukocyte-expressed IL-7Rα in the regulation of psoriasis-like skin inflammation. Sci Rep. 2019;9:11714. doi:10.1038/s41598-019-48046-y.
42. Schubert ML, Schmitt M, Wang L, Ramos CA, Jordan K, Müller-Tidow C, Dreger P. Side-effect management of chimeric antigen receptor (CAR) T-cell therapy. Ann Oncol. 2021;32:34–48. doi:10.1016/j.annonc.2020.10.478.
43. Schmidts A, Wehrli M, Maus MV. Toward Better Understanding and Management of CAR-T Cell-Associated Toxicity. Annu Rev Med. 2021;72:365–82. doi:10.1146/annurev-med-061119-015600.
44. Guo Y, Luan L, Patil NK, Sherwood ER. Immunobiology of the IL-15/IL-15Rα complex as an antitumor and antiviral agent. Cytokine Growth Factor Rev. 2017;38:10–21. doi:10.1016/j.cytogfr.2017.08.002.

Figures

Figure 1

CD19 specific armored CAR-T cells are developed. (**A**) Schematic diagram of third different CD19 specific CARs. It consists of the third-generation CD19-CAR, P2A, IL-15 and IL-15Ra. (**B**) Flow cytometry was used to detect the expression of CD19 specific CARs by using goat anti-mouse IgG (Fab specific). (**C**) Total RNA was extracted from different CD19-CAR-T cells to detect the relative expression of IL-15, IL-15Ra via PCR. SD, splice donor; SA, splice acceptor.

Figure 2

IL-15 armored CAR-T cells exhibit higher proliferative and less-differentiated phenotype in vitro. (**A**) The extent of T cell proliferation was reflected through direct cell counting with time. CAR-T cells were stimulated every 7 days with fresh NALM-6 cells, and T cells were counted before the addition of NALM-6 cells. (**B**) CAR-T cells were co-cultured with NALM-6 (2:1) without IL-2 in the culture medium for 24 h. The concentration of IL-2 in the supernatant was measured via ELISA. (**C**) CAR-T cells were co-cultured with NALM-6 (10:1) for 7 days, the T subsets were detected using flow cytometry. (**D**) CAR-T cells were co-cultured with NALM-6 (2:1) for 24 h. The supernatant was collected to detect the concentration of IFN-γ via ELISA. (**E**) CAR-T cells were co-cultured with NALM-6 (10:1) for 7 days, the percentages of apoptotic cells and cell viability were detected through Annexin V and 7-ADD staining. Results were analyzed by student's t-test. * $p < 0.05$; ** $P < 0.01$; *** $p < 0.001$.

Figure 3

IL-15Ra reduces the IL-15-induced toxicity in vitro. (**A**) CAR-T cells were co-cultured with NALM-6 (2:1) for 24 h. The supernatant was collected to detect the concentration of IL-15 via ELISA. (**B**) CAR-T cells were co-cultured with NALM-6 (10:1) for 7 days, the expression of CD132 on the cell surface was detected using flow cytometry. (**C**) CAR-T cells were co-cultured with NALM-6 cells at indicated ratios for 24 h. Luciferase activity was used to determine the cell lysis rates. (**C**) The supernatants of different CD19-CAR-T cells were collect to measure the concentration of IL-15.

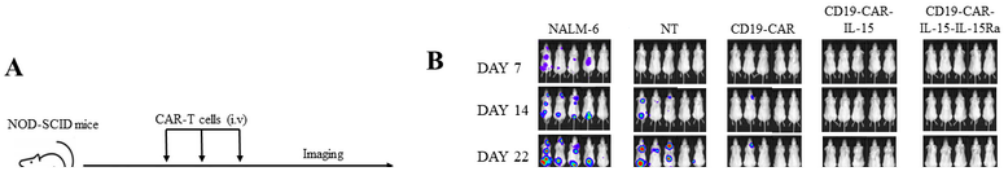

Figure 4

IL-15 armored CAR-T cells expressed with IL-15Ra exhibit enhanced anti-tumor activity and reduced toxicity in vivo. (**A**) Schematic diagram of mouse experimental processes. 1×10^6 NALM-6-GFP cells were injected into NOD-SCID mice intravenously to construct the xenograft mouse model. One days after tumor cell injection, 1×10^7 CAR-T cells were injected into tail vein once a day for three days. Tumor development was monitored using IVIS. (**B, C** and **D**) Quantitative bioluminescence

(radiance=photons/cm^2/sr) imaging data for all mice are shown. Overall survival of mice with NALM-6 xenografts are shown. (**E**) Sera from tumor-bearing mice were extracted and the concentrations of human IL-15 were measured using ELISA kit.

Supplementary Files

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