ABSTRACT

Second generation CD19 targeted chimeric antigen receptor (CAR) T cell therapies have been approved by the FDA thanks to great clinical success. However, these clinical trials suggest that the outcome of younger patients is better than that of older patients. We hypothesize it is due to age-related changes in the T cells collected from patients. To investigate our hypothesis, we compared the function of CAR T cells derived from young or aged B6 mice. T cells isolated from the spleen of aged B6 mice were significantly fewer than those of young B6 as well as activated T cells with CD3/CD28 beads. The immune phenotype subsets of CAR transduced T cells showed that aged CAR T cells skewed toward CD8+ and effector-like phenotypes at the expense of CD4+ and memory-like phenotypes. Furthermore, compared to young CAR T cells, aged CAR T cells exhibited superior cytotoxicity. Using in vivo murine model, aged CAR T cells were short-lived and expanded poorly despite their superior in vitro cytotoxicity. To evaluate for potential mechanisms involving preferential production of effector-like CAR T cells from aged mice we performed gene-expression, as well as single-cell secretory polyfunctional analyses. While the polyfunctional strength index (PSI) of CD8+ aged CAR T cells was higher for aged CAR T cells the increased score was due mostly to abundant secretion of cytokines.

INTRODUCTION

CD19 CAR T cell therapies have been approved by the FDA for children and young adults with relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (B-ALL) and adults with r/r large B-cell lymphoma. Recent reports about long-term follow-up of CD19 CAR T cell therapy in B-ALL suggest that the median event-free survival of children and young adult patients is longer than that of adult patients (Over 11 months versus 6.1 months)\(^7\). The reason for the difference between survival of pediatric and adult patient is unclear, but we hypothesize it is due to age-related changes in the T cells collected from patients. Therefore, we compared the function of CAR T cells derived from young or aged mice as donors.

OBJECTIVES

To clarify the distinct clinical outcome between young B-ALL patients and adult B-ALL patients who received CAR T cell therapy.

RESULTS

To clarify the distinct clinical outcome between young B-ALL patients and adult B-ALL patients who received CAR T cell therapy.

CONCLUSIONS

CAR T cells derived from aged mice exhibited enhanced cytotoxicity but shorter persistence and less memory-like phenotypes. In our future directions we are extending these observations to human CAR T cells and identifying potential methods to improve the function of aged CAR T cells.

MATERIALS AND METHODS

Mice

Young C57BL/6J (B6) mice (6-12 weeks) and aged B6 (≥ 72 weeks) were used as donors for CAR T cell preparation. B6.129S7-Eµ-myc/Myr (Rag1 KO) and B6.PL-Thy1.2 (Thy1.2) were used as recipients.

CAR constructs and mouse T cell transduction

4 types of mouse specific CD19 CAR encoded GFP fusion proteins were evaluated with all having the same anti-CD19 scFv and CD8 hinge and transmembrane domains but differing in their intracellular domain (m19A: lacks the CD3ζ signaling domain, m19C: CD3ζ signaling domain only, m19B: CD28 and CD8ζ signaling domains, m19B: CD8ζ and CD28 signaling domains). Retroviral supernatants were prepared and used to transduce activated T cells as described\(^5\).

Cell line

Es-MMC cells were derived from Eμ-myc transgenic mice as described\(^3\).

Real-Time cell analysis cytotoxicity assay

Cytotoxicity assays were run on an xCELLigence RTCA (real time cell analyzer) instrument according to the manufacturer’s instructions. Briefly, 3T3-mCD19 cells were seeded at 10,000 per well in an E-Plate 96. The next day CAR T cells were resuspended in fresh complete medium without IL2 and added onto 3T3-mCD19 cells at 10,000 cells per well. Cell growth was monitored for 4 days.

Single-cell secretory polyfunctional analysis

The viable mouse CAR T cells were enriched, and then divided into CD4+ cells and CD8+ cells using anti-mouse CD49a beads. These cells were stimulated with 3T3 or 3T3-mCD19 for 24 hours and analyzed by isolight\(^3\).

RNA-sequencing

The single live GFP+ T cells were sorted by BD FACSAria III. These RNA were extracted and treated with DNase, and then analyzed by RNA-DESeq.

REFERENCES


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