Upregulating the Expression of F-CAR-T Cells by Increasing the Amount of Tcm Cells in F-CAR-T Cells

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Abstract

CAR-T cells are most known for protecting one's immune system by attacking cancer cells. Usually, it is infused into the patient's body to provide treatment. When comparing F-CAR-T cells with C-CAR-T cells, F-CAR-T cells typically have more Tscm cells. Thus, this study investigates the effect of upregulating Tscm cells in F-CAR-T cells using FACS for CD45RO- and CD62L+. The experiments will use the expression of Tim3, LAG3, and PD1 and measure if F-CAR-T cells can kill CD19-expressing RAJI cells by FACS for CD19 and Annexin V/PI through various durations and with various numbers of injected CarT. Then Tscm cells are measured by FACS for CD45RO- and CD62L+. Moreover, two special diets will be used as an experimental control to regulate the increase and decrease of Tscm cells. There are two most possible results: (1) Both the Tscm receptor and the diet will have a positive effect in terms of upregulating Tscm cells in F-CAR-T cells; (2) Both the mouse peritoneal macrophage and the diet will inhibit the growth of Tscm cells. The result of our study will provide important information for the future clinical trial of Tscm cell application and improve CAR-T cells' persistence. Future studies should focus on shortening the CAR-T cells manufacturing processes as well as exploring more applicable usages of Tscm cells in detail.

Keywords: ***Tscm cell, CD19 expressing RAJI, CAR-T cell***

1. Introduction

It is generally known that the CAR-T cells are capable changing a person's cancer's immune system and therefore can attack and destroy the cancer cells. A more thorough overview starts with the T cells are taken from one individual's blood, and a receptor called chimeric antigen receptor (CAR) is used to bind to a certain protein on the patient's cancer cells. Typically, CAR-T cells are grown in the laboratory and then infused to the patients to provide treatment. However, current CAR-T cells manufacturing process requires a large amount of time, usually result in a minimum of 7-14 days of waiting time and two recent large-scale CD19-targeted CAR-T clinical trials reported that 20-30% of enrolled patients died because of the time-consuming process of T cells manufacturing process [1].

For Tscm cells which stand for Stem memory T cells, they represent the earliest and long-lasting developmental stage of memory T cells, displaying stem cell-like properties, and exhibiting a gene profile between naïve and central memory T cells. On the other hand, they have a significant role in the adaptive immune response to infectious diseases and cancer.

From "Next-day manufacture of a novel anti-CD19 CAR-T therapy for B-cell acute lymphoblastic leukemia: first-in-human clinical study", researchers have shown that F-CAR-T cells express more abundant T stem cell memory (Tscm) than C-CAR-T cells. Moreover, a higher percentage of T stem central memory cells (Tscm) was detected in F-CAR-T cells. Thus, my research question is to investigate the therapeutic effect of Tscm cells, especially how it can affect F-CAR-T cells' long-lasting function. Furthermore, my hypothesis is that if more Tscm cells are present in F-CAR-T cells, and so when it comes to comparing F-CAR-T cells to C-CAR-T cells, F-CAR-T cells are going to be less exhausted and express "better" phenotypes. Through this way, F-CAR-T cells can be more persistent and stand a better chance against CD19+ cells in vitro [2,3].

In a research paper, "Improving CAR T-cell Persistence" written by Violena Pietrobon et al, it was demonstrated that the clinical benefits of CAR T therapies are still limited. Patients that respond to CAR T therapies remain at risk due to the lack of long-term persistence after adoptive transfer. Furthermore, it indicated that by promoting the persistence of CAR T-cells through a logical therapeutic approach, CAR-T cells are going to be less exhausted [4].

Research question: I predict that if more Tscm cells are present in F-CAR-T cells, when it comes to comparing F-CAR-T to C-CAR-T, F-CAR-T cells are going to be less exhausted through reduced expression of Tim3, LAG3, and PD1 and be able to kill CD19 expressing RAJI cells by FACS for CD19 and Annexin V/PI through various durations and with various numbers of injected CAR-T [5].

2. Methods

2.1. *Materials*

This experiment will use central memory T cells (Tscm) as the independent variable and use F-CAR-T cells and C-CAR-T cells as two dependent variables. The two diets are used as experiment controls to compare its effect on the number of Tscm cells.

Peripheral blood mononuclear cells (PBMC) were obtained from 40 patients and within 30 hours after harvest, T-cells were isolated from the PBMC using Dynabeads CD3/CD28 CTS (Thermo Fisher Scientific, USA), and transduced next day with CD19+ CAR lentiviral vectors in X-vivo culture medium containing IL-2. Then the CAR-T cells were collected the next day without an expansion step and washed with saline.

Human anti-programmed death-1 (PD-L1) was used as the positive control, CD4(+) and CD25(+) were used as the negative control. Then through reduced exhaustion by reduced expression of Tim3, LAG3, and PD1, increased CD19 cell killing by FACS annexin V/PI of CD19 cells, and increased Tscm cells from F-CAR-T cells, the amount of Tscm cells from F-CAR-T cells were measured again. This process was repeated for a total of 4 times.

Table 1. Group A and Group B

Group A	Uses human anti-programmed death-1 (PD-1) as positive control
Group B	Uses CD4(+) and CD25(+) as negative control

In table 1, the positive control in group A human anti-programmed death-1 (PD-L1) antibody possesses the capability to revitalize host T cells. Under normal physiological conditions, PD-L1 regulates the activity of effector T-cells in peripheral tissues in response to infection. When cancer cells are attacked by immune system, they start overexpressing PD-L1. Thus, it is used to activate as well as the concentration of central memory T cells. [6,7]

In table 1, the negative control in group B CD4(+) and CD25(+) T cells are used as T cell suppressors as it is already known that they play a critical role in the prevention of organ-specific autoimmunity and allograft rejection. Moreover, they also inhibit the induction of tumor immunity.

2.2. *Supplemental Tables and Figures*

Table 2. Possible Result Combinations

Outcomes	Reduced exhaustion by reduced expression of Tim3/LAG3/PD1	Increased CD19 cell killing by FACS annexin V/PI of CD19 cells	Increased Tscm cells from F-CAR-T cells	Supported/Rejected by hypothesis
Possible Result 1	+	+	+	Fully supported
Possible Result 2	+	-	+	Partially supported
Possible Result 3	+	+	-	Partially supported
Possible Result 4	+	-	-	Partially supported
Possible Result 5	-	+	+	Partially supported
Possible Result 6	-	-	+	Partially supported
Possible Result 7	-	+	-	Partially supported
Possible Result 8	-	-	-	Rejected

Note: "+" represents a positive result/presence. "-" represents a negative result/presence.

For table 2, it shows a combination of 8 possible results for 8 different situations and whether it is supported, partially supported, or rejected by the hypothesis.

3. Results

Possible Result 1: There is a reduced exhaustion by reduced expression of Tim3, LAG3, and PD1, as well as an increased CD19 cell killing. Moreover, the amount of Tscm cells from F-CAR-T cells are increased, indicating that this result is fully supported by the hypothesis.

Possible Result 2: There is a reduced exhaustion by reduced expression of Tim3, LAG3, and PD1, and a positive increase Tscm cells from F-CAR-T cells, yet there is no increase in CD19 cell killing by FACS. Thus it is partially supported by the hypothesis.

Possible Result 3: There is no increase in the amount of Tscm cells in F-CAR-T cells yet there is a reduced exhaustion of reduced expression of Tim3/LAG3/PD1, indicating that this is partially supported by the hypothesis.

Possible Result 4: In this scenario, this result failed to increase Cd19 cell killing by FACS and so making it partially supported by the hypothesis.
Possible Result 5: The reduced expression of Tim3, LAG3, and PD1 couldn't limit the amount of Tscm cells, yet it indeed increased CD19 cell killing by FACS and increased the amount of Tscm cells from F-CAR-T cells. Therefore, this result is partially supported by the hypothesis.
Possible Result 6: The reduced expression of Tim3, LAG3, and PD1 was not able to suppress the number of Tscm cells, nor did the CD19 cell killing by FACS. In this case, it is partially supported by the hypothesis.
Possible Result 7: In this scenario, this result only managed to increase CD19 cell killing by FACS but was not able to reduce exhaustion through reduced expression of Tim3/LAG3/PD1 nor increased the amount of Tscm cells from F-CAR-T cells. Thus, it is partially supported by the hypothesis.
Possible Result 8: In this case, none of the three criterions are met and so it is rejected by the hypothesis.

4. Discussion

The main reason that leads to Possible Result 1 is that all three criterions are met. Possible Result 1 indicates that reduce exhaustion through reduced expression of Tim3/LAG3/PD1 as well as increase CD19 cell killing by FACS to upregulate the amount of Tscm cells in F-CAR-T cells are achieved. Thus, the hypothesis is fully supported by the hypothesis in this case.
The outcome in Possible Result 2 is most likely to be the FACS annexin V/PI of CD19 cells is not working in the patient's body or the patient has built an antibody already. In this case, the result partially supports the hypothesis.
For Possible Result 3, it indicates that there is no increase in the amount of Tscm cells in F-CAR-T cells. In this scenario, the result partially supports the hypothesis.
For Possible Result 4, it indicates that there is only reduced exhaustion through reduced expression of Tim3/LAG3/PD1 but there is neither increase in the amount of Tscm cells nor Cd19 killing by FACS. In this case, the hypothesis is partially supported by the outcome.
For Possible Result 5, it indicates that the reduced expression of Tim3, LAG3, and PD1 is not in terms of upregulating the growth of Tscm cells. In this case, the hypothesis is partially supported.
For Possible Result 6, it indicates that the reduced expression of Tim3, LAG3, and PD1 is not valid approach in terms of upregulating the amount of Tscm cells in F-CAR-T cells. However, the amount of Tscm cells from F-CAR-T cells did increase. Thus, this result is partially supported by the hypothesis.
For Possible Result 7, it indicates that the reduced exhaustion of reduced expression of Tim3/LAG3/PD1 is limiting the upregulation of Tscm cells yet the amount of Tscm cells increased in F-CAR-T cells. In this scenario, the hypothesis is partially supported by the result.
For Possible Result 8, none of the methods are working to stimulate the growth of Tscm cells in F-CAR-T cells. In this case, the hypothesis is fully rejected by this outcome.

5. Conclusion

Generally, this study explores the therapeutic effect of Tscm cells in F-CAR-T cells, as well as the persistence of F-CAR-T cells. The result of our study will indicate whether or not Tscm cells have an impact on the persistence of F-CAR-T cells. The possible controversial results on the concentration of Tscm cells will also indicate the potential relationship between the amount of Tscm cells and the phenotypes of F-CAR-T cells. Since people have not yet fully understood the effects and usages of Tscm cells until the recent years, more specific targeting proteins and mechanisms of Tscm cells still need to be investigated in clearer detail in order for scientists to get a better overview of the Tscm cells. Furthermore, more studies can be done to shorten the manufacturing time for CAR-T cells so that more patients can get the treatment they need.

References

[1] Flynn JK, Gorry PR. Stem memory T cells (TSCM)-their role in cancer and HIV immunotherapies. Clin Transl Immunology. 2014 Jul 18;3(7): e20. doi: 10.1038/cti.2014.16. PMID: 25505968; PMCID: PMC4232066.
[2] Gardner RA, Finney O, Annesley C, Brakke H, Summers C, Leger K, et al. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. Blood. 2017; 129:3322–31.
[3] Park JH, Rivière I, Gonen M, Wang X, Sénéchal B, Curran KJ, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N. Engl J Med. 2018; 378:449–59.
[4] Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisa-genlecleucel in children and young adults with B-cell lymphoblastic leukemia. NEngl J Med. 2018; 378:439–48.
[5] Pietrobon, V.; Todd, L.A.; Goswami, A.; Stefanson, O.; Yang, Z.; Marincola, F. Improving CAR T-Cell Persistence. Int. J. Mol. Sci. 2021, 22, 10828.
[6] Chakravarti N, Prieto VG. Predictive factors of activity of anti-programmed death-1/programmed death ligand-1 drugs: immunohistochemistry analysis. TransI Lung Cancer Res 2015; 4(6): 743-751. Doi: 10.3978/j.issn.2218-6751.2015.12.10
[7] Tumaini B, Lee DW, Lin T, Castiello L, Stroncek DF, Mackall C, et al. Simplified process for the production of anti-CD19-CAR-engineered T cells. Cytotherapy 2013; 15:1406–15.