Modulating CAR T Cell Metabolism to Favor a Memory Phenotype: Enhancing Anti-tumor Activity

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Abstract

The immune system is the body's defense against disease. T cells are involved in the immune response to disease and play a major role in detecting and eliminating pathogen infected cells and tumor cells. CAR T cell therapy is a novel form of immunotherapy which utilizes a host's own T cells and provides them with a chimeric antigen receptor (CAR) protein thereby enabling them to target specific antigen, or molecules, found on tumor cells which normally evade the immune system. It is suggested that a more stem-like phenotype in CAR T cells may improve long term viability *in vivo* and can enhance their anti-tumor potential. Glycolytic modulator X has been shown to favor a memory phenotype when cultured with T cells by modulating pathways associated with metabolism. We explored the modulatory effects of glycolytic modulator X on CAR T cells with respect to phenotype and effector function. Human T cells were expanded *ex vivo* and then co-cultured with tumor *in vitro*. Our results demonstrated that in a dose-depending manner glycolytic modulator X upregulated markers associated with a stem-like phenotype, augmented tumor killing at different T cell-to-tumor cell ratios, and enhanced cytokine secretion over 10 days in tumor co-culture.

Introduction

CAR T cell therapy is a promising mode of combatting cancer that treats a hosts own immune cells as a living drug. This therapy has been effective with treating refractory B cell lymphomas but faces challenges with solid tumors and other hematological disorders. Various areas of CAR production are under optimization and development; preclinical data in animal models coupled with retrospective analysis from clinical trials has shown that use of less differentiated T cells positively correlates with T cell persistence and clinical outcomes (Priceman et al., 2015). Stem cell memory (T_{SCM}) and central memory (T_{CM}) subsets have shown the greatest response in patients and animal models. Expansion of T cells inexorably leads to differentiation. Understanding that a stem-like phenotype at the time of engraftment favors long term persistence of CAR T cells and yields better sustained anti-tumor activity (Gattinoni et al., 2012) facilitates the need to find methods of preserving stemness in T cells without hindering their ability to proliferate. T cells favor a glycolytic metabolism when in an effector state and it is thought that modulating the metabolism of T cells to favor fatty acid oxidation and lipid metabolism can impact their phenotype during expansion. Our goal was to explore glycolytic modulator X as a modulator of T cell stemness and this was tested by comparing CAR T cells cultured at 1 mM and 2 mM concentrations with an untreated control and using these manufactured cells for *in vitro* assays.

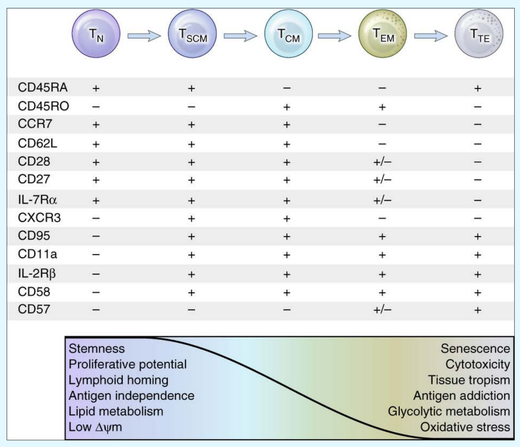

Figure 1. The progression of T cell differentiation with associated markers (Gattinoni et al., 2017)

Methods

PBMCs were isolated from a healthy donor by ficoll density centrifugation and T cells were activated for 7 days via CD3/CD28 stimulation beads. On day 2 cells were lentivirally transduced with a CD19-CAR cassette coupled with addition of protamine sulfate. Protamine sulfate was diluted on day 3. Cells were cultured in X-VIVO contained 10% FBS, 50 U/mL IL-2, and 0.5 ng/mL IL-15 at 37º C with 5% CO2, and medium was changed every 2-3 days. Glycolytic modulator X was added at 1 mM and 2 mM concentrations from day 0 and reintroduced with each media change through expansion. CAR T cells were positively selected for EGFRt, which is found in the lentiviral cassette, on day 11 of expansion. T cells were phenotyped via flow cytometry and frozen at days 18 and 25; cells were later thawed for use *in vitro* functional assays.

To determine cytotoxicity, CAR T cells were co-cultured at varying effector:target (E:T) ratios for different time durations in X-VIVO + 10% FBS with different tumor types (Sup-B15,K562, RAJI-WT, RAJI-PDL1) in 96-well plates for 1 – 15 days and analyzed via flow cytometry. Percent killing was determined by comparing CD45-negative cell counts relative to those observed by mock (untransduced) T cells. Supernatants were collected from the co-culture assays at different time points and ELISA was ran to detect IFNg secretion.

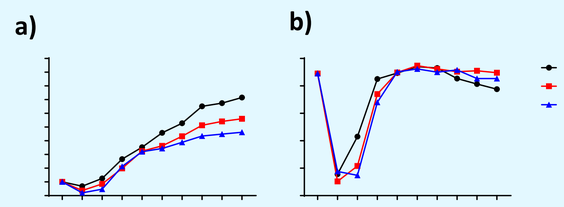

Figure 2. Expansion and viability of T cells treated with glycolytic modulator X at 1 mM, 2 mM plus untreated control. ***(a)*** *Fold expansion and* ***(b)*** *viability of T cells isolated from a healthy donor over the course of 25 days in culture.*

Phenotype

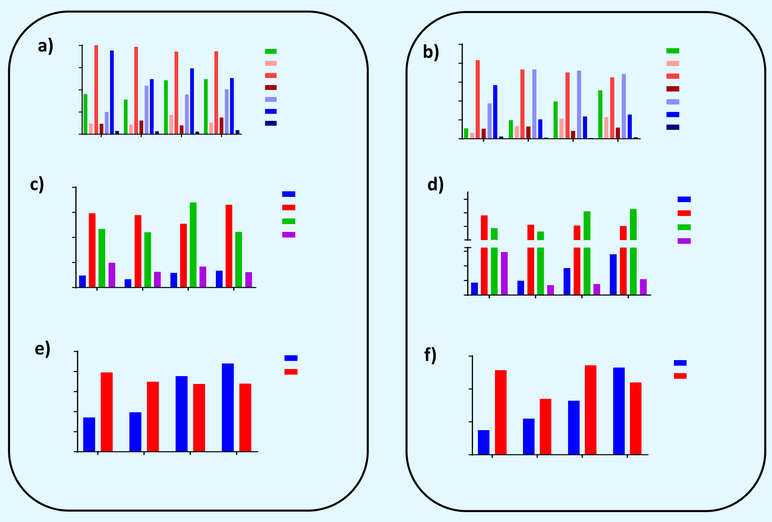

Figure 3. Expression of memory markers in T cells modulated by glycolytic modulator X. *Shown is frequency of CCR7, CD45RA, CD45RO, and CD62L expression at* ***(a)*** *18 days and* ***(b)*** *25 days of culture, mean fluorescent intensity (MFI) of CCR7, CD45RA, CD45RO, and CD62L at* ***(c)*** *18 days and* ***(d)*** *25 days of culture, and MFI of CD27 and CD28 at* ***(e)*** *18 days and* ***(f)*** *25 days of culture.*

Tumor Killing and Cytokine Secretion

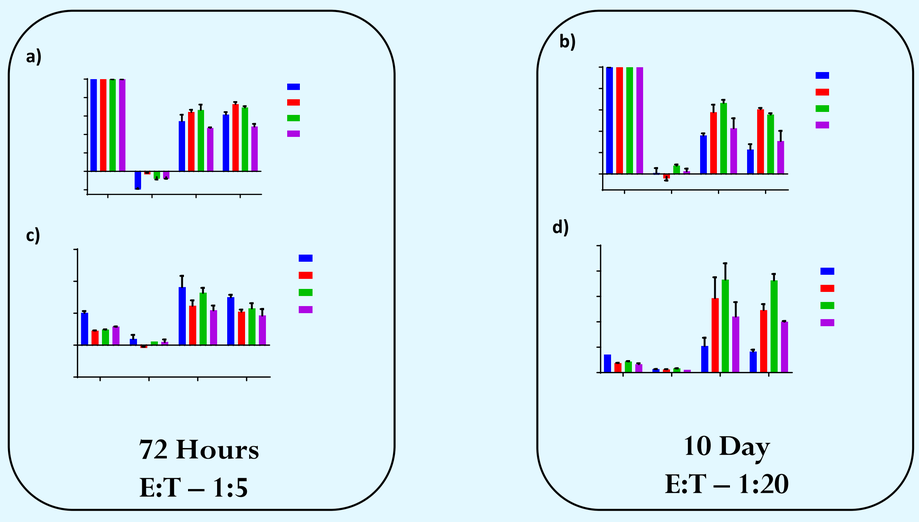

Figure 4. Quantification of tumor killing and IFNg production by CAR T cells treated with glycolytic modulator X. *CAR T cells treated with glycolytic modulator X at 1 mM and 2 mM concentrations taken from day 18 of expansion (plus 2 mM treated cells from day 25) were co-cultured with tumors Sup-B15, K562, RAJI-WT, and RAJI-PDL1 over 72 hours and 10 days with percent of tumor killed shown at* ***(a)*** *72 hours,* ***(b)*** *10 days at their corresponding E:T ratios. Also shown is the IFNg content in pg/mL from supernatants collected from co-culture at (c) 72 hours, (d) 10 days. Data shown reflects n=2 for each data point with ± standard deviation shown.*

Conclusions and Future Directions

T cells cultured *ex vivo* with glycolytic modulator X experienced a minor reduction in proliferation but no negative impact on viability. Glycolytic modulator X upregulates stem markers CCR7, CD45RA, and CD27 over 18 days and in a dose-dependent manner upregulates CCR7, CD45RA, CD27, and CD28 over 25 days of culture. *In vitro* co-culture assays demonstrated that CAR T cells treated with glycolytic modulator X experienced a dose-dependent improvement in tumor killing with the greatest response shown in T cells cultured 18 days prior to plating. Production of IFNg was superior in cells exposed to glycolytic modulator X over 10 days.

Future experiments employing *in vivo* animal models will be valuable in elucidating the functionality of CAR T cells manufactured in the presence of glycolytic modulator X in living systems. Repeated trails of expansion and killing assays will also be necessary to discern the potential influence of donor-contingent responses to modulation.

References

1. Gattinoni L., et al. (2012). Paths to stemness: building the ultimate antitumour T cell. Nat Rev Cancer 12(10): 671-84

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3. Priceman SJ, et al. (2015). Smart CARs engineered for cancer immunotherapy. Curr Opin Oncol 27(6): 466-474.