Centrum für Integrierte Onkologie Köln Bonn

Glucose- and co-stimulatory domain-dependency of CAR T cell activation

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Introduction

Chimeric antigen receptors (CARs) redirect T cell cytotoxicity against cancer cells providing a promising approach to cancer immunotherapy. However, until now, encouraging results could only be achieved in the hematologic malignancy context. One reason for immunotherapy failure is the oxygen- and nutrient-deficient microenvironment in solid tumors. Since both, tumor cells and activated T cells, rely on glycolysis to meet their energetic and biosynthetic demands for growth, proliferation, survival and T cell effector function, tumor-infiltrating T cells and cancer cells metabolically compete in the tumor microenvironment. However, by maximizing glucose uptake and glycolysis, cancer cells outsmart T cells for glucose resulting in impaired T cell effector responses.

The features of CAR co-stimulatory domains remain largely undefined, but seem to have impact on the persistence and resistance to exhaustion of CAR T cells in the tumor context. These attributes are also metabolism-mediated. Therefore, we tried to define the effect of prolonged glucose-deprivation on the effector function of CAR T cells with either CD28 or 4-1BB co-stimulatory domains. This would untie metabolic T cell impairment and open a new and more effective path for the CAR T cell therapy of solid tumors.

Methods

Production of CAR T cells:

HEK293T cells were utilized for the production of gamma-retrovirus-containing supernatants. In doing so, PEIpro-mediated transfection of the cells was performed with 10 µg CAR vector DNA and 5 µg DNA of each helper plasmids. Two gamma-retroviral helper plasmids were used for safety reasons: one encoding the envelop protein GAL-V and the other encoding the group-specific antigen, the reverse transcriptase and a polymerase.

Peripheral blood mononuclear cells were isolated from buffy coats via density gradient centrifugation. The T cells were activated with the agonistic anti-CD3- and anti-CD28-antibodies OKT3 (0.2 µg/ml) and 15E8 (0.05 µg/ml), and with 1000 U/ml IL-2.

Finally, the T cells were transduced under usage of gamma-retrovirus-containing supernatants of HEK293T cells.

IFN-γ-ELISAs:

CAR T cells were cultivated under different glucose concentrations for 8 days. IFN-γ-ELISAs were performed with the supernatants of activated CAR T cells on day 1 and 8. For the activation, equal numbers of CAR T cells with equalized transduction rates were incubated on antibody-coated plates (isotype control: 9G10, TCR stimuli: OKT3 + 15E8, or CAR stimulus: BW2064/36) over night.

Results

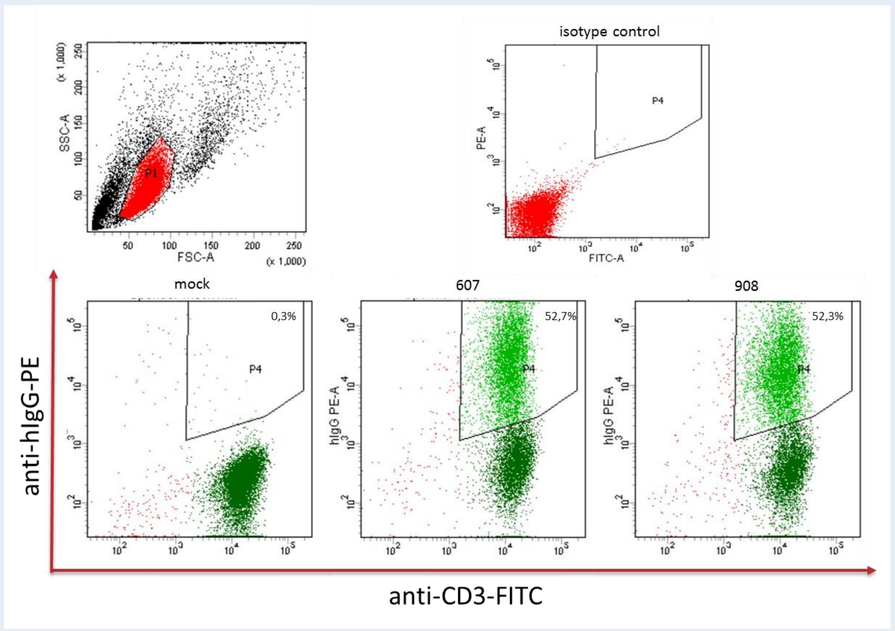

Figure 1:

Flow cytometrical analysis of the CAR T cell transduction rates.

The CAR expression was measured via PE-conjugated anti-hIgG mAbs among the CD3 positive T cell population (anti-CD3-FITC positive).

mock: control T cells w/o CAR

#607: anti-CEA-CD28-CD3zeta-CAR

#908: anti-CEA-4-1BB-CD3zeta-CAR

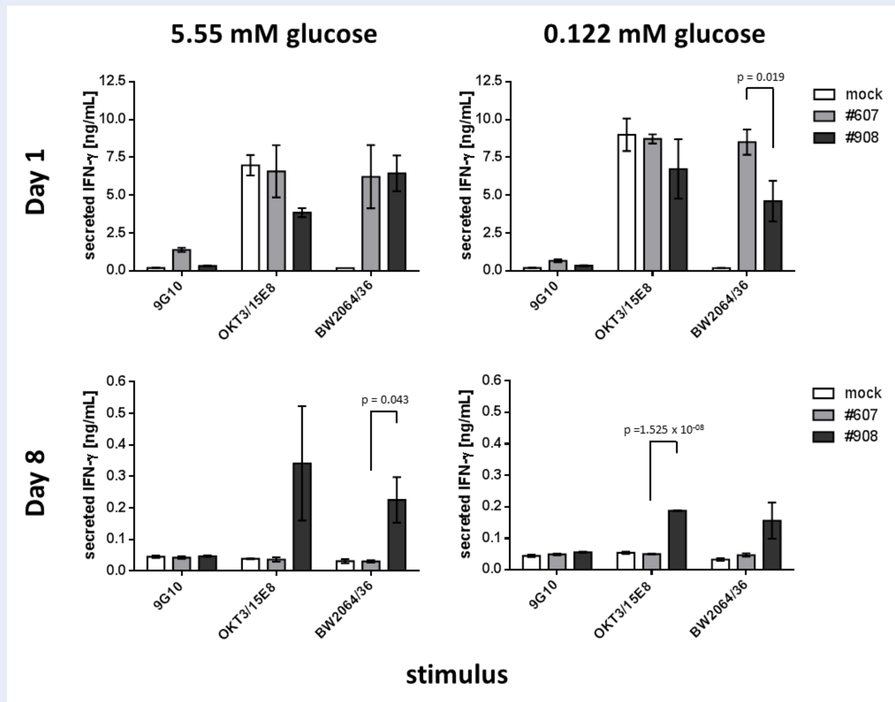

Figure 2:

Glucose-concentration dependent IFN-γ secretion of CD28- and 4-1BB-CAR T cells.

T cells were isolated, transducted and cultivated under 5.55 mM and 0.122 mM glucose for 8 days in total. Antibody-mediated T cell activation was performed over night before harvesting the supernatants.

Isotype 9G10: 5µg/ml

Agonistic antibody concentrations:

- OKT3: 1µg/ml
- 15E8: 5µg/ml
- BW2064/36: 5µg/ml

#607: anti-CEA-CD28-CD3zeta-CAR

#908: anti-CEA-4-1BB-CD3zeta-CAR

Conclusion

On the one hand, the CD28-CAR T cells exhibit higher activation rates on day 1 under low glucose conditions than 4-1BB-CAR T cells, on the other hand, CAR T cells with the 4-1BB co-stimulatory domain perform better after 8 days under low glucose conditions.

These results indicate a lower glucose-dependency of 4-1BB-CAR T cells compared to CD28-CAR T cells. From this point of view one would prefer 4-1BB CAR T cells over CD28-CAR T cells for the therapy of solid tumors.

However, the amount of secreted IFN-γ by 4-1BB-CAR T cells is massively diminished after 8 days compared to day 1. Therefore, an even lower glucose-dependency would be more preferable for immunotherapeutic treatment. The achievement of this attribute would be the goal of further investigations.

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