HYPERMETABOLIC EXPANSION CONDITIONS IMPRINT LASTING DYSFUNCTION ON ADOPTIVE CELL THERAPIES

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Background Adoptive cell therapies, like chimeric antigen receptor (CAR) T cell therapy act by redirecting and enhancing a patient’s immune response to tumor cells, with success in hematologic malignancies. However, many patients relapse due to failure of persistence of cells. Further, efficacy of cellular therapies is limited by barriers like poor tumor infiltration and an immunosuppressive tumor microenvironment. In addition, generating required numbers is a limiting factor in CAR T cell production, leading to the development of bioreactors designed for high scale proliferation. Culture media conditions commonly used for T cell expansion are extremely hypermetabolic and can stress rapidly expanding T cells. Commonly used expansion media are often at least 2 to 10 times richer in fuel sources like glucose, oxygen and amino acids compared to physiological levels. This may potentially result in dysfunctional CAR T cells that are unable to persist and carry out effector functions in in vivo environments that carry physiologic levels of carbon sources. In this study, we aimed to directly compare the efficacy of commonly used expansion media to expand T cells, both in traditional culture flasks and gas-permeable Rapid expansion (G-Rex)R bioreactors.

Methods Human Peripheral blood mononuclear cells cells from healthy donors were used to generate anti-CD19 CAR-T cells in different media formulations (standard RPMI, hyperglycemic RPMI, X-VIVO 15) in (G-Rex)R bioreactors and flasks. Resulting cells were analyzed for metabolic/functional parameters at the end of expansion.

Results CAR-T cells expanded in rich media (X-VIVO 15) grew at increased rates compared to identical cells cultured in RPMI. However, X-VIVO15-expanded T cells showed decreased mitochondrial mass and glucose uptake compared to cells expanded in RPMI, indicative of metabolic insufficiency and poor in vivo persistence of therapeutic T cells. Further, RPMI-expanded CAR-T cells were more polyfunctional when activated with NALM6 compared to T cells expanded in rich media. Notably, these differences were exacerbated during culture in hypermetabolic Grex flasks. Our results indicate that culture conditions impact metabolic and functional potential of therapeutic cells, representing a window of opportunity that can be harnessed to better equip these cells for success.

Conclusions Our data suggests that use of culture conditions to specifically drive rapid expansion may carry liabilities for long-term persistence of engineered therapeutic T cells. Hypermetabolic culture conditions may imprint an unappreciated form of dysfunction; our data suggest that modifying or engineering cell culture systems to more adequately mimic physiologic metabolic conditions may better prepare T cells to eradicate cancer in patients.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0273