Elucidating the relationship between mechanosensing and metabolism in T cells

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Statement of Purpose: Adoptive immunotherapies have leveraged the ability of T cells to recognize a specific antigen via their T cell receptor (TCR) and to exert various effector functions for targeting diseases such as cancer [1]. During activation and differentiation T cells are metabolically reprogrammed to meet the requirements for proliferation, differentiation and effector functions. While research has been mainly focused on the metabolic changes associated with T cell activation, the emerging field of mechanoimmunology and the role of mechanotransduction during T cell activation receive increasing attention [2]. The group of Lance Kam was able to demonstrate that T cell activation and proliferation respond to the rigidity of an underlying substrate, which will inspire the next generation of biomaterials aiding the ex vivo expansion required for immunotherapy [3]. The overarching hypothesis driving this project is that the impact of mechanical stiffness of an activating substrate on T cell activation and subsequent function is mediated by changes in cell metabolic state. To elucidate the relationship between T cell metabolism and mechanosensing, hallmarks of cellular metabolism in response to substrate rigidity were measured. Furthermore, traction force microscopy using modified poly-acrylamide gels was employed to quantify the force generation via the TCR in response to different manipulations of the cellular metabolism.

Methods: CD4+ and CD8+ human T-cells were activated on polyacrylamide (pAA) gels of varying stiffnesses coated with activating antibodies to CD3 and CD28 at different densities. For investigating the impact of substrate stiffness on activation and cellular metabolism (feedforward mechanism), T cells were cultivated on pAA gels for three days and the glucose consumption and lactose secretion into the media during the cultivation was evaluated via an enzymatic nutrient consumption assay while key markers of T cell activation, metabolism and nutrient transport (CD25, CD134, GLUT-1, 4-EBP1, pPDHK-1) were quantified via flow cytometry. To explore the impact of the metabolic state of T-cells prior to activation on cellular traction force generation (feedback mechanism), the pAA platform was modified to incorporate a thin layer of fluorescent beads on their surface to enable tracking the gel deformation by T cells during activation. pAA gels were modified to include a monolayer of densely spaced fluorescent beads (polystyrene microspheres) trapped on top of the hydrogels acting as fiduciary markers to enable traction force microscopy.

Results: T cell activation (as measured by CD134 expression) experienced a biphasic response to substrate stiffnesses when stimulated with 0.3x or 1x anti-CD3/CD28 antibody density. This behavior was shifted when stimulating T cells with higher antibody concentrations (3x), confirming previous results by Yuan et al. [3]. The activation level correlated with GLUT-1 expression and phosphorylation levels of the downstream mTOR target 4EBP-1 as shown in figure 1. Measuring the glucose consumption and lactate secretion revealed that the metabolic switch towards a mainly glycolytic metabolism is affected by pAA stiffness (see figure 1).

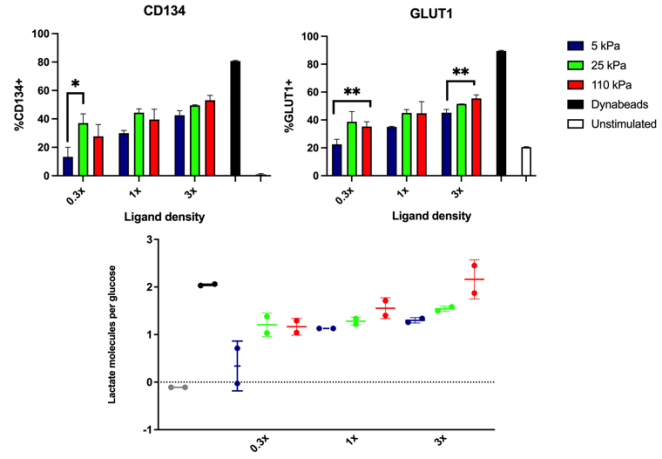

Figure 1. Percentage of CD134+ and GLUT+ T cells and nutrient consumption for 5, 25, and 110 kPa pAA gels with 0.3x, 1x, or 3x anti-CD3/CD28 antibody density. One-way ANOVA was performed (CD134: $F(8,9)=11.08$, $p=0.0008$; GLUT1: $F(8, 9)=10.67$, $p=0.0009$, NC: $F(8, 9) = 6.946$, $p=0.0044$) and if significant, statistical significance within groups was assessed via Šidák's multiple comparison test ($\alpha=0.05$); * $p < 0.05$, ** $p < 0.01$.

A platform using activating pAA substrates and biotinylated fluorescent beads was developed to track the force generation of T cells via their TCR during the initial stages of activation. Optimization of the system was required to allow proper adhesion of the cells and induce sufficient activation levels to be able to measure displacement of the substrate surface.

Conclusions: The preliminary results of this study demonstrate that substrate rigidity impacts the metabolic reprogramming and activation of human T cells. Furthermore, a biomaterial approach was developed to track the force generation of T cells on a micrometer scale in response to metabolic modulation. In future experiments, various feeding and metabolic inhibition studies will be performed to elucidate how the metabolic state affects traction forces of T cells. In summary, these findings will help to develop biomaterial approaches for the ex vivo expansion and metabolic priming of T cells for adoptive immunotherapies.

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References:

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