Assessment of molecular mechanisms involved in T-cell receptor (TCR)-dependent metabolic switch into aerobic glycolysis

Poster# B623

Gisele V. Baracho[1], Ali Rezvan[2], Sam X. Shi[1], Aruna Ayer[2], Stephanie Widmann[1], Aaron J. Tyznik[1]

[1]BD Biosciences, San Diego, CA [2] BD Biosciences, San Jose, CA

Abstract

Upon activation, T cells undergo metabolic adaptations to fulfill the energy and biosynthetic requirements for their rapid growth and proliferation. TCR engagement and CD28 co-stimulation trigger the initial metabolic shifts that enable T cell activation including increased glucose uptake. PI3K signaling plays a pivotal role in regulating GLUT1, the primary glucose transporter in T cells. Using high-dimensional spectral flow cytometry, we found correlated expression between GLUT1 and granzyme B (GZMB) in subsets of *in-vitro* activated human T cells. Notably, the PI3K inhibitor Ly294002 abrogated TCR-dependent upregulation of GLUT1 abolishing GLUT1^{high}GZMB^{+} T cells in the cultures. A comprehensive single-cell targeted mRNA profiling of over 56, 000 immune cells obtained with the BD Rhapsody™ HT Xpress System further showed a TCR-dependent upregulation of *GZMB* and various metabolic regulators such as *GAPDH*, *HMGB2 and TK1*. The results provide additional details on the involvement of the glycolytic pathway in T cell activation and offer a deeper insight into the molecular mechanisms associated with TCR-dependent cell activation.

Glucose metabolism and interconnected pathways

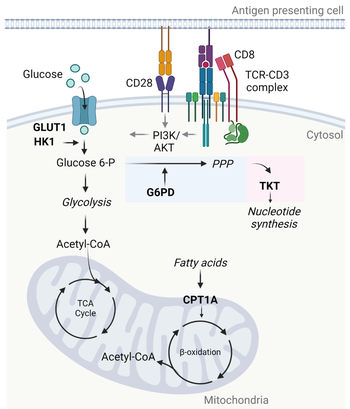


Figure 1A: Graphic illustration of major metabolic pathways: glycolysis, non-oxidative pentose phosphate (PPP), TCA cycle/Krebs cycle and fatty acid beta oxidation depicting PI3K-AKT signaling pathway activation downstream CD28 and TCR complex. APC: antigen- presenting cell.

Methods

Single-cell multiomics using the BD Rhapsody™ HT Xpress System

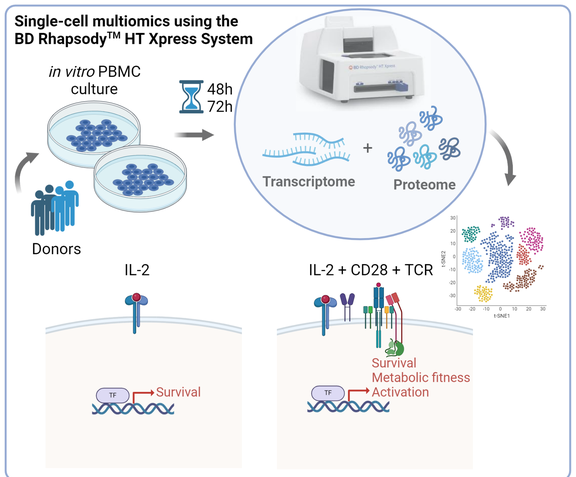


Figure 1B: Workflow for T cell *in vitro* activation and single-cell mRNA and protein analyses. PBMCs from two healthy and two Systemic Lupus Erythematosus (SLE) individuals were cultured in human recombinant IL-2 or IL-2 plus Dynabeads Human T-cell Activator CD3/CD28 (Thermo Fisher Scientific) for 48 or 72 hours. Single cells were loaded into an 8-lane BD Rhapsody™ cartridge: 4 lanes at 48h and 4 lanes at 72h followed by library preparations for deep sequencing.

Spectral flow cytometry using the BD FACSymphony™ A5 SE Cell Analyzer

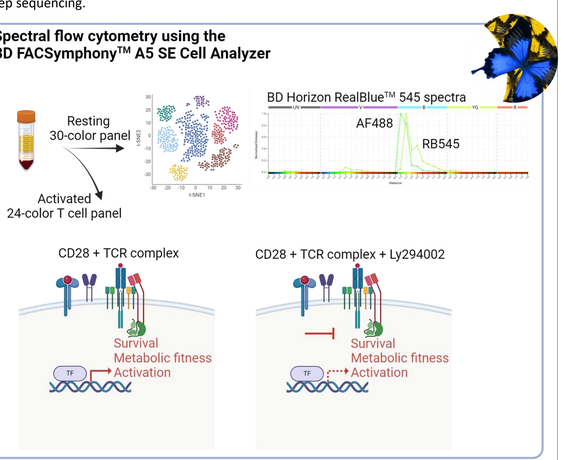
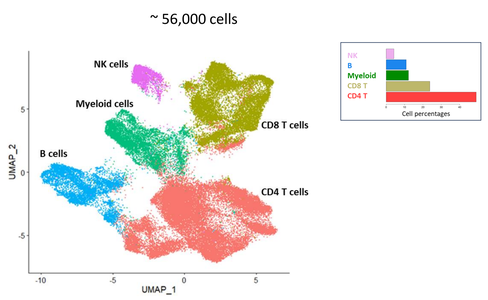


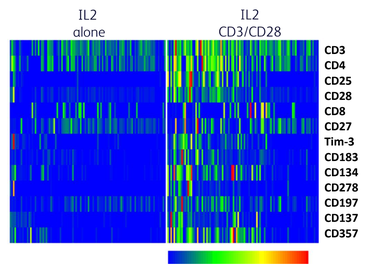
Figure 1C: Assessment of metabolic proteins in resting and activated T cells. Freshly isolated PBMCs and *in vitro* activated T cells were stained respectively with a 30-color and a 24-color panel (Results). Both panels included reagents optimized for intracellular and spectral flow cytometry such as BD Horizon™ RealBlue and Real Yellow reagents. BD® Spectrum Viewer facilitated the choice of reagents based on their spectral profiles (RB545 and Alexa Fluor 488 illustrated). PBMCs stimulation with anti-CD3/CD28 in the presence or absence of a PI3K inhibitor (Ly294002) enabled examining metabolic pathways components.

Results - Single-cell multiomics approach for evaluation of metabolic mediators in TCR-induced responses

2A Annotation of immune cell populations in cell cultures enriched with CD4 and CD8 T cells



2B Heatmap displaying proteins upregulated upon T cell activation



2C Top mRNA transcripts induced in response to T-cell activation includes metabolism regulators

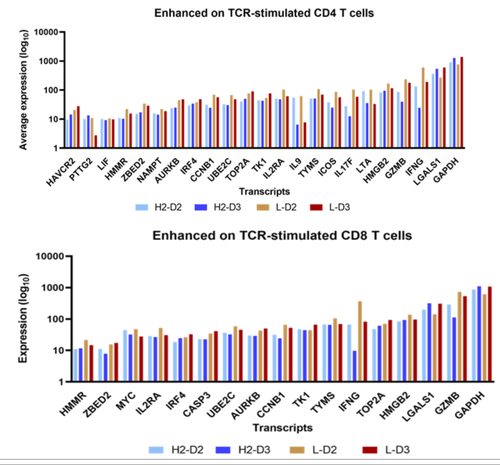


Figure 2. Assessment of mRNA transcripts and proteins, revealing the expression of metabolic mediators in early T cell activation. A. Uniform Manifold Approximation and Projection (UMAP) was applied for dimensionality reduction and analysis of 259 targeted-mRNA transcripts and 35 proteins. **B.** In contrast to IL-2, IL-2 plus anti-CD3/CD28 treatment induced the upregulation of activation markers (CD25, Tim-3, CD137, GITR) on T cell subsets expressing CD3, CD4, CD8 **C.** Differential gene expression analyses unveiled metabolic mediators among the top expressed genes in CD4 or CD8 T cells such as *GAPDH, HMGB2, LGALS1, TYMS,* and effector molecules like *GZMB and IFNG.* H: healthy donor; L: lupus donor; D2: 48h; D3: 72h.

Broadly, stimulation of the TCR complex, CD28 receptor and IL-2 receptor promotes signaling through different pathways including glucose metabolism. Glucose catabolism drives the initial transcriptional programs for T cells to transition to another state, survive and/or proliferate. The BD Rhapsody™ Targeted mRNA T cell panel supported the identification of *GAPDH,* a major enzyme involved in glucose consumption and featured as a top TCR-induced gene in both CD4 and CD8 T cells (Figure 2C). Notably, the *GZMB* gene was also identified demonstrating its role as a connection between aerobic glycolysis and T cell effector functions. The selected genes were equally expressed at 48h or 72h of cultures in both healthy and SLE groups. Because the targeted mRNA panel hindered the analysis of *GLUT1* expression, the main glucose transporter in T cells, we next explored high-dimensional flow cytometry to further elucidate this pathway.

Results - Using spectral flow cytometry for profiling metabolic mediators in resting and activated T cells

3A Expression of GLUT1 and metabolic enzymes in resting T cells, B cells and myeloid cell subsets

Fluor	Specificity	Clone
BUV395	G6PD	EPR6292
BUV496	HLA-DR	G46-6
BUV563	CD45	HI30
BUV615	CD45RA	HI100
BUV661	HK1	EPR10134(B)
BUV737	CD27	M-T271
BUV805	CD19	HIB19
BV421	CD197	2-L1-A
V450	CPT1A	8F6AE9
V480	CD38	HB7
BV510	TCR Cb1	JOVI.1
BV570	CD3	UCHT1
BV605	CD56	R19-760
BV650	CD127	HIL-7R-M21
BV711	IgM	G20-127
BV750	CD16	3G8
BV786	CD279	NAT105
AF488	GLUT1	EPR3915
RB545	GZMB	GB11
BB630	CD4	SK3
BB660	IgD	IA6-2
PerCP	CD8	SK1
BB700	CD123	7G3
RB780	TKT	7H1AA1
PE	CD11c	B-ly6
RY586	CD25	2A3
APC	CD14	M5E2
R718	CD28	L293
APC-H7	CD20	2H7
Viability	FVS440UV	

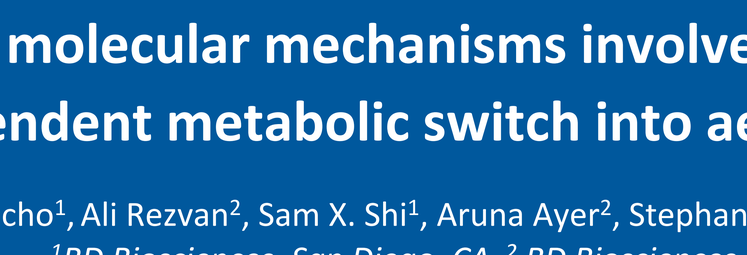
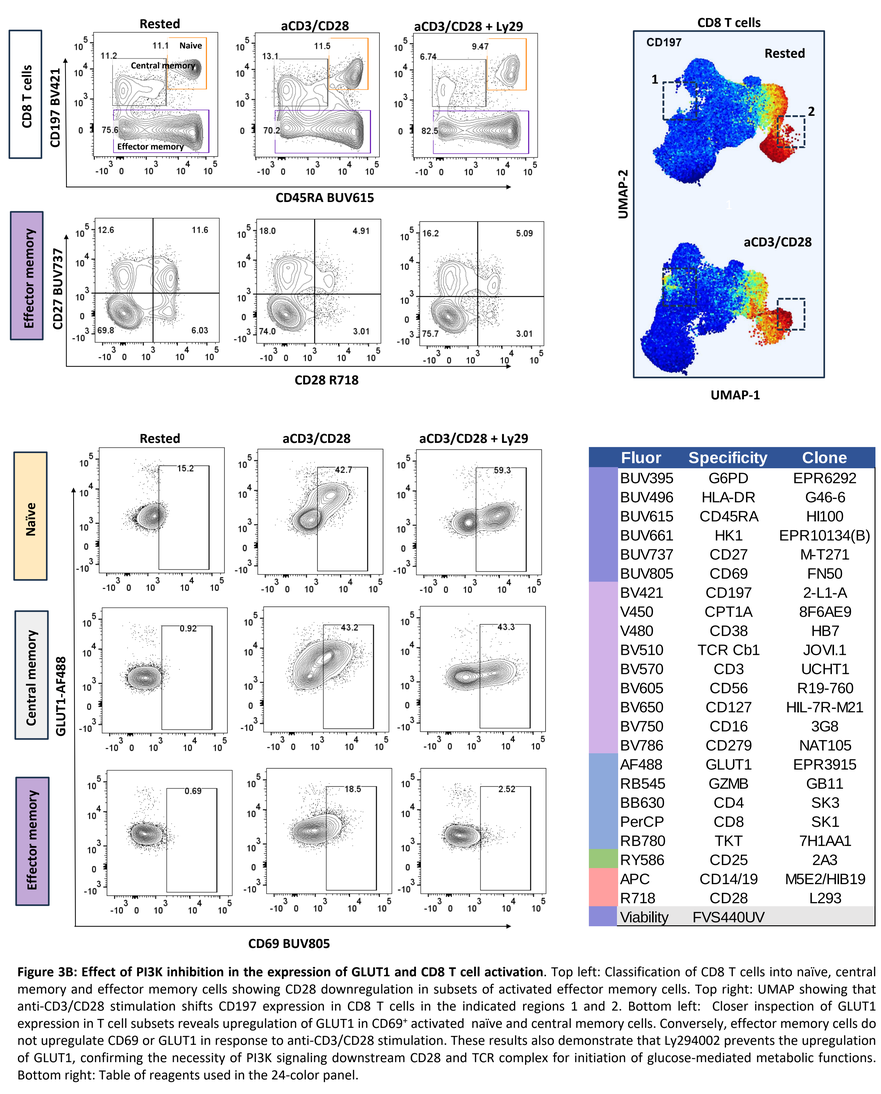


Figure 3A: Using validated fluorochrome-conjugated antibodies and matching isotype controls to explore the expression of metabolic proteins representing different metabolic pathways . The table shows a 30-color panel used to measure metabolic proteins in different immune cell populations. As noted in the first histogram overlay, GLUT1 is highly expressed in pDC but weakly expressed in resting T cells. BUV, BD Horizon Brilliant™ Ultraviolet; BV, BD Horizon Brilliant™ Violet; V, BD Horizon™ Violet; BB, BD Horizon Brilliant™ Blue; RB, BD Horizon RealBlue™; RY, BD Horizon RealYellow™; R, BD Horizon™ Red; AF, Alexa Fluor™, FVS, BD Horizon™ Fixable Viability Stain

To explore GLUT1 expression and activation of the glycolytic pathway in activated T cells, PBMCs were stimulated with anti-CD3/CD28 Dynabeads for 24h. In parallel, stimulated cells were cultured with a PI3K inhibitor (Ly294002), therefore, blocking PI3K/AKT signaling downstream the CD28 and T cell receptors.

3B Impact of PI3K inhibition on glycolysis and CD8 T cell activation



Fluor	Specificity	Clone
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BUV805	CD69	FN50
BV421	CD197	2-L1-A
V450	CPT1A	8F6AE9
V480	CD38	HB7
BV510	TCR Cb1	JOVI.1
BV570	CD3	UCHT1
BV605	CD56	R19-760
BV650	CD127	HIL-7R-M21
BV750	CD16	3G8
BV786	CD279	NAT105
AF488	GLUT1	EPR3915
RB545	GZMB	GB11
BB630	CD4	SK3
PerCP	CD8	SK1
RB780	TKT	7H1AA1
RY586	CD25	2A3
APC	CD14/19	M5E2/HIB19
R718	CD28	L293
Viability	FVS440UV	

3C Selective loss of naïve and central memory GLUT1^{+}GZMB^{+} CD8 T cells occurs upon PI3K inhibition

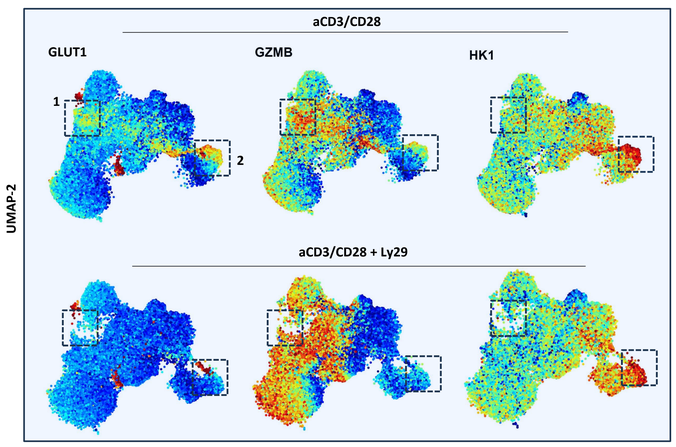

Figure 3C. PI3K signaling downstream CD28 and TCR stimulation leads to GLUT1 and GZMB upregulation in newly activated cells. . UMAP displaying GLUT1, GZMB and Hexokinase 1 (HK1) expression in CD8 T cells activated with anti-CD3/CD28 alone (top images) or in the presence of Ly294002 (bottom images). The highlighted regions 1 and 2 correspond to CD197^{+} cells (Figure 3B), in which GLUT1 is strongly expressed in anti-CD3/CD28 activated cells. GZMB is also induced in regions 1 and 2, however, PI3K inhibition with Ly294002 prevents the formation of GLUT1^{+}GZMB^{+} cells. Notably, HK1 expression is not affected by PI3K inhibition.

Figure 3B: Effect of PI3K inhibition in the expression of GLUT1 and CD8 T cell activation. Top left: Classification of CD8 T cells into naïve, central memory and effector memory cells showing CD28 downregulation in subsets of activated effector memory cells. Top right: UMAP showing that anti-CD3/CD28 stimulation shifts CD197 expression in CD8 T cells in the indicated regions 1 and 2. Bottom left: Closer inspection of GLUT1 expression in T cell subsets reveals upregulation of GLUT1 in CD69^{+} activated naïve and central memory cells. Conversely, effector memory cells do not upregulate CD69 or GLUT1 in response to anti-CD3/CD28 stimulation. These results also demonstrate that Ly294002 prevents the upregulation of GLUT1, confirming the necessity of PI3K signaling downstream CD28 and TCR complex for initiation of glucose-mediated metabolic functions. Bottom right: Table of reagents used in the 24-color panel.

Conclusions

- Cellular metabolism is crucial for the activation and specialized functions of immune cells. Here we offer tools and strategies for a comprehensive analysis of metabolic proteins in a variety of cell subsets:
  1. New instrumentation (BD Rhapsody™ HT Xpress System) enabling sample multiplexing and higher throughput for simultaneous analysis of cells from various donors, cultured in different conditions and captured in different days for broad assessment of mRNA transcripts and proteins regulated during T cell activation.
  2. Five clones for flow cytometry applications and specific for catabolic or anabolic pathways (GLUT-1 - EPR3915; G6PD - EPR6292; HK1 - EPR10134(B); CPT1A - 8F6AE9 and TKT - 7H1AA1) and fluorochromes (BD Horizon™ RB545, RB780 and RY586) for panel expansion from 24 to 30 colors.
- T cell activation leads to dramatic shifts in cell metabolism from oxidative phosphorylation to aerobic glycolysis. To measure such changes *in vitro*, we evaluated specially the expression of the glucose transporter GLUT1. We detected GLUT1 upregulation in activated cells. This process was disrupted by PI3K inhibition demonstrating the relevance of this signaling pathway for the uptake of nutrients (glucose) during cell activation. Importantly, PI3K inhibition led to the loss of a GZMB^{+} functional CD8 T cell population.