The mTORC1 inhibitors, Temsirolimus and Everolimus, suppress HIV patient-derived CD4+ T cell death and activation in vitro

Palmer CS[1], Ostrowski M[2], Zhou J[1], Linda Lam [1], Landay A[3], Jaworowski A[1,4], McCune JM[5], Crowe SM[1,4,6]

[1]Centre for Biomedical Research, Burnet Institute, Melbourne, Australia, [2]Instituto de Investigaciones Biomédicas en Retrovirus y SIDA. Facultad de Medicina, Buenos Aires, Argentina, [3]Department of Immunology/Microbiology, Rush University Medical Center, Chicago, IL, USA, [4]Department of Medicine, Monash University, Melbourne, Australia, [5]University of California, San Francisco, Division of Experimental Medicine, Department of Medicine, CA, USA, [6]Infectious Diseases Unit, The Alfred, Melbourne, Australia

Introduction

Glucose transporter 1 (Glut1) is the main glucose transporter on T cells. We have shown that Glut1 level is increased on activated CD4 T cells in people infected with HIV and this was associated with immune activation and low CD4 T cell count (***Palmer et al, AIDS 2014***).

In this activated state, instead of breaking down glucose through oxidative phosphorylation to form ATP, glucose is broken down vis aerobic glycolysis to lactic acid, and generating precursors for cell wall synthesis and growth.

However, glycolysis is only moderately efficient in energy production so we postulated that a metabolic shift from oxidative phosphorylation to glycolysis results in metabolic exhaustion and death of CD4 T cells. We postulate that inhibition of glycolysis would suppress activation-driven CD4 T cell death.

CD4 T cell activation is associated with increased glucose uptake and aerobic glycolysis

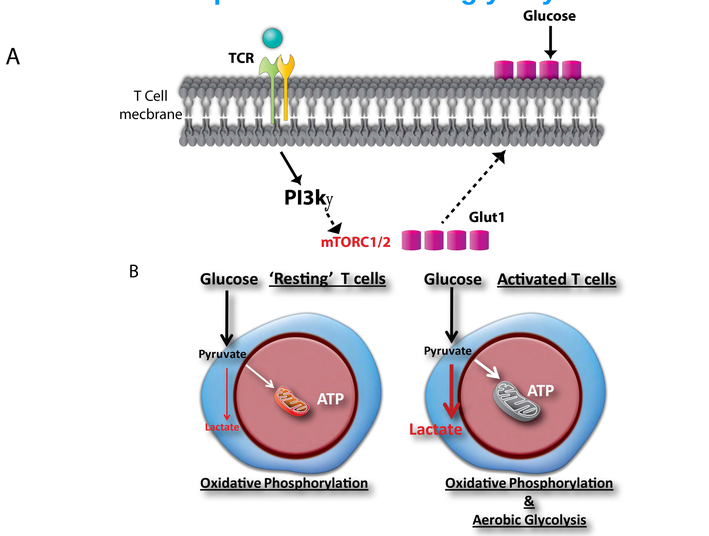

Figure 1. (A) Increased Glut1 expression on activated T cells. (B) Aerobic glycolysis is increased in activated T cells (Palmer et al, Frontiers in Immunology, 2015).

Materials and methods

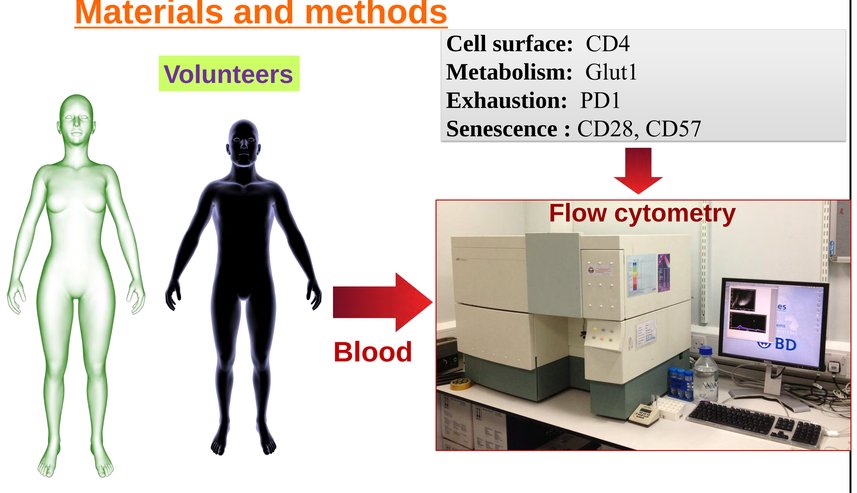

Figure 2. Immunometabolic analysis using flow cytometry

Results

1 GLUT1 IS ELEVATED IN IMMUNOLOGICAL NON-RESPONDERS TO cART

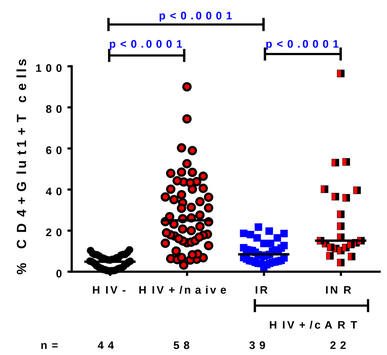

Figure 3. Glucose metabolic activity in CD4+ T cells is increased during HIV infection and remains elevated during cART especially in immunological non-responders (INR; CD4 T cell count <350, and >2 years on cART.

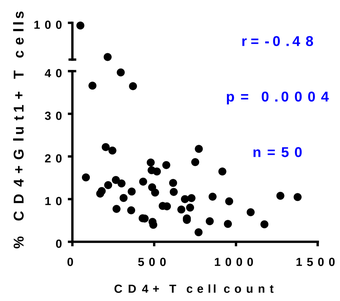

Figure 4. There is an inverse relationship between the percentage of CD4+Glut1+ T cells and CD4 cell count in HIV+/cART patients.

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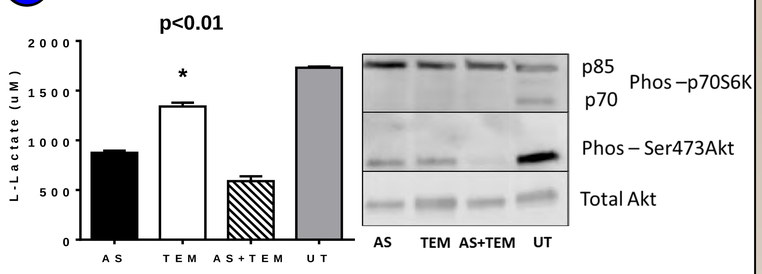

Figure 5. Metabolic inhibitors (AS, TEM) suppress lactate secretion of CD4 T cells activated with PMA and IL-2. AS; PI3K, inhibitor; TEM; temsirolimus and mTOTC1 inhibitor. UT; activated but not treated with inhibitors.

Figure 6. Metabolic inhibitors suppress PI3 kinase (pSer473) and mTOR (p70S6K) signalling.

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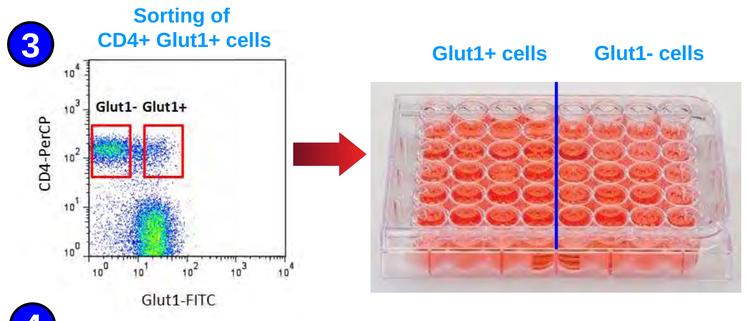

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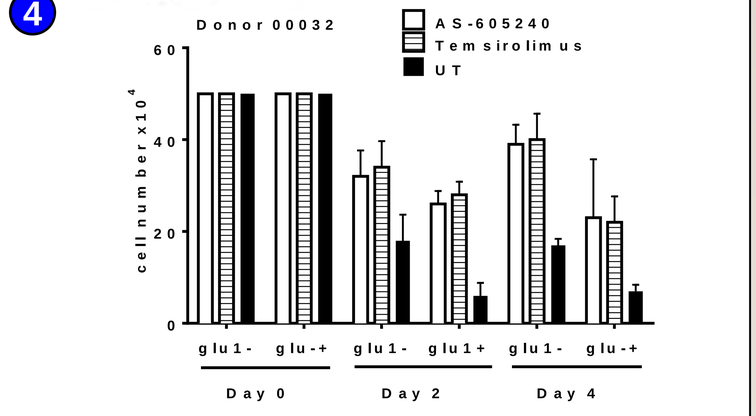

Figure 7. Representative donor CD4+Glut1- and CD4+Glut1+ cell death in the presence or absence of the mTORC1 inhibitor temsirolimus.

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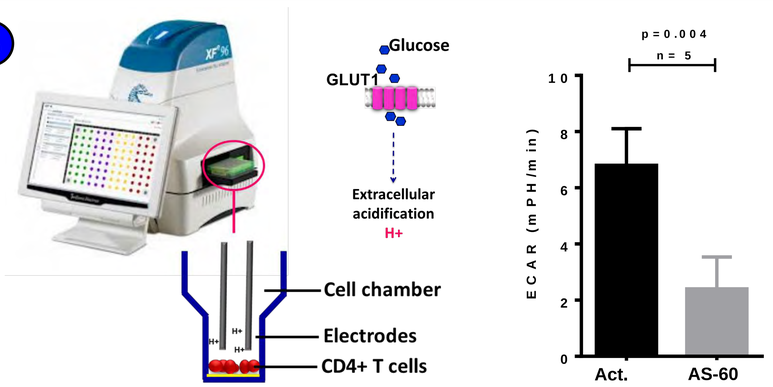

Figure 8. XF-24 Extracellular Flux Analyser measures extracellular acidification rate (ECAR), which is a proxy for glycolytic metabolism. Activated CD4+ T cells have suppressed glycolytic metabolism in the presence of the PI3K$_\gamma$ inhibitor AS-605240 (AS-60).

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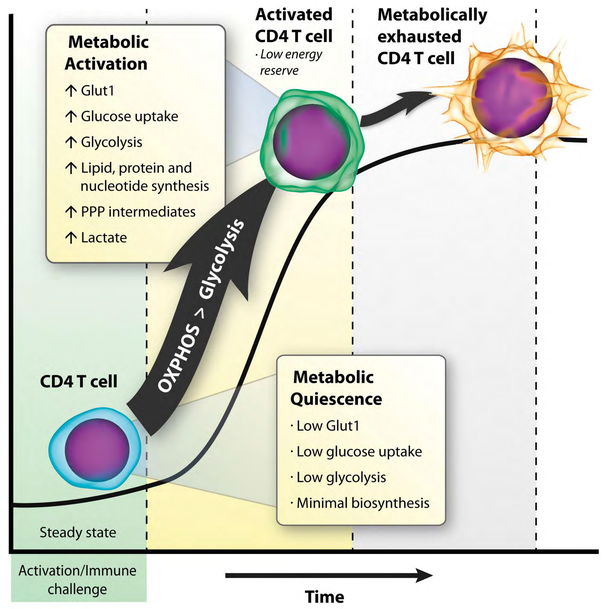

Figure 9. Naïve and memory T cells have low glucose uptake and rely primarily on OxPhos to produce energy. Activated T cells increase glucose uptake and switch to glycolysis. The activated state is maintained by glycolysis and our premise is that, prolonged activation results in metabolic exhaustion and cell death. PPP, pentose phosphate pathway. OxPhos: Oxidative phosphorylation, PPP: Pentose phosphate pathway.

Conclusions

We propose a new paradigm by which CD4+ T homeostasis in HIV+ subjects is controlled by their metabolic phenotype, irrespective of treatment status. "CD4+ T cell metabolic exhaustion" is a potential mechanism by which CD4 T death occurs.

Therapies that specifically target glucose metabolic pathways in CD4+ T cells could be exploited to improve immunological recovery in HIV+/cART subjects or enhance longer term remission without cART.

Reference:

1. **Palmer CS,** et al. (2014). Increased glucose metabolic activity is associated with CD4+ T cell activation and depletion during chronic HIV infection. AIDS 2014,; 28(3):297-309.

2. **Palmer CS,** et al. (2015). Glucose metabolism regulates T cell activation, differentiation, and functions. Frontiers in Immunology. doi: 10.3389/fimmu.2015.00001

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Graphics

We acknowledge the graphics done by Mr Wallace Wainhouse at **NICE-consultants.com**. **Contact: info@naturalimmunity.com.au**