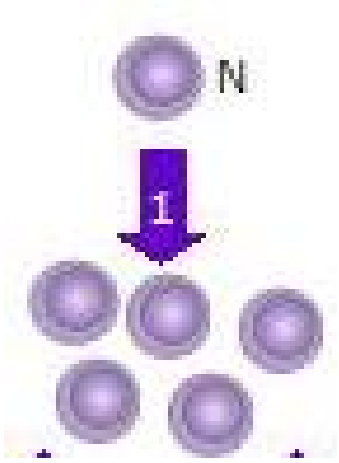
Measuring T cell proliferation using the “Warburg” effect.



T cell proliferation. After the naive T cell (N) encounters an antigen it becomes activated and begins to proliferate (divide) into many clones or daughter cells, which either become memory or effector cells. Adapted from Wikimedia, Commons [Author: Ciar at English Wikipedia]

Researchers from the University of Cambridge aimed to use naturally occurring changes in cellular metabolism to measure of T cell proliferation. When naïve and memory T cells are resting, they primarily use oxidative phosphorylation (TCA cycle) to generate energy. Upon stimulation, these cells switch to glycolysis to provide more energy for cellular activation, biosynthesis of effector molecules and cell proliferation. This shift in metabolism to glycolysis results in accumulation of lactate which is excreted out of the cell, similar to the Warburg effect that occurs in cancerous cells.

The two most commonly used T cell proliferation methods are thymidine DNA incorporation and cell proliferation dye dilution assays. These assays require specialised training to use radioactive material and the flow cytometer, respectively. Grist *et al.* aimed to use lactate accumulation in response to high rates of glycolysis in replicating and proliferating cells as a proxy for T cell proliferation.

To measure lactate, Grist *et al.* harvested supernatant from the T cell proliferation assay and froze the

supernatant at -20°C. This was followed by detection of lactate using a spectrophotometry, at a later time point. Researchers showed that lactate assay was as sensitive as the thymidine incorporation assay at later time points, 5 days post start of proliferation, but not at early time points of the T cell proliferation assay (2 days). Additionally, the lactate assay showed comparable results to cell proliferation dye dilution assays, in a T-reg suppression of effector T cell proliferation assay. In fact, the lactate assay could be more favourable than cell proliferation dye dilution assay because no phenotyping of cells using flow cytometry is required to distinguish proliferating effector and T-regs memory cells, as T-regs do not use glycolysis to generate energy, thus detected lactate in the assay will be from replicating effector cells only.

In summary, Grist *et al.* showed the utility of the lactate assay as a measure of T cell proliferation. The assay is safe, sensitive, reliable, cost effective and requires minimal manipulation. An added advantage of the lactate assay is that it can be easily incorporated into any long term T cell stimulation assays, that is followed by storage of cell culture supernatant.

Journal Article: Grist *et al.*, 2018. Extracellular Lactate: A Novel Measure of T Cell Proliferation. Journal of Immunology

Journal Article: Cheleka AM Mpande