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Strapline: Glucose inhibits Treg cell function in tumours

Title: Going sugar free: Treg cells avoid glucose to maintain functional fitness

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Running title: Metabolic rewiring of tumour Treg cells

Regulatory T (Treg) cells play a critical role in suppressing self-reactive T cells to preserve immune homeostasis (1). In the context of cancers however, Treg cells dampen the function of anti-tumour immune cells to promote cancer progression (1). Altered cellular metabolism

is one of the hallmarks of cancer cells. The high glycolytic activity and amino acid metabolism of cancer cells creates a hostile tumour microenvironment (TME) that is depleted of glucose and amino acids. In addition, high glucose utilization of tumours also makes the TME acidic due to the accumulation of lactic acid, a glycolytic by-product (2). Cellular metabolism and T cell development, maintenance and function are closely linked. $CD8^+$ T cells are potent in killing tumour cells and share many metabolic similarities with cancer cells. The high glycolytic demand of $CD8^+$ T cells for their effector function detriments them in the TME where they are outcompeted by tumour cells for available glucose (3). Besides limited glucose availability, lactate build-up in the TME also impedes the function of tumour infiltrating $CD8^+$ T cells (4). Treg cells in contrast are traditionally known to rely on oxidative phosphorylation (OXPHOS) to generate energy (5). However, the link between altered TME and increased suppressive activity of tumour infiltrating Treg cells remained unknown until now.

A string of papers published recently in the journal *Nature* reveal unique metabolic features of tumour infiltrating Treg cells. In particular, two papers from Watson *et al*. and Zappazodi *et al.* investigated the impact of glucose availability and lactate build up on the suppressive function of Treg cells in the TME. Both these papers used distinct approaches to highlight that Treg cells are functionally impaired in high concentrations of glucose. Watson *et al*. assessed the suppressive function of infiltrating Treg cells from tumours with different glycolytic potential. They discovered that the suppressive function of Treg cells was inversely proportional to the glycolytic ability of tumours (Figure 1). Interestingly, even in the same TME, using fluorescent glucose tracer, Watson *et al*. were able to predict Treg suppressive function depending on their glucose avidity (6). Treg cells with high tracer uptake showed impaired suppressive function. In keeping with the functional defect, transcriptional profiling of tumour infiltrating Treg cells with high and low glucose avidity revealed downregulation of genes responsible for immune suppression in Treg cells with high glucose avidity (6). Interestingly, low glucose avidity Treg cells showed upregulation of lactate dehydrogenase (*ldha*) and the monocarboxylate transporter MCT1 (*Slc16a1*) (6). Lactate uptake is mediated through MCT1 and therefore possibly enables Treg cells to use it as an alternative energy source in highly glycolytic TME with high lactate concentrations. Tracing experiments with labelled lactate demonstrated that tumour Treg cells readily metabolized lactate into metabolites higher up in the glycolytic pathway via gluconeogenesis – which is the reversed form of glycolysis. Watson *et al.* ablated MCT1 in Treg cells using

Foxp3^{Cre} and implanted B16 melanoma tumours in these mice. Mice with Treg cells deficient for the lactate transporter MCT1, showed less tumour burden supported by elevated activation of tumour infiltrating $CD8^+$ T cells. Interestingly, intratumoral Treg cells from these mice became more glucose avid and developed an inflammatory phenotype by producing IFNγ. Despite the functional defect in tumours, these mice did not develop autoimmunity suggesting context specific usage of fuel source by Treg cells (6).

Zappazodi *et al.* took a different approach to discover a destabilizing role of glycolysis in tumour infiltrating Treg cells. Using transcriptional profiling data from melanoma patients before and after ipilimumab (α-CTLA4) treatment, these authors discovered that tumour glycolysis negatively correlated with immune infiltration in tumour (7). Interestingly this correlation diminished in ipilimumab treated patients but remained for LDHA and MCT1 suggesting that highly glycolytic tumours cannot be treated with ipilimumab single therapy. To fully understand the link between tumour glycolysis, CTLA4 blockade and immune infiltration, Zappazodi *et al.* made LDHA deficient 4T1 mammary carcinoma cell lines. CTLA4 blockade promoted the survival of mice carrying LDHA deficient tumours compared to controls with LDHA intact 4T1 tumours. In line with earlier studies, immune infiltrates in tumours of the same size revealed elevated numbers of $CD8^+$ T cells and Treg cells in LDHA deficient tumours (7, 8). Interestingly, when LDHA deficient tumour bearing mice were treated with CTLA4 blockade, the infiltrating Treg cells acquired an inflammatory phenotype akin to $CD8^+$ T cells and produced IFNγ. This inflammatory phenotype correlated with downregulation of CTLA4 and CD25 in tumour infiltrating Treg cells (7). These authors discovered that CTLA4 blockade promoted glycolysis in Treg cells leading to the acquisition this inflammatory phenotype. In line with the findings from Watson *et al*., lactate reversed the inflammatory features. CD28 is known to induce glycolysis in T cells (9). By ablating CD28, these authors also showed that CTLA4 mediated upregulation of glycolysis is dependent on CD28 signalling. To unequivocally demonstrate the inhibitory effect of glycolysis on Treg cells, Zappazodi *et al.* generated LDHA and Glut1 (glucose transporter) deficient Treg cells. When LDHA deficient tumours were implanted in these mice and treated with α-CTLA4, Treg cells showed no more downregulation of CTLA4 and CD25 as well as no upregulation of IFN-γ expression suggesting that truly glycolysis is responsible for the loss of Treg cell suppressive function. However, these Treg cells also lost functional fitness

in the absence of LDHA or Glut1 suggesting a broader role for glycolysis in tumour infiltrating Treg cells (7).

While these two papers highlight the negative role of glycolysis on the function of tumour infiltrating Treg cells, Watson *et al.* emphasize on the usage of lactic acid as an alternate fuel source for tumour infiltrating Treg cells (Figure 1). Both studies show that forcing intratumoural Treg cells into glycolysis via either loss of lactate uptake (Watson *et al.*) or treatment with α-CTLA4 antibodies (Zappazodi *et al*.) leads to a loss of their suppressive function (Figure 1). Despite the extraordinary attempt from these two groups to unravel the metabolic competition between tumours and Treg cells, there are several questions that remain unanswered. Whereas increased glycolysis is bad for Treg function, it remains unclear what impact dampened glycolysis has on intratumoural Treg cell function and maintenance. Zappazodi *et al.* used Glut1 and LDHA deficient Treg cells to demonstrate impaired glycolysis and short-term restoration of Treg cell function, however, the long-term maintenance was impaired. Given the importance of LDHA in the lactate metabolic pathway it seems counterintuitive why deletion should promote Treg cell function. A possible explanation to this conundrum might come from the proposed glucose-lactate axis by Watson *et al*. Glycolysis is not a one-way street and gluconeogenesis, the reversed reaction of glycolysis, seems to be particularly important in intratumoral Treg cells. Depending on changes in glucose/lactate concentration, LDHA either converts pyruvate into lactate or the other way around (10). Effector T cells show high glucose uptake and lactate production (11), and activation of memory $CD8^+$ T cells leads to a rapid lactate production needed for their effector function (12). These studies suggest a preference of glycolysis over gluconeogenesis in conventional $CD4^+$ T cells and $CD8^+$ T cells. These differences offer new approaches to pharmacologically inhibit Treg cells specifically in the TME. While inhibiting lactate uptake or lactate conversion might look like an obvious target it should be noted that the lactate transporter MCT1 also transports acetate, succinate, propionate and butyrate (13). It also remains to be determined how pharmacological inhibition/deletion of LDHA, as an important part of the glycolytic pathway, is impacting the intratumoural $CD4^+$ and $CD8^+$ T cell responses. Dampening of the effector response is likely to outweigh the beneficial effects of impaired Treg cell function in tumours. Pure gluconeogenesis enzymes seem to be more appropriate targets. However, Watson *et al*. couldn't show any beneficial effects on tumour growth by inhibiting phosphoenolpyruvate carboxylase (PEPCK), the rate limiting enzyme in

gluconeogenesis (6). Further studies are required to define the specific metabolic requirements of tumour infiltrating T cells and will offer possibilities to target T cell subsets in a context specific manner.

Author contributions

Patrick M Gubser - Conceptualization; writing-original draft & editing.

Ajithkumar Vasanthakumar - Conceptualization; writing-original draft & editing.

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Figure caption:

Figure 1. The influence of low (left) and high glycolytic tumour (right) on glucose uptake and function of Treg cells in the respective tumour microenvironment. Treg cells with low glucose avidity are highly functional compared to high glucose avidity Treg cells. Tumour derived lactic acid is sensed by low glucose avidity Treg cells through MCT1. Upregulation of CTLA4 dampens glycolysis and improves the functional fitness of Treg cells. CTLA4 blockade and CD28 co-stimulation are both critical to induce glycolysis in Treg cells, which consequently promote loss of function.