CPD Evidence 2 (2022)

1. Registrant Information

1.1 Full Name: Chen Liu, AMRSB
1.2 Profession: BMS
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Standard 3 – A registrant must seek to ensure that their CPD has contributed to the quality of their practice and service delivery
Standard 4 – A registrant must seek to ensure that their CPD benefits to the service user

2. Details

I presented a paper in the journal club hosted by Prof. Rudd's Lab at the University of Montreal.

Details start from the next page.

CPD Evidence (version 3, created 08/2022)

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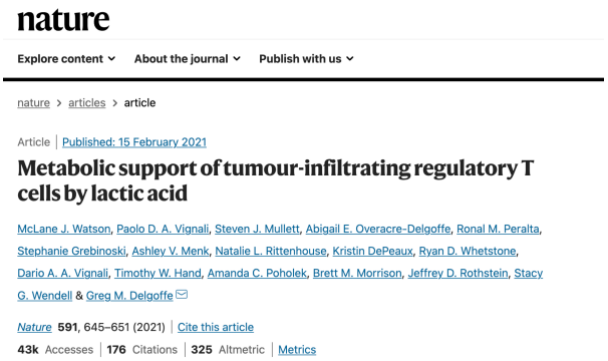
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Metabolic support of tumour-infiltrating regulatory T cells by lactic acid

McLane J. Watson, Paolo D. A. Vignali, Steven J. Mullett, Abigail E. Overacre-Delgoffe, Ronal M. Peralta, Stephanie Grebinoski, Ashley V. Menk, Natalie L. Rittenhouse, Kristin DePeaux, Ryan D. Whetstone, Dario A. A. Vignali, Timothy W. Hand, Amanda C. Poholek, Brett M. Morrison, Jeffrey D. Rothstein, Stacy G. Wendell & Greg M. Delgoffe

Nature **591**, 645–651 (2021) | Cite this article

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Chen Liu
Lab meeting journal club, 24/11/2022

Hello, today I will be presenting a paper by Dr McLane Watson *et al.* from the University of Pittsburgh, published in Nature. The title is “Metabolic support of tumour-infiltrating regulatory T cells by lactic acid.”

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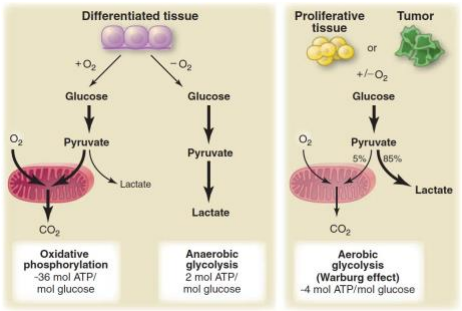
Introduction



Warburg effect [1]

- Deregulated metabolism within the tumour microenvironment (TME): metabolite-depleted, hypoxic and acidic.
- Infiltrated effector T cells have to compete with the tumour cells for metabolites => impaired function.

Hypothesis: **Altered metabolic landscape profile of the TME and increased activity of Treg are linked.**

[1]: Science. 2009 May 22; 324(5930): 1029–1033

Before we talk about the results, I would like to make a brief introduction on tumour metabolism. Unlike differentiated tissues, tumour cells prefer anaerobic respiration. This is known as the “Warburg effect”. Tumour infiltrating T cells have to compete with tumour cells for glucose, and

this is one of the contributing factors that is why the tumour microenvironment (TME) is immunosuppressive. However, the inhibitory function of regulatory T cells was hardly affected, suggesting Tregs are labouring a different metabolic profile from other T cells. In this study, the researcher investigated how the distinct metabolism of Tregs supports its suppressive function in the TME.

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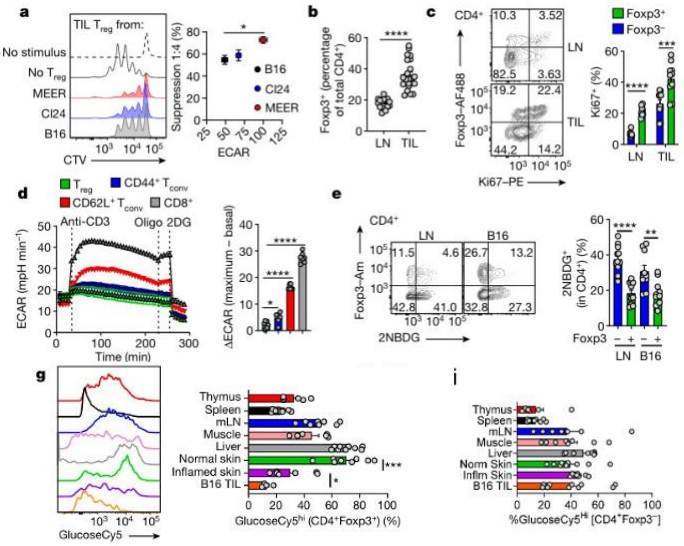

- Suppressive function of Treg correlated with tumour cell glycolysis activity.
- Reduced glycolysis and glucose uptake in Treg compared to Tconv.
- The uptake of glucose by Treg is tissue-heterogeneous.

[a] Firstly, they measured the suppression capacity of the Tregs isolated from different tumour models. Apart from B16, they had the Clone 24 melanoma and the MEER, a head and neck small cell carcinoma model. Remember from the previous presentation, the extracellular acidification rate measures the rate of glycolysis. They found that the suppression function of intratumoural Tregs is correlated with glycolysis by tumour cells. **[b]** They used CD4 and FOXP3 as markers to confirm that more Tregs were observed in the TIL than in lymph nodes. And those Tregs in TIL is actively proliferating as well. **[c]** They compared the glycolysis between activated Tregs and other conventional T cells. As you can see, TCR triggering induced less glycolytic activity and capacity (between oligomycin and 2-DG) in Tregs. This suggests that glucose transport may be limited in the Tregs. **[e]** Indeed, there was a significant reduction in TIL Tregs in glucose uptake when measuring with 2-NBDG. **[g]** They used a glucose monitor called GlucoseCy5 to measure the glucose uptake by infiltrating Tregs in different tissues, but notably low in the B16 tumour and

inflamed skin. They used imiquimod to induce ear skin inflammation. These results suggested that inflammation and tumour may drive lower glucose uptake in Tregs.

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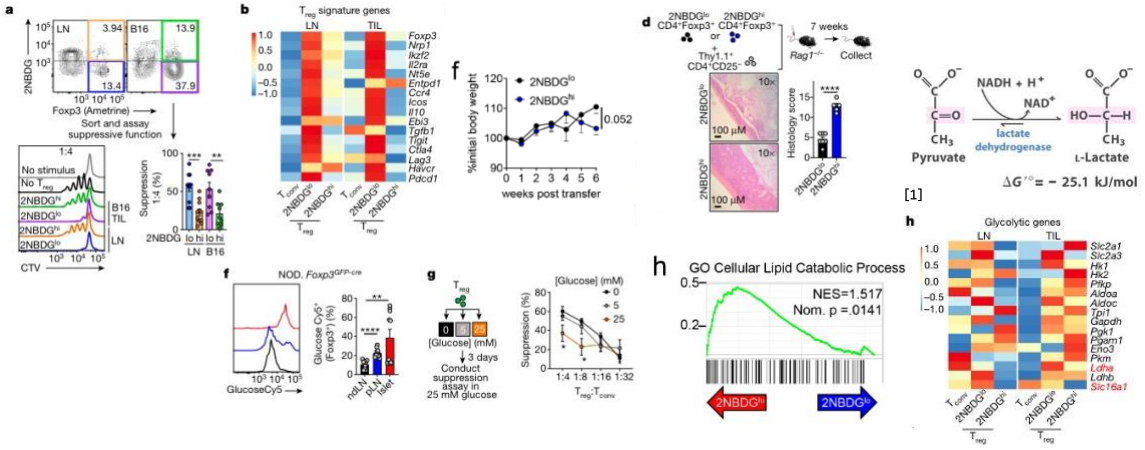

- Glucose avidity in Treg correlates with poor suppressive capacity.
- Treg upregulates genes involved in lactate metabolism: lactate dehydrogenase (*Ldha*) and monocarboxylate transporter MCT1 (*Slc16a1*).

[1]: Protoepedia

[a] Next, they purified two subsets of Tregs based on their glucose avidity. 2-NBDG avid, or higher uptake Tregs showed significantly reduced suppressive capacity compared to 2-NBDG low Tregs. Thus, glucose uptake may predict poorly suppressive Tregs. **[b]** RNA sequencing revealed that high 2-NBDG has reduced expression of *Foxp3*, *Il2ra* (CD25), *Nrp1* and other Treg signature genes. Although those glucose-avid Tregs are still FOXP3+, they are considered to be *weaker*. **[f]** We mentioned that Tregs had lower glucose uptake under inflammatory conditions than other T cells. They transferred glucose high or low Foxp3+ Tregs along with Foxp3- conventional T cells into *Rag1* deficient mice (incapable of forming mature lymphocytes) to induce colitis. At seven weeks, mice receiving 2-NBDG high Tregs began to lose weight due to the increment of colitis. **[d]** As you can see in the H&E stain, the mucus layer of the 2-NBDG high group was thickened, indicating a more robust induction of inflammation. The histology score quantified the extent of inflammation, damage to the anatomical structure and hyperaemia. **[f]** To investigate whether increased glucose uptake could be observed in an autoimmunity setting, they chose the NOD (non-obese diabetic)-*Foxp3* model. Tregs from pancreatic islets and draining lymph nodes were considered to be dysfunctional. In comparison, non-draining lymph nodes were functional and had

significantly lower glucose uptake. Thus, glucose avidity in Tres correlates with poor suppressive capacity. The next question was: if high glucose uptake could make a Treg dysfunctional, can a high-glucose environment, regardless of uptake, do the same job? **[g]** The answer is yes. Conditioning Tregs in a high glucose environment dampened their suppressive function, as shown here. **[h, GSEA]** So the Tregs are not much relying on glucose to be functional, then what is the profile to support the metabolism of a Treg? Lipid catabolism was enriched to the 2-NBDG high group, indicating that lipid catabolism was not contributing to its inhibitory function. **[h, heatmap]** Interestingly, the 2-NBDG low subpopulation showed enriched expression of enzymes involved in the terminal steps of glycolysis, specifically the lactate dehydrogenase (*Ldha*) and the monocarboxylate transporter 1 MCT1, encoded by *Slc16a1*. The MCT1 brings import lactate to the cell. **[reaction]** The reaction is described here. Although lactate is the end-product of glycolysis, it represents a major energy source for many cells, including tumour cells. Under aerobic conditions, glucose is fully burnt by the cells via the Kreb cycle, eventually to carbon dioxide. Alternatively, glucose may undergo glycolysis leads to the production of lactate anaerobically. Hence, these results suggested that lactate supported the function of Tregs.

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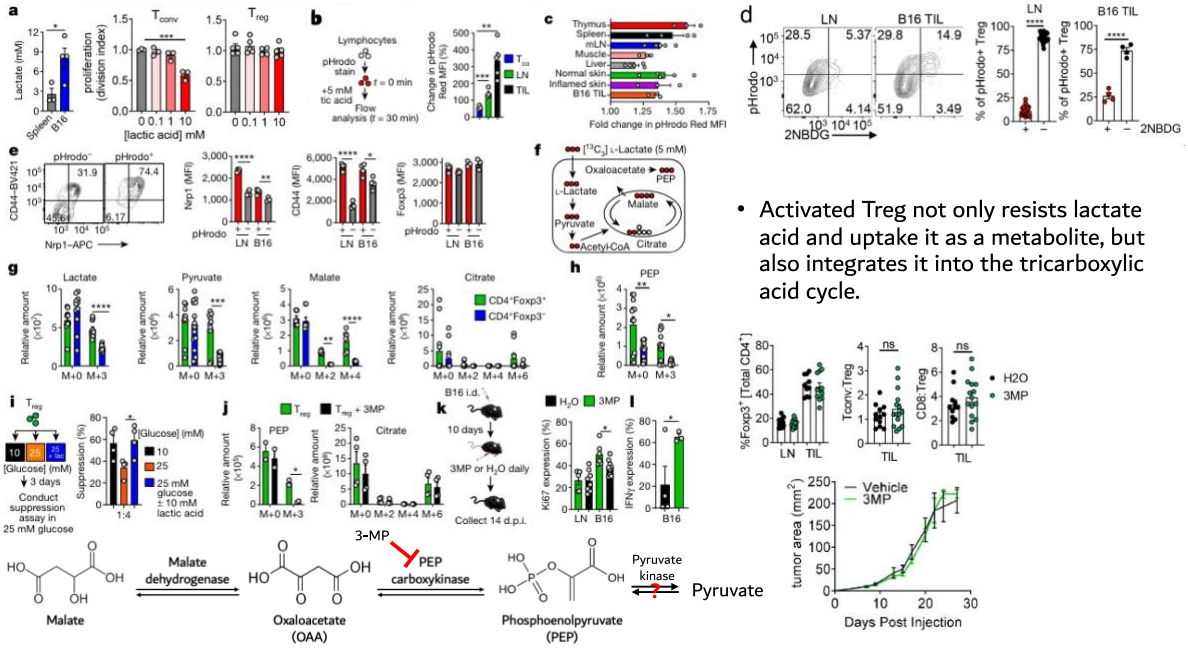

[a] The combination of glycolytic cancer cells and cancer-associated fibroblasts increases lactate levels in the TME as you can see here. The consequence is that it makes the TME

immunosuppressive. The proliferation of conventional T cells was significantly curbed under high lactate concentrations. In contrast, Tregs were resistant to tumour-equivalent concentrations of lactate. **[b]** In this study, they used an intracellular pH change monitor called "pHrodo". The higher the pH dropped, the brighter the pHrodo. In this case, intratumoural Tregs took up lactate, but not conventional T cells. **[c]** This assay also revealed that lactate uptake was heterogeneous among tissues, notably low in glucose-rich organs, such as the liver. **[d]** Indeed, most 2-NDBG-positive Tregs do not take up lactate. **[e]** Those lactate-uptaking Tregs also showed increased CD44 and Nrp1 expression, while maintaining similar Foxp3 level. The CD44 co-stimulation promotes Foxp3+ regulatory T-cell persistence and function via the production of IL-2, IL-10 and TGF-β. Nrp1 could facilitate the interaction with dendritic cells and promote the migration of Treg into the TME or inflammatory sites. Thus, Tregs are not only resistant to lactate, but also take it up as a metabolite. To investigate what happened after lactate was imported into the cell, they treated activated Tregs and conventional T cells with carbon-13 labelled lactate. **[f]** The pathway for lactate metabolism is shown here. Lactate enters the cell via MCT1. It is firstly converted to pyruvate by lactate dehydrogenase. Then pyruvate enters mitochondria and undergoes decarboxylation, NAD+ reduction and attachment of the enzyme A to form the acetyl CoA. And from here, acetyl CoA joins the tricarboxylic acid cycle or the Kreb cycle to complete the rest of cell respiration. **[g]** With the help of mass spectrometry, it is possible to differentiate carbon-12 and carbon-13, which allows them to discover how lactate was used in the cell. The "M+n" denotes molecular mass plus the number of heavy carbons. M+3 (pyruvate) means the number of pyruvates, which has carbon 13 in all three carbons. Malate is another intermediate in the Kreb cycle, which has four carbons. They found that in Tregs, heavy-carbon labeled lactate was taken up by the cell and used to produce pyruvate and malate. **[reaction]** In liver cells, the Kreb cycle intermediate, oxaloacetate, could leave the mitochondria and be recycled via this pathway. With the help of phosphoenolpyruvate carboxykinase (PEPCK), to yield **[h]** PEP, phosphoenolpyruvate. And PEP could be converted back to pyruvate via a dephosphorylation step. Now the question is, did this reaction occur in Tregs? **[j first]** From the mass spec it's impossible to differentiate the pyruvate was directly from lactate or PEP. So, they used a PEPCK inhibitor called 3-mercaptopicolinic acid (3-MP), and the result showed that there was a reduction in PEP after 3-MP treatment. The amount of citrate was not affected, suggesting that the Kreb cycle was not significantly affected. **[i]** From previous results, we know that high glucose could curb

the function of Treg. Conditioning Tregs with glucose and lactate confirmed that their function could be rescued by lactate. **[k, l]** Treating B16-bearing mice with 3-MP increased the expression of Ki-67 and IFNγ production in TIL Tregs. **[right figures]** However, it did not overtly affect the percentage of TIL Tregs, nor change the ratio of Tregs to CD4 or CD8 cells. The tumour area remains unchanged between the two groups. From the above experiments, they confirmed that Tregs not only use lactate to feed the Kreb cycle, but also used to generate PEP to provide fuel in TIL Tregs.

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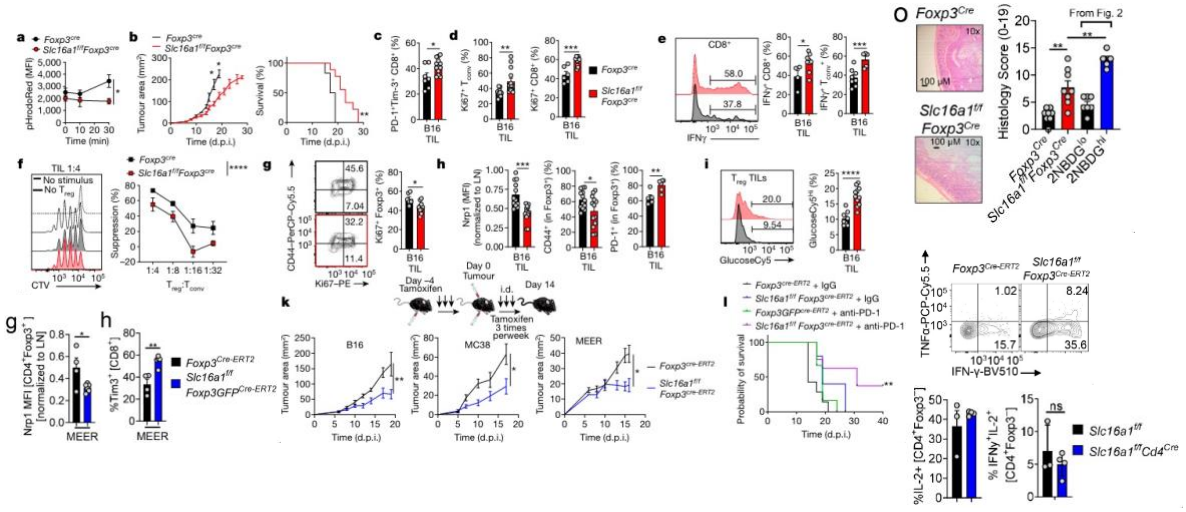

- Treg-specific deletion of MCT1 (Slc16a1) mice had slowed B16 tumour growth and prolonged survival.
- Dysfunctional intratumoural Treg became glucose-avid and developed a fragile phenotype which produced IFN-γ.
- Deletion of MCT-1 in Treg shifted the TME to anti-PD-1 immunotherapy-conducive without affecting the functionality of conventional T cells.

The next step is to investigate the significance of lactate metabolism in Tregs. They produced the MCT1, or Slc16a1 knockout transgenic mice. Only the MCT1 in mature Tregs were deleted. **[a]** As shown here, the deletion of MCT1 resulted in the loss of lactate uptake. **[b]** The MCT1 knockout mice also showed slower tumour growth and prolonged survival. **[c, d, e]** MCT1 KO mice had a higher amount of PD-1, Tim-3, Ki-67 and IFNγ+ CD8 T cells, suggesting decreased suppressive function by MCT1 deficient Tregs. **[f, g]** In ex vivo experiments, MCT1 KO TIL Tregs also had reduced suppressive function and were less proliferative. **[h]** We mentioned earlier that lactate-uptaking Tregs had upregulated CD44 and Nrp1 expression, however in MCT1 deficient cells they were downregulated. Meanwhile has higher PD-1 expression. **[i]** Indeed, those weaker Tregs became glucose-avid. To remove any unforeseen effects of MCT1 knockout, they

introduced another model, the *Slc16a1/Foxp3 Cre-ERT2*, it's a tamoxifen-inducible Tregs-specific model. They repeated all the *in* vivo experiments and deleted the MCT1 just before implanting the tumour cells. **[k]** They found that knockout of MCT1 in Tregs has slowed the growth of B16, MC38 adenocarcinoma and MEER head and neck small cell carcinoma. **[MEER g, h]** In the TIL from the MEER model, MCT1 KO Tregs had lower Nrp1 and higher Tim-3 expression. Those data indicated that they had generated a fragile Tregs phenotype by knocking out MCT1. **[l]** MCT1 knockout synergised with anti-PD-1 immunotherapy, leading to a stronger tumour clearance. 37.5% of B16-bearing mice had complete tumour regression. **[right middle figure]** Interestingly, they spotted that those less reactive Tregs started to produce IFNγ, which was beneficial in immunotherapy. **[right bottom figure]** Meanwhile, if MCT1 were deleted in the CD4 conventional T cells, it would not affect their ability to produce IL-2 or IFNγ. Thus, the expression of MCT1 and consequent uptake of lactate are dispensable for most tissue-derived Tregs, but required to maintain their high suppressor activity in the TME.

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Conclusion and perspectives

- Regulatory T cells employ lactate to protect their suppressive function against the negative effect from glucose.
- MCT-1 is required to maintain the high suppressive activity in the TME. Deletion of MCT-1 in Tregs synergised anti-PD-1 immunotherapy.
- Other tolerogenic cells, eg tumour-associated macrophages, *may* share similar lactate metabolism to Tregs in the TME.

- **Targeting lactate metabolism, by inhibiting MCT-1 or lowering tumoural acidity may break the barrier to cancer immunotherapy.**

In conclusion, they found that Tregs had a distinct metabolic profile. Unlike effector T cells that use glucose, glucose is deleterious to Tregs. And they utilise lactate as one energy source to ensure their suppression function. The lactate transporter, MCT1, is required to maintain the inhibitory function of regulatory T cells. Deletion of MCT1 could promote tumour clearance and attenuates the TME to be checkpoint therapy-conducive. Therefore, they proposed that other

immunosuppressive cells in the TME, such as the tumour-associated macrophages, may share a similar metabolic profile as tumour-infiltrating Tregs.