**Model Refinement and Evaluation**

**Refinement:**

- Metabolic tasks that all cells should be able to perform

- When evaluating the models with tasks, boundary reactions (any reaction that consumes or introduces mass into the system) are removed.

- Tasks used in model validation are those from *Richelle A. et al (2019)*.

- Gap-fill…

- dead-end reactions…

**Evaluation: T-cell specific functions**

- Base medium to use when evaluating the reconstructed T-cell models:

- H2O; O2; H; O2S; CO2; Pi; H2O2; HCO3; H2CO3; CO and RPMI-1640 medium elements are left unconstrained, unless otherwise stated

- Glutamine ideal concentration range is 0.6-2.0 mM for lymphocytes (Oliveria DC, 2016)

-> First test whether when simply maximizing the biomass, the medium values fall in the normal blood levels. If so, set these for the remaining tests?

-> Some of the following functions are evaluated by simply maximizing the biomass. Others, specific conditions must be set.

- Naïve models (naïve\_CD4 and naïve\_CD8): They need a metabolic balance that favours energy production over biosynthesis to move through tissues and prevent cell death, without leaving a quiescence state.

- High FAO

- High TCA

- High OXPHOS

- Pyruvate and glutamine oxidation via the TCA cycle

- Cholesterol sulphate production must occur (reaction(s): ). It competitively replaces TCR-binding cholesterol, reducing TCR avidity and signalling

- Mimic naïve cells activation (proliferation phase) using the naïve models (Also: do naïve models in activated conditions resemble their effector counterparts?)

- To mimic:

- Glut1 transporter is upregulated to increase glucose uptake (reaction: *GLCt1r*, with positive flux)

- Increased methionine uptake (reaction: *METtec*, with positive flux)

- Upregulation of vitamin D uptake (reaction: VITD3t2). Switch from naïve T cells to effector T cells requires the presence of sufficient extracellular vitamin D and upregulation of the vitamin D receptor occurs (Konijeti GG et al, 2015)

- High Glycolysis

- High FAS

- High glutaminolysis

- Low FAO

- calcium

- All Effector T cells (CTL, Th1, Th2, Th17) [set certain boundaries, as we want to model when they are proliferating and not the steady state?]

- Low TCA and OXPHOS

- High Glycolysis (secrete lactate from pyruvate)

- High glutaminolysis

- Glut1 transporter is upregulated to increase glucose uptake (reaction: *GLCt1r*)

- High levels of aminoacids

- increase of glycolytic activity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

- Thelper subsets

- Th17

- oxphos even lower than other Thelper subsets

- de novo FAS rather than acquisition of extracellular FA

- increased glutamine utilisation by highly expressed glutamate oxaloacetate transaminase 1 (negative fluxes from *ASPTA* reaction) -> generates aspartate and alpha-ketoglutarate, converted to 2-hydroxyglutarate (using reactions *HMR\_0718* and/or *HMR\_0719*)

- Inhibition of glycolysis is capable of inhibiting the cells, especially Th17

- Selenium supplementation may enhance Th1-type immune responses to a greater extent than Th2-type responses (Hoffmann PR et al, 2018)

- Vitamin A is essential for T cell activation and differentiation into T helper subsets Th1, Th2 and Th17 cells (Ross CA, 2012)

- Regulatory T cells

- low glycolysis (but independent, i.e., no glycolysis won’t necessarily lead to cell death)

- High FAO (but do not depend entirely, as glycolysis can serve as an alternative energy source + deletion of CPTI transporter does not affect their development and function)

- High OXPHOS

- cholesterol biosynthesis required for suppressive function

- Glut1 levels (reaction: *GLCt1r*, with positive flux) lower than Thelper subsets

- Mevalonate pathway increases proliferation

- Memory T cells

- Low glycolysis

-High OXPHOS

- High FAS

- Central Memory

- FAS <-> FAO futile cycle: FA formed are broken down by lisossomal-acid-lipase-mediated lipolysis to liberate FA from storage to be used as substrates in FAO

- Effector Memory

- import FA and rely on glycolysis. (May resort to the futile cycle if no availability of FA in the environment??)

- Increased expression of phosphoenolpyruvate carboxykinase (*PEPCK*/ *PEPCKm* reactions)

- Increases glycogen biosynthesis, and posterior glutathione production through the pentose phosphate pathway

- IL7 increases expression of glycerol channel AQP9 (*H2OGLYAQPt* reaction)

- Increases triglyceride synthesis (this does not happen in effector or naïve cells – ‘absence’ of receptor or increase of transportation does not increase triglyceride synthesis?)

**References**

Hoffmann PR, Berry MJ*. The influence of selenium on immune responses*. Mol Nutr Food Res. 2008;52(11):1273-80.

Konijeti GG, Arora P, Boylan MR, et al. *Vitamin D Supplementation Modulates T Cell-Mediated Immunity in Humans: Results from a Randomized Control Trial.* J Clin Endocrinol Metab. 2015;101(2):533-8.

Oliveira DC, da Silva Lima F, Sartori T, Antunes Santos AC, Rogero MM and Fock RA. *Glutamine metabolism and its effector on immune response: molecular mechanism and gene expression.* Nutrire. 2016; 41:14. doi 10.1186/s41110-016-0016-8

Richelle A, Chiang AWT, Kuo CC, Lewis NE (2019) *Increasing consensus of context-specific metabolic models by integrating data-inferred cell functions.* PLOS Computational Biology 15(4): e1006867. <https://doi.org/10.1371/journal.pcbi.1006867>

Ross CA. *Vitamin A and retinoic acid in T cell-related immunity*. Am J Clin Nutr. 2012;96(5):1166S-72S.