Immunometabolism in cancer

Society for Immunotherapy of Cancer Annual Meeting
SITC Primer

UPMC | HILLMAN CANCER CENTER

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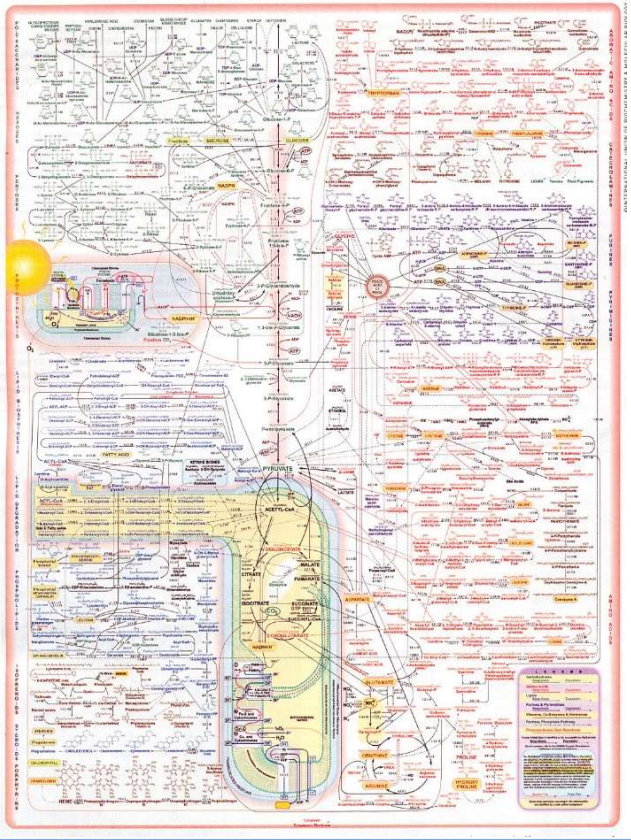
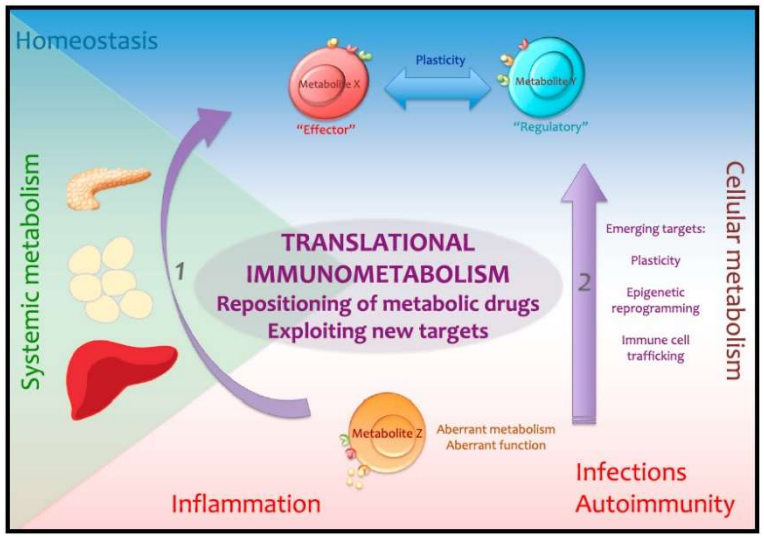
Metabolism in immunity



- The term ‘metabolism’ is so broad that effectively it is almost impossible to *not* study it, because essentially all cells need to maintain homeostasis [cell biology]
- A subset of folks think about how the immune system changes can have effects on the systemic metabolism of an individual: chronic inflammation, for instance, can deplete blood glucose levels
- But, essentially, for our purposes today, immunometabolism refers broadly to how metabolic pathways affect immune cell function
  - These pathways can be cell intrinsic (how does a T cell process glucose?)
  - Or cell extrinsic (how does a T cell function in a glucose poor environment?)
- Most of the time when people are discussing metabolism, they actually mean ‘energetics’ : how do cells meet their metabolic needs? [catabolism]
- Others explore how cells exchange various carbon sources to produce macromolecules (build proteins, generate nucleotides, and build fats for cell membranes) [anabolism]

Why study immunometabolism?

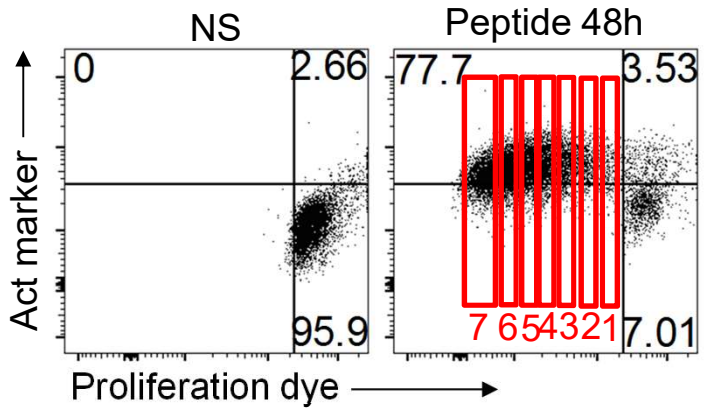


- Immunologists explore how their cell type of interest responds to signals
  - Does a macrophage elaborate different amounts of IL-6 in response to Gram- vs Gram+ bacteria?
  - Does ligation through CD28 induce the expression of my gene of interest and through what transcription factor?
  - If cell type X lacks gene Y, what is are the cellular and disease model consequences?
- However, almost *all* cells need to also sense how much fuel is in their environment before any decision is made
- Likewise, the products of various metabolic pathways (ATP, ROS, DAG, acetyl groups, NAD+, O-GlcNAc, aKG, O_2) are essential for propagating such signals
- How cells meet their metabolic needs is almost always taken for granted: when we stimulate our cells *in vitro,* **we effectively do so in a sickeningly sweet, salty, and umami broth** at hyperoxic conditions that do not mimic any physiologic environment
- In other words, there are regulatory pathways at work at a primordial level that may define whether a cell lives, dies, proliferates, arrests, differentiates to A vs B, or remains stem like

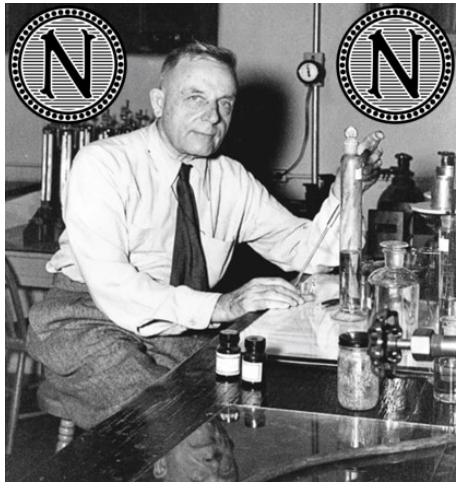
Isn't metabolism effectively homeostasis? Homeostasis is not interesting…

- As it turns out, meeting your metabolic needs is important, but as cells change function, their needs change
- This is increasingly important in immunology, as almost all cells in immunity have periods of extreme quiescence and extreme activity
- This is of course no better evident than in T cells, that, in naivete or memory, simply *wait,* yet in the effector phase proliferate to an almost unfathomable degree
- There are thus major energetic demands that must be met in order to carry out an immune response

- Fluorescently label T cells with a dye
- Activate them in some way
- Measure how the dye dilutes



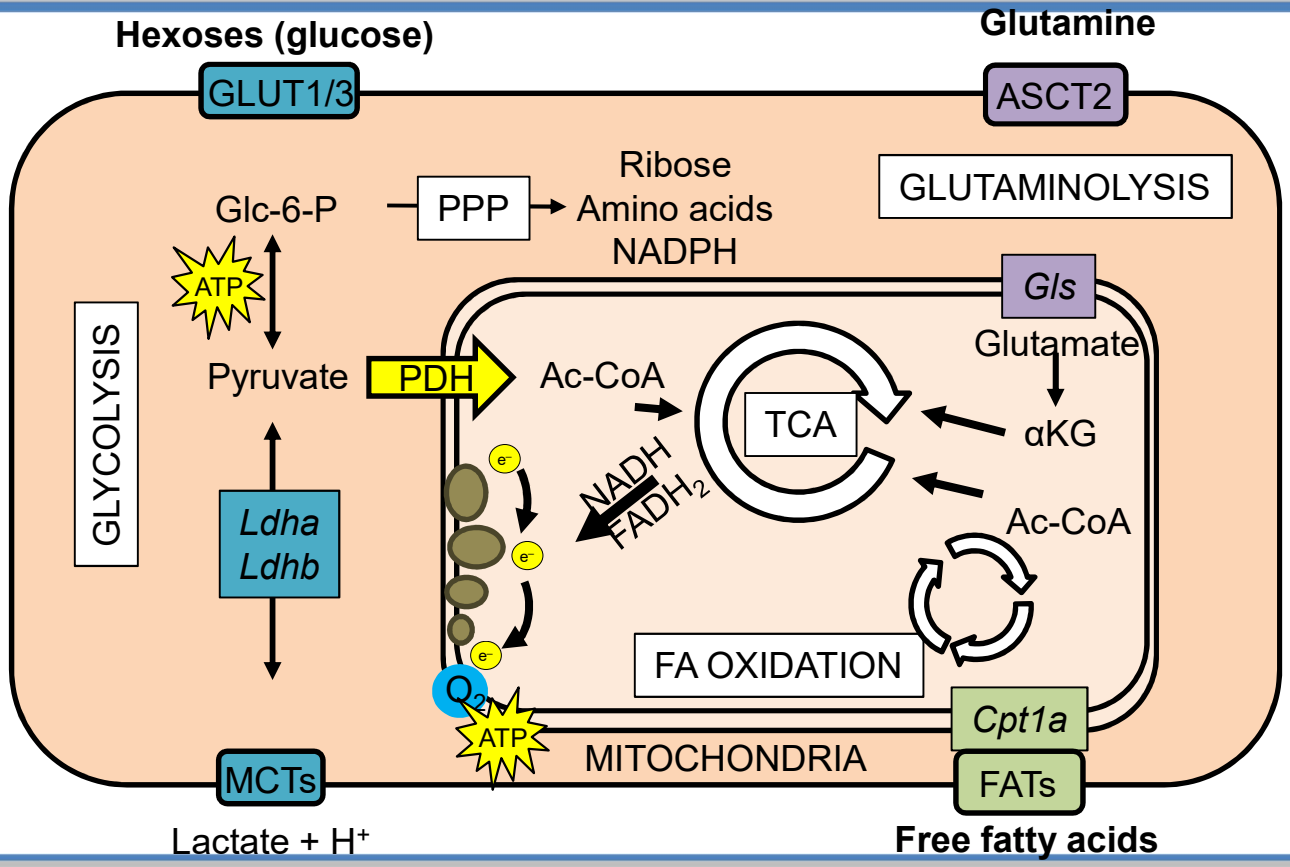
Immunometabolism started in cancer metabolism



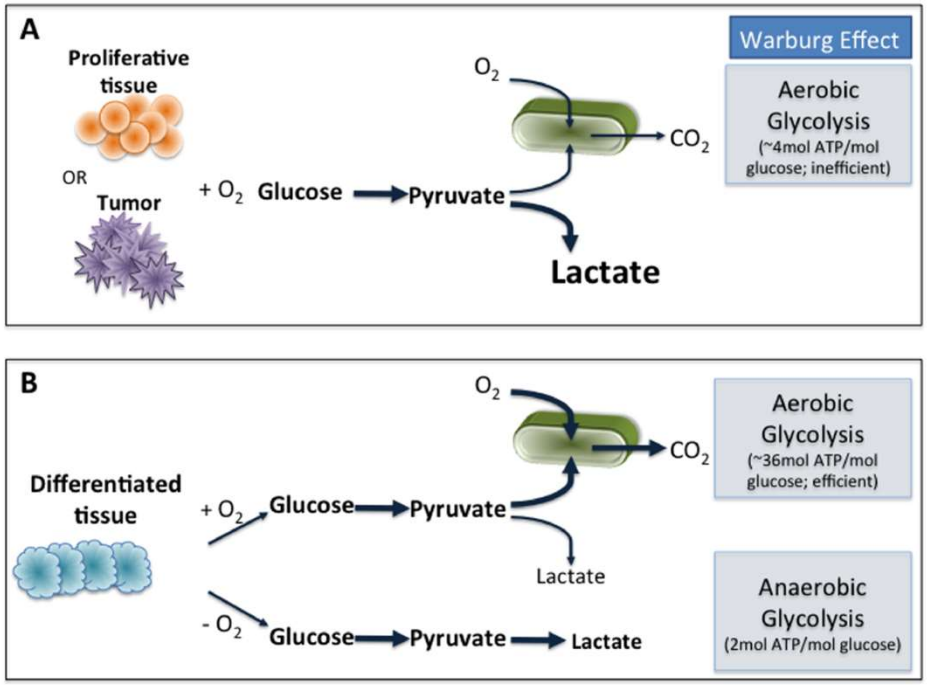
Otto Von Warburg

- Warburg studied metabolic reactions in cancer versus normal tissue
- He reasoned that deregulated energetics contributed to the transformed phenotype (he thought that it was actually causative)
- His work helped delineate a number of intricate metabolic pathways
- Nobel prize for connecting cancer cell metabolism and proliferation

The study of cancer cells opened the door for much metabolic understanding



Aerobic glycolysis



- Normally, cells ferment glucose into lactate when oxygen is limiting
- Mitochondrial oxphos can't occur so the cell makes ATP rapidly and regenerates NAD+ by converting pyruvate to lactate
- Warburg found that cancer cells perform this even in the presence of oxygen ('aerobic')
- In subsequent decades, it was found that really any highly proliferative cell tends to undergo aerobic glycolysis
- But now we know even this is not the whole story…

Warburg metabolism ('aerobic glycolysis') also occurs in T cells upon activation

Experimental Cell Research 77 (1973) 127–135

CHANGES IN THE CARBOHYDRATE METABOLISM OF MITOGENICALLY STIMULATED HUMAN PERIPHERAL LYMPHOCYTES

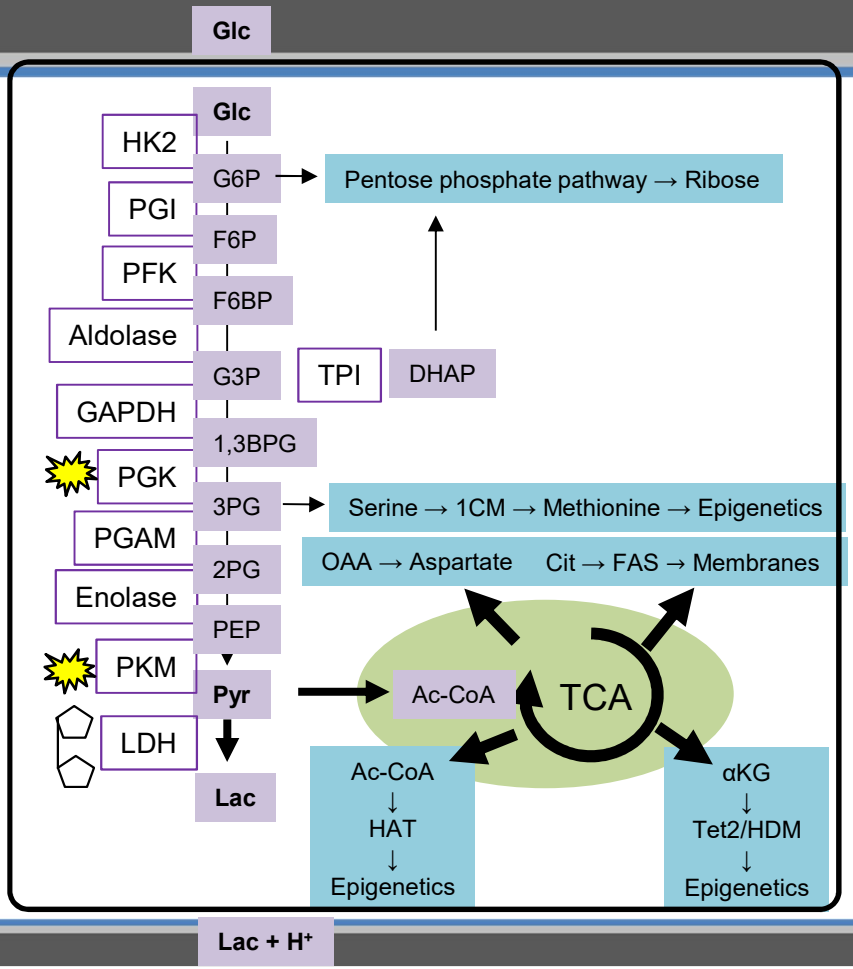
II. *Relative Importance of Glycolysis and Oxidative Phosphorylation on Phytohaemagglutinin Stimulation*

D. ROOS and J. A. LOOS

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands

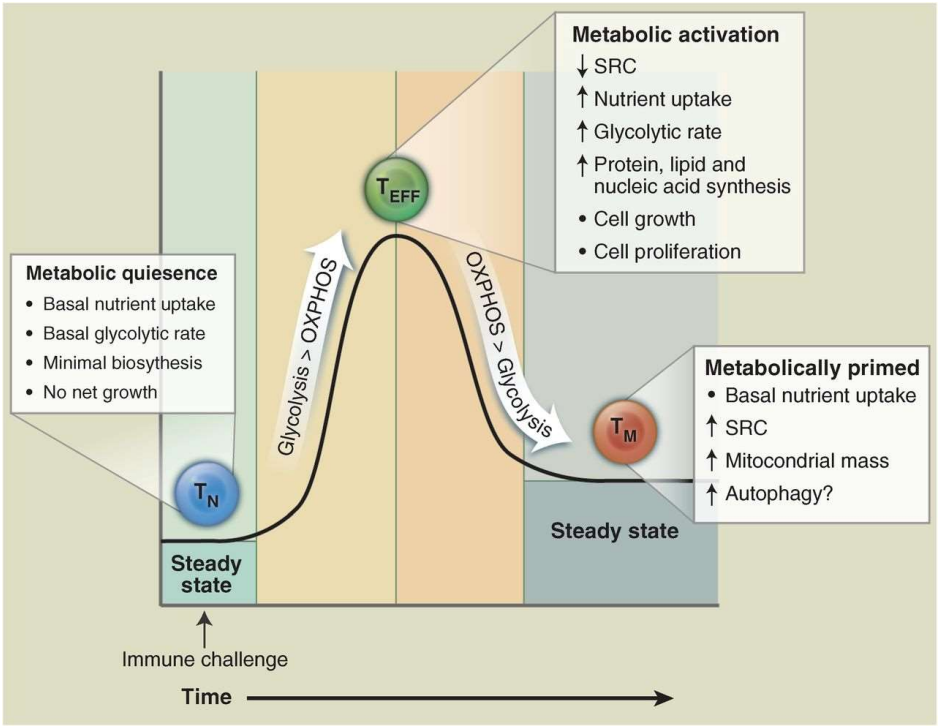
Cells preserve biosynthetic pathways by inducing aerobic glycolysis

- Activated T cells ferment glucose into lactate rather than oxidize it in the mitochondria (aerobic glycolysis)
- Glycolysis serves many purpose for cells
  - Generates ATP
  - It's very fast: 110X faster than TCA, ETC, ATP synthesis
  - Regenerates NAD+
- Glycolysis frees up intermediates for anabolic cell growth rather than simply burning them
  - PPP: Nucleotides
  - NEAA synthesis
  - Fatty acid synthesis
  - Epigenetic modifications



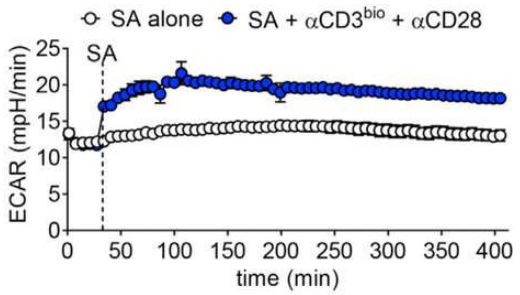
Metabolic changes in T cell activation

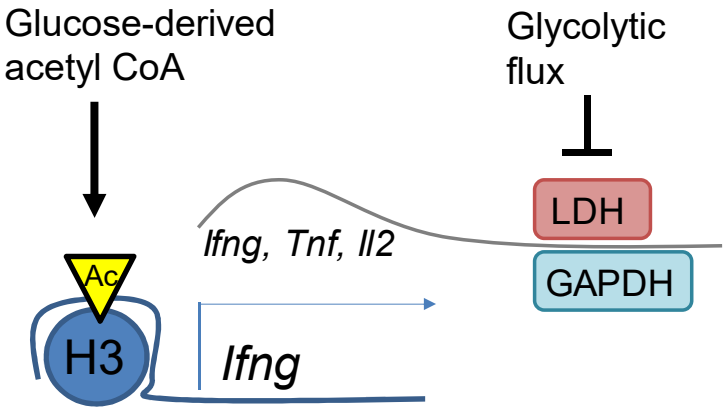
- T cells must persist for a lifetime but also rapidly proliferate to meet demands
- After an effector response, T cells must not only contract but remain primed, ready to re-engage antigen upon activation
- Metabolic reprogramming is key for these changes in T cell function
- Encounter with metabolic stress can have lasting effects on T cell function, long after nutrient balance has been restored



Glycolysis supports many aspects of inflammatory T cell function

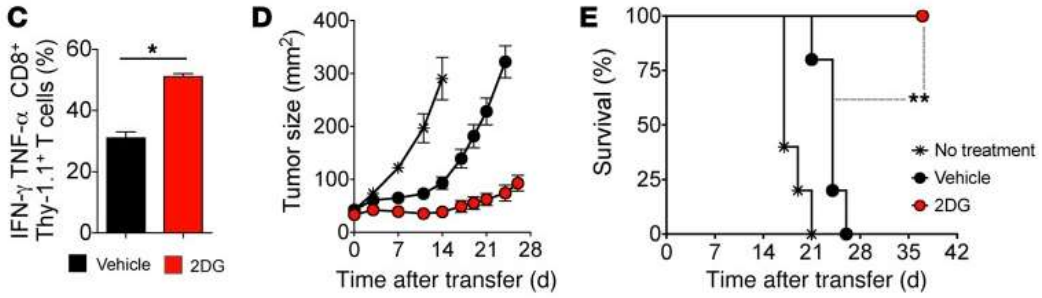
- Glucose is a primary fuel for T cells, and how it is handled has major effects on T cell biology
- T cells begin fermenting glucose just minutes after activation
- Glycolysis has been shown to be key for effector functions of T cells
  - Type 1 differentiation – acetylation of the *Ifng* locus and consequent cytokine transcription
  - Cytokine translation – GAPDH and LDH act as RNA binding proteins
- Proliferation – glucose shunted to the PPP generates nucleotides, 1CM supports amino acid synthesis, NADPH for membranes
- Proposed mechanisms are many and varied, but as glycolysis is key for so many pathways, it is unlikely there is some unified central mechanism behind glycolytic control of T cell function





Limiting glycolysis may benefit T cell therapies for cancer

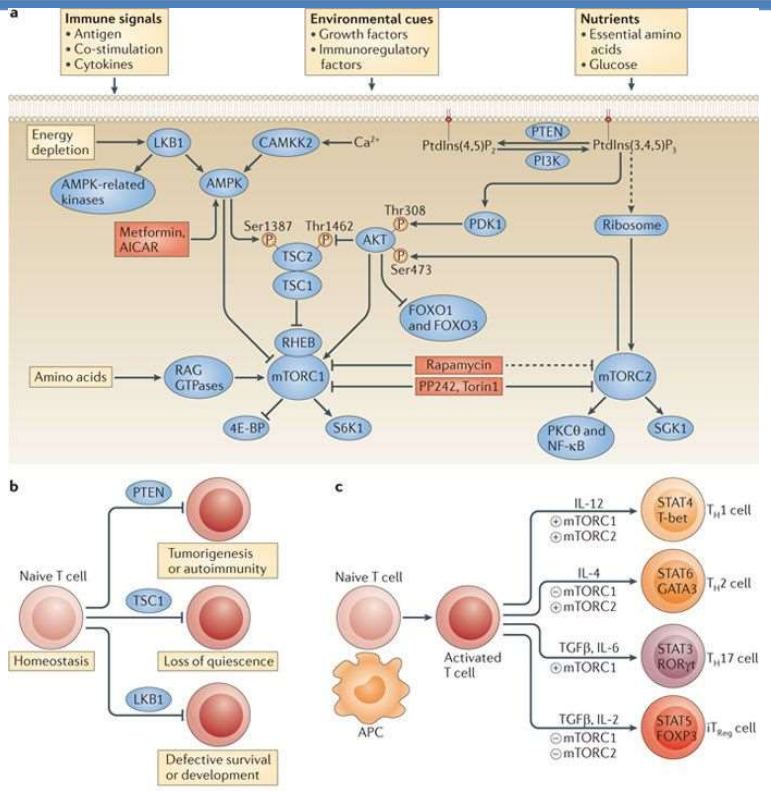
- Glycolysis is associated with enhanced effector functions, but may also promote terminal differentiation
- This process may be accelerated *in vitro*
- Cell therapies generated in the presence of a glycolysis inhibitor (2-deoxyglucose) have higher potency
- However, 2DG may prevent cell proliferation in vitro, so one must find a 'happy medium' (pun TOTALLY intended)



Sukumar et al, JCI 2013

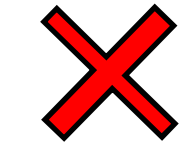
Sensing nutrients represents an important immune checkpoint

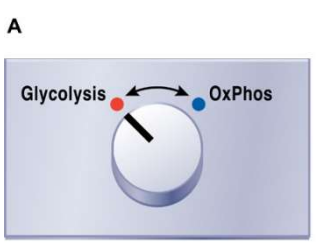
- Cells possess biochemical machinery to sense nutrient availability in the milieu
- Key among these is the mTOR signaling complex
- Strong mTOR inhibition can induce immune tolerance and rapamycin and its orthologs are used to treat transplant recipients and some autoimmune diseases
- But mTOR signaling has many subtle effects on T cell fate and function, and alterations in nutrient sensing can fine-tune T cell differentiation
- Indeed, low doses of rapamycin may potentiate memory formation and thus may boost immunotherapy

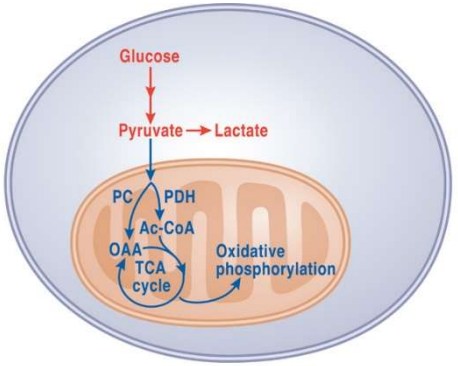


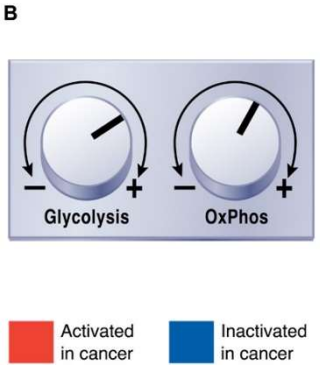
Debunking myths about aerobic glycolysis

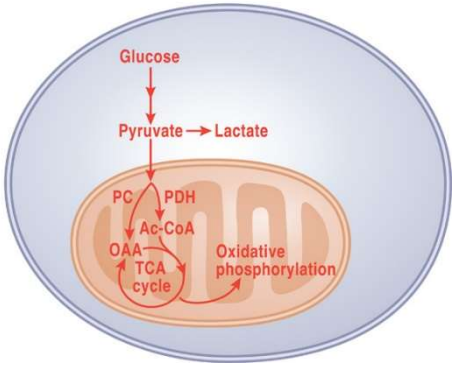
- Glycolysis is often referred to as a ‘switch’, that cells ferment glucose at the expense of their mitochondrial activity
- However, this is not the case, while glucose is certainly diverted away from mitochondrial oxidation, mitochondria still remain active, oxidizing other fuel sources and performing other vital chemistry
- Further, *in vivo* 13C glucose tracing has recently revealed T cells are probably not as fermentative as we observe *in vitro*





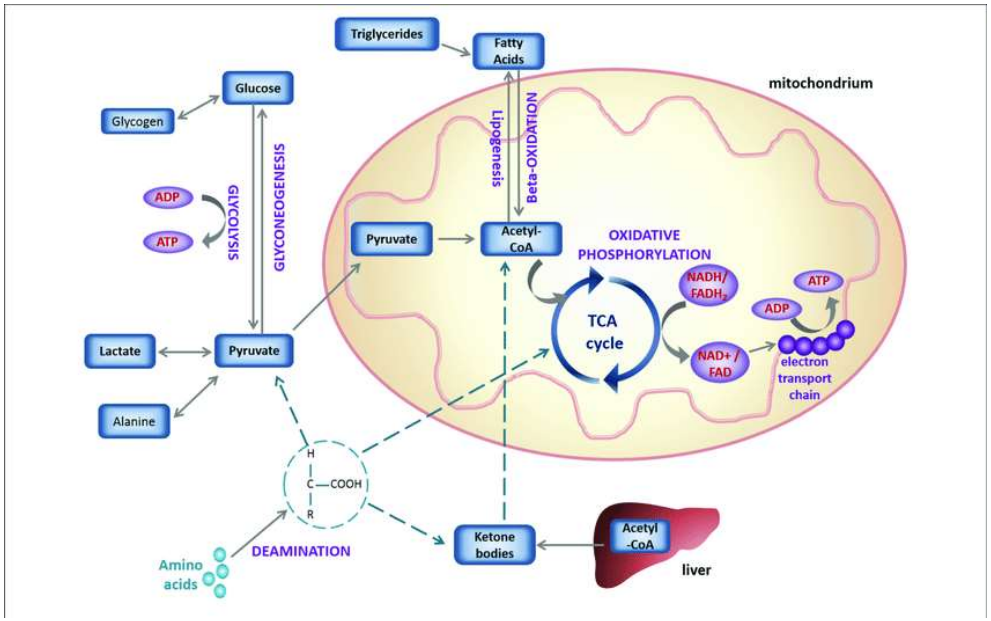






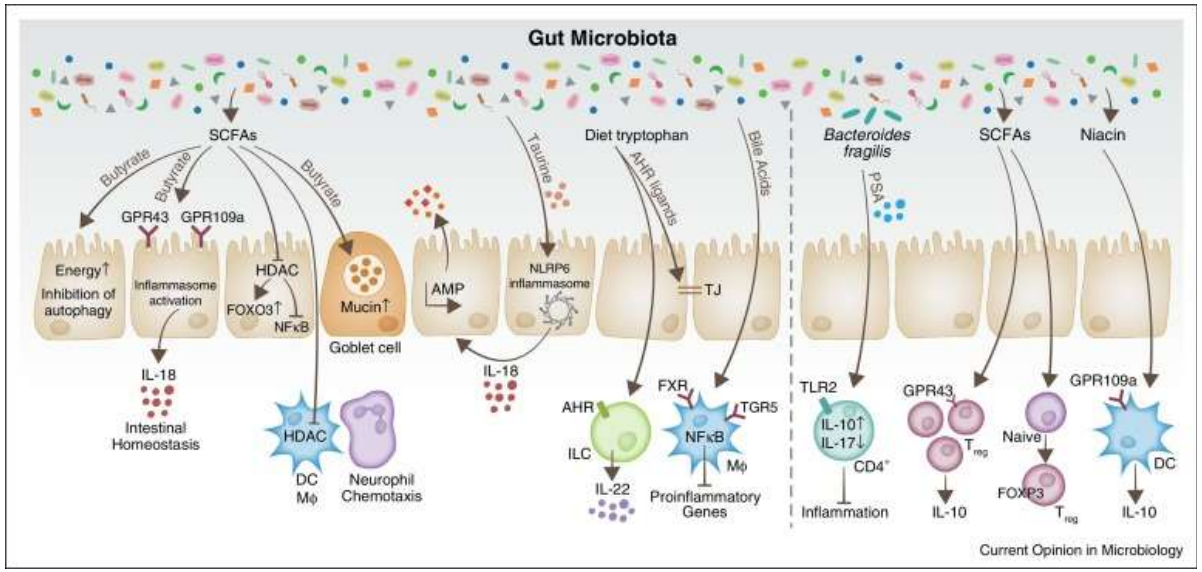
Mitochondrial OXPHOS is driven through multiple pathways

- While we learn about glycolysis feeding mitochondrial respiration in school, mitochondria are driven by multiple sources
- Glucose provides pyruvate as one input into the TCA cycle, but fatty acids can provide acetyl-CoA directly through beta oxidation
- Glutamine and other amino acids can be converted into alpha-ketoglutarate, another TCA cycle intermediate
- Ketones can also be directly interconverted into acetyl-CoA for driving TCA cycle



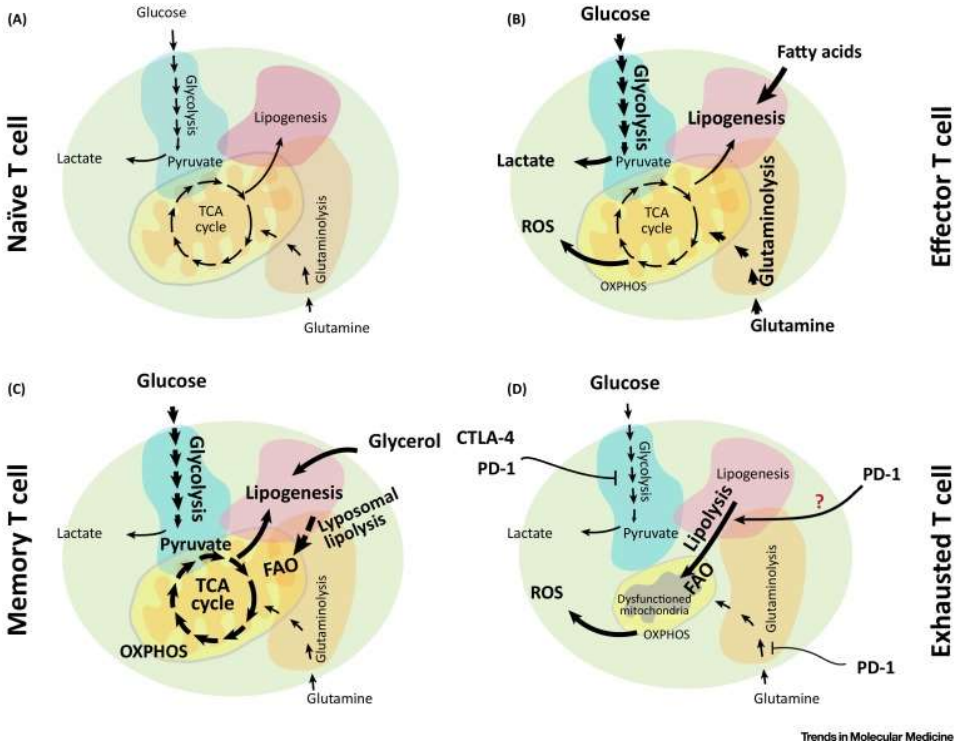
Many metabolites do not arise from within…

- While diet and cellular metabolism generates many of the intermediates our immune system needs to function, a significant proportion of metabolites are derived from the microbiome
- Butyrate, for instance, is produced by bacteria in the gut, which feeds colon cells but also modulates the immune system (promoting Treg cells, etc.)
- Gut-derived metabolites can have systemic effects, so it is critical to further understand how microbial interactions with immune cells may modulate the state of the immune system



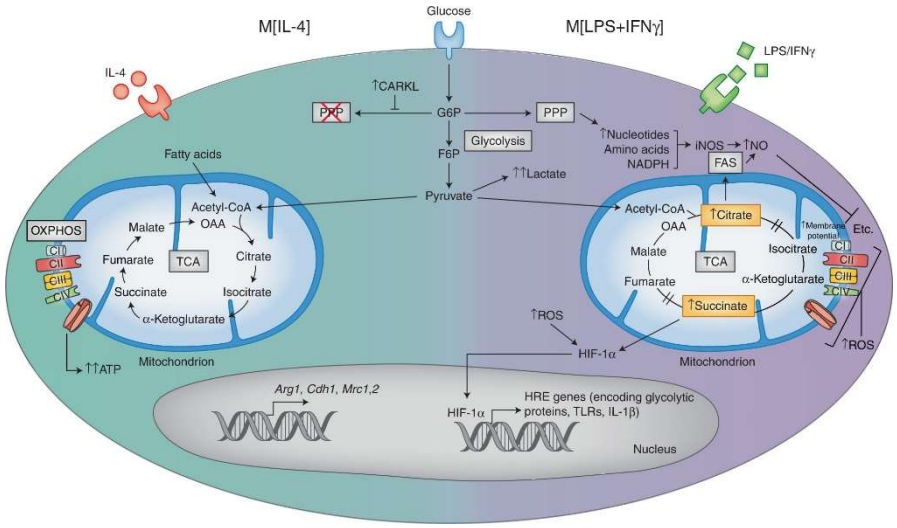
Relative importance of fuel source choice for various cell pouplations

- There are many ways for cells to meet their energetic and synthetic needs, and cells will change rapidly to avoid catastrophe
- There are many reviews and associated interpretations of how subsets of immune cells have different preference for one fuel or another, especially in T cells
- However, I believe the data favor a more general model in which common cellular processes are (generally) linked to typical sources of fuel
- Nothing is absolute: if a cell ‘prefers’ its acetyl groups from glucose but those levels drop, cells will rapidly change their appetite



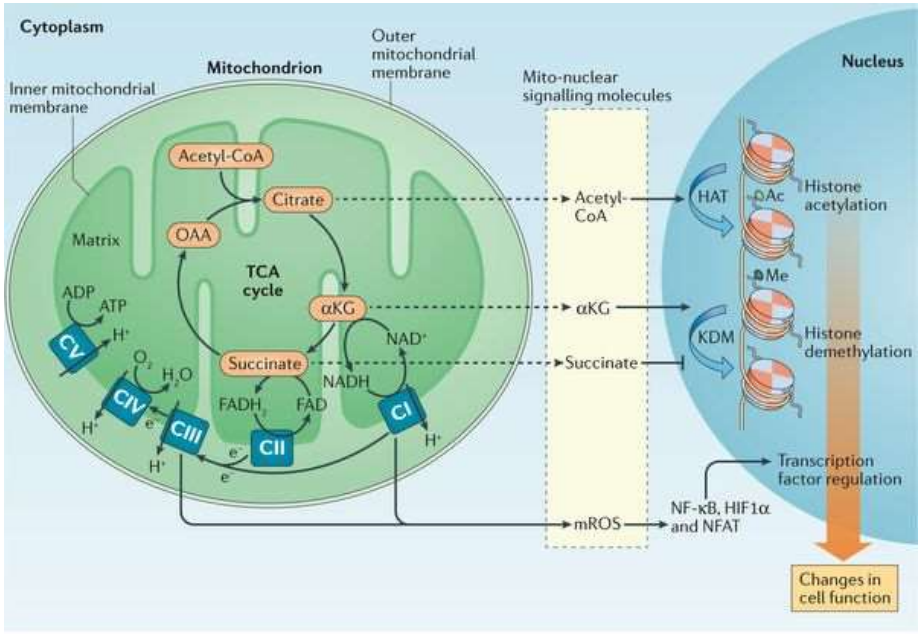
Similar paradigms exist in other immune cells

- Macrophages change their metabolism in response to cytokine cues and PAMPs: thus various states of macrophage differentiation carry alterations in their metabolic programs
- Short lived or fast acting inflammatory cells generally perform glycolysis during that phase
  - Neutrophils
  - Mast cells
  - NK cells and type 1 ILCs
- Data suggest that mitochondrial metabolism and antigen presentation may have some interactions as well



That TCA cycle you memorized is more important than just generating ATP

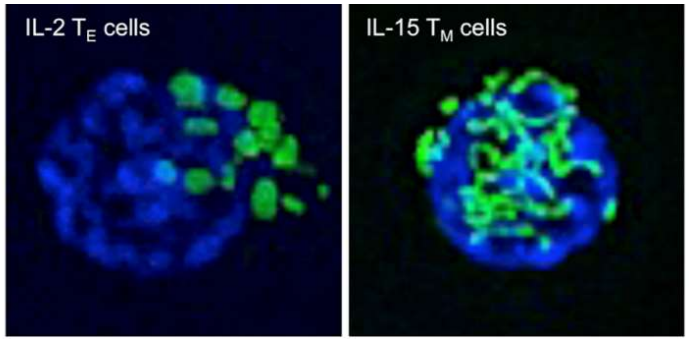
- Epigenetics and metabolism are intrinsically linked
  - Modifications of histones are the product of cellular metabolism, including acetyl, phospho, and methyl groups
  - Histone and DNA demethylation are dependent on alphaketoglutrate (TCA), O2, and iron (mitochondria)
  - Metabolic repression can result in resistance to epigenetic changes
- Transcriptional activity can also be controlled through metabolic products
  - Many transcription factors are NAD+ dependent, getting crucial signals from redox balance
  - Acetylation controls txn factor localization and activity
  - Methylation (derived from one-carbon glucose metabolism) can suppress function

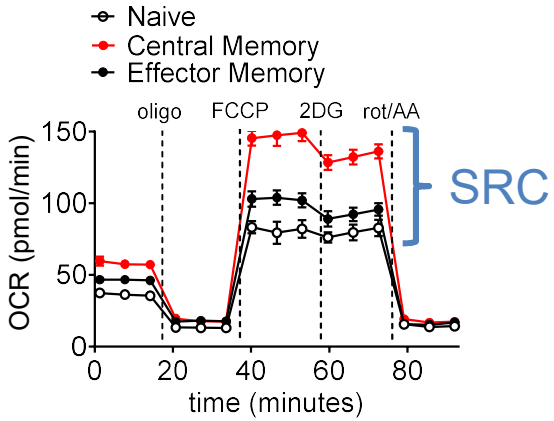


Nature Reviews | Immunology

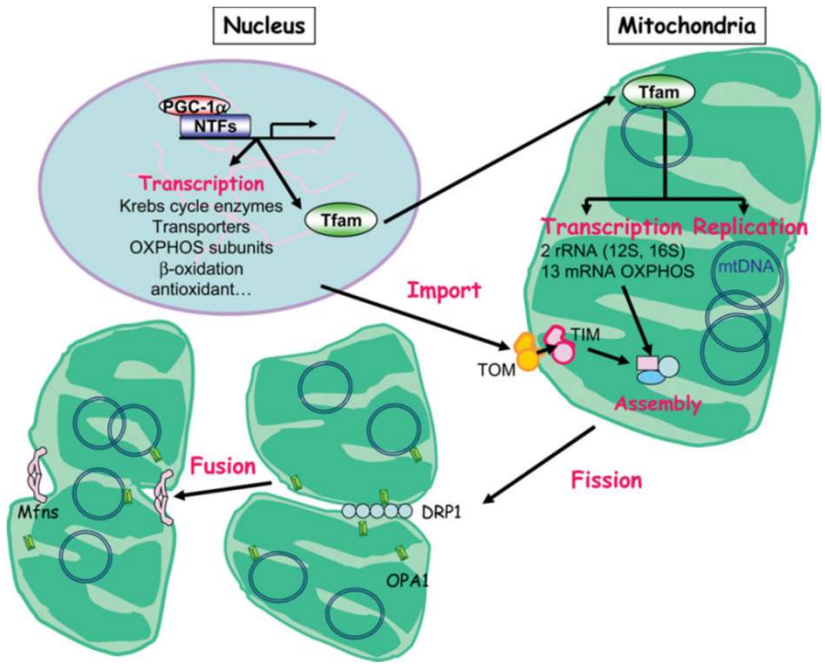
Long lived cells are characterized by mitochondrial reserve

- Generally speaking, cells that persist generally possess higher mitochondrial reserve (respiratory capacity)
- In immunity, this translates to stemlike/memory populations
- Mitochondria can buffer signaling (especially calcium), prevent oxidative damage, and provide and efficient source of energy for work in metabolically dearth conditions



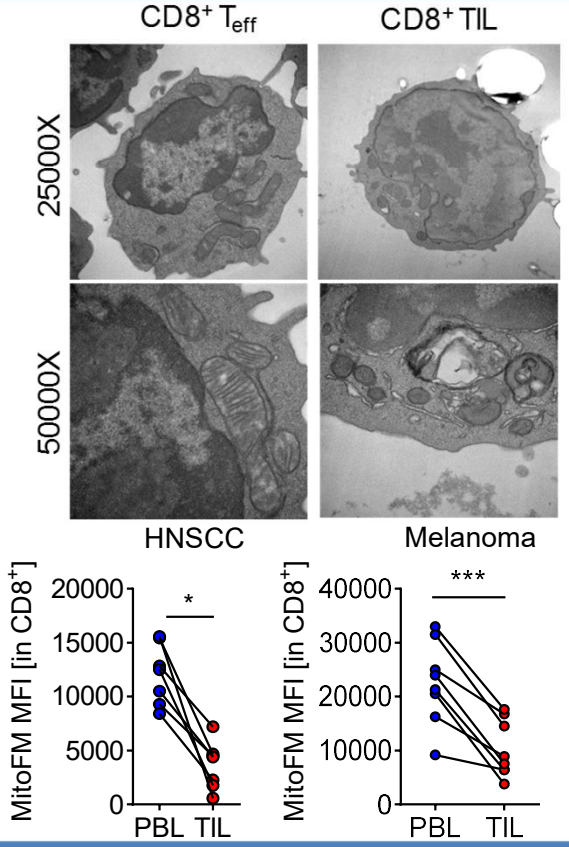


Taking care of your mitochondria

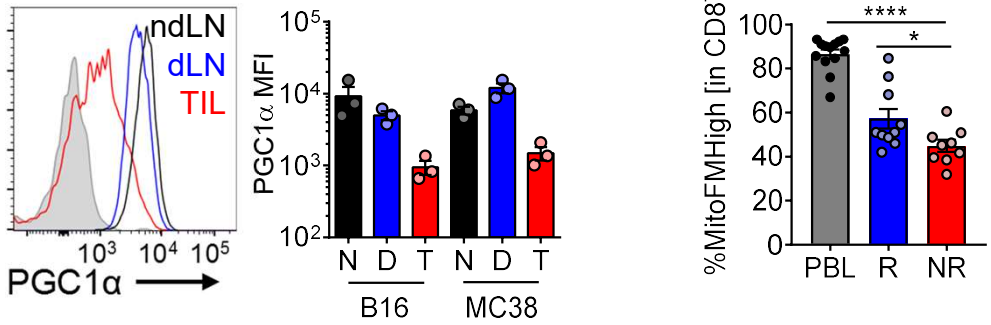


- Mitochondria may define long-lived cells but they themselves are not long-lived
- OXPHOS produces reactive oxygen species which damage mitochondrial DNA and proteins
- Thus, antioxidant activity and mitochondrial turnover are critical for continued use of mitochondria for energy and biosynthetic intermediates
- This process is a balance: mitochondrial fission vs fusion, mitochondrial biogenesis vs mitophagy
- As mitochondria have their own (but insufficient) genomes, this is an energetically demanding process that is a coordinated effort from nucleus and mitochondrial sources

Mitochondrial defects underlie T cell dysfunction in cancer



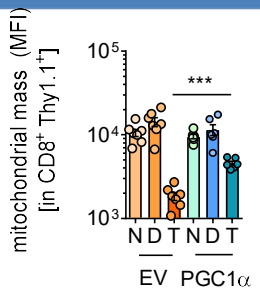
- T cells harvested from tumors display repressed glucose uptake and dramatically reduced mitochondrial mass
- Human tumor-infiltrating T cells succumb to similar metabolic insufficiency
- Associated with the development of T cell ‘exhaustion’ but is not rescued by PD-1 blockade
- Rather, T cells in tumors repress the expression of PGC1α, a transcriptional co-activator that facilitates mitochondrial biogenesis and fusion
- Our data suggest that patients that do poorly on PD-1 blockade repress the metabolic machinery to a greater extent than those who respond to PD-1 blockade

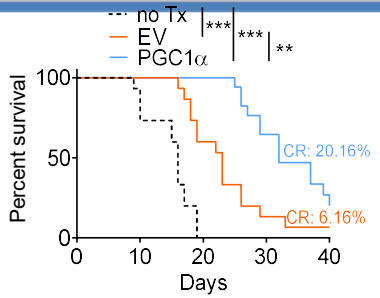


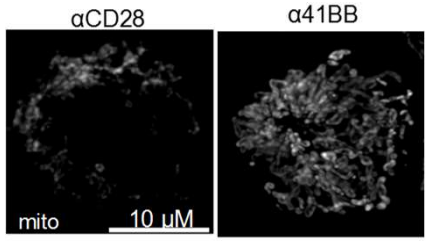
Scharping et al, *Immunity* 2016

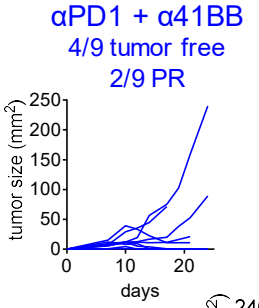
Metabolic support can be delivered to the immune system in many ways

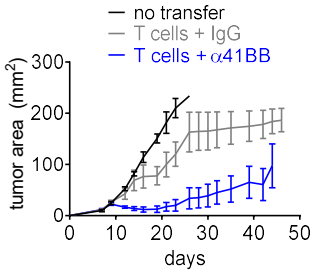
- Enforcing expression of PGC1α to improve adoptive cell therapies
  - Increases mitochondrial mass in T cells
  - Superior antitumor efficacy
- Immunotherapeutic stimulation of 41BB
  - Promotes mitochondrial biogenesis
  - Increases T cell function in the tumor
  - Improves mouse models of PD-1 blockade and adoptive T cell therapy
- Oncolytic virus-mediated deliver of metabolic modulation
  - Virus inflames tumor but also delivers the gene for leptin into tumor cells
  - Local leptin levels rise and support T cell metabolism

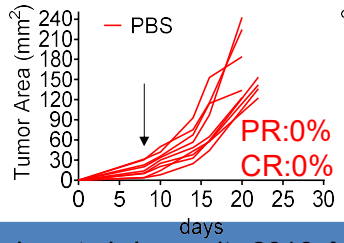


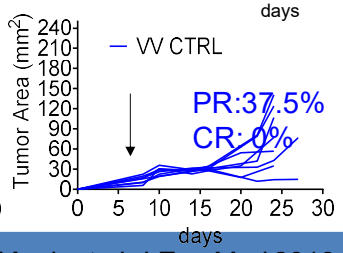


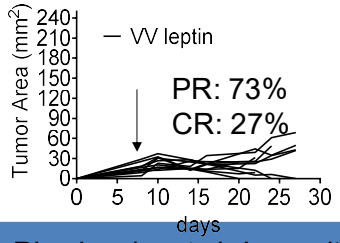












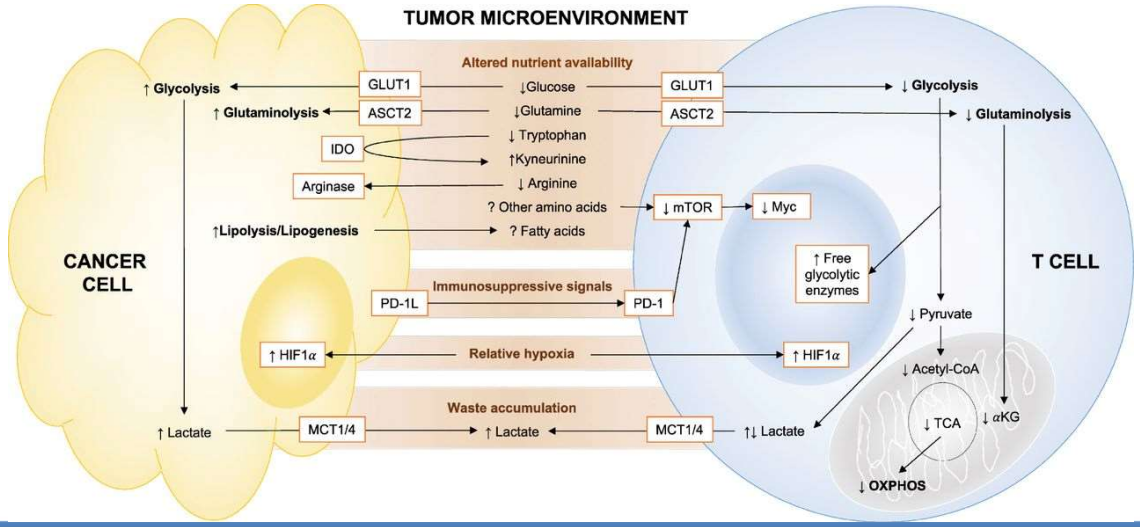
Scharping et al, *Immunity* 2016, Menk et al *J Exp Med* 2018, Rivadeneira et al, *Immunity* 2019

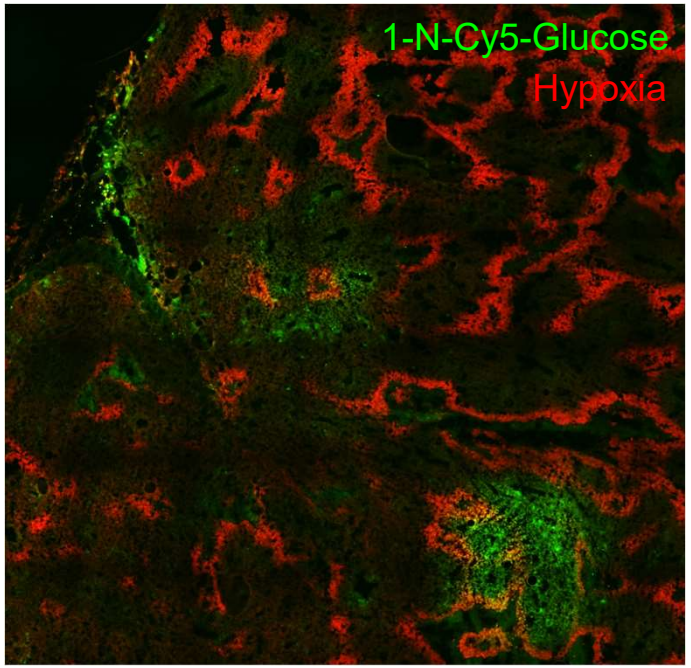
Because metabolism is central to cellular function, modulation of metabolic pathways has wide-reaching effects

This is most certainly the case in immune-mediated diseases like cancer

Nutrients are limiting within the tumor microenvironment

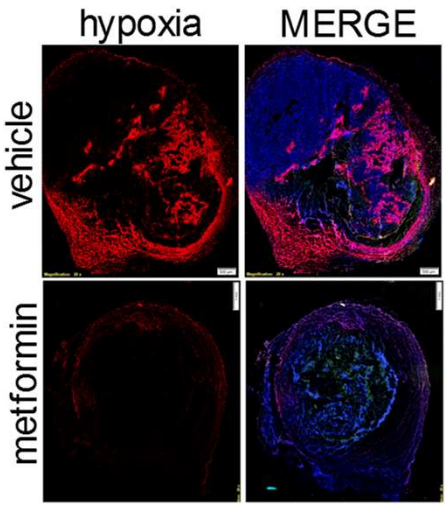
- Tumor cells, being rapidly proliferative, are metabolically deranged, upsetting the nutrient balance in the tumor microenvironment
- Thus, infiltrating immune cells often due so at a metabolic disadvantage
- Can metabolite balance be restored, rescuing proper T cell function?

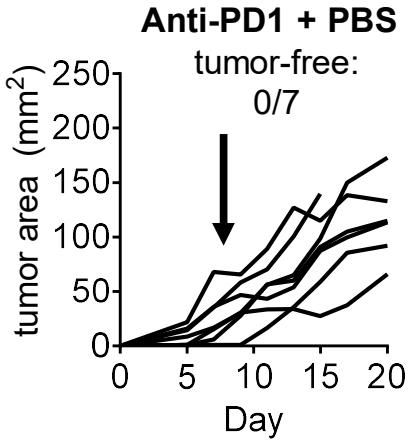


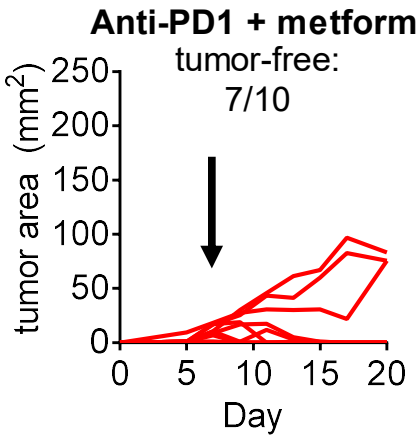


Restoration of nutrient balance need not be heroic

- We have hundreds of repurposable agents used in other diseases that modulate metabolism
- One example: the type II diabetes drug metformin can target tumor cell OXPHOS and normalize tumor oxygen tension
- This tips the balance in favor of T cells, rendering the environment more sensitive to immunotherapy

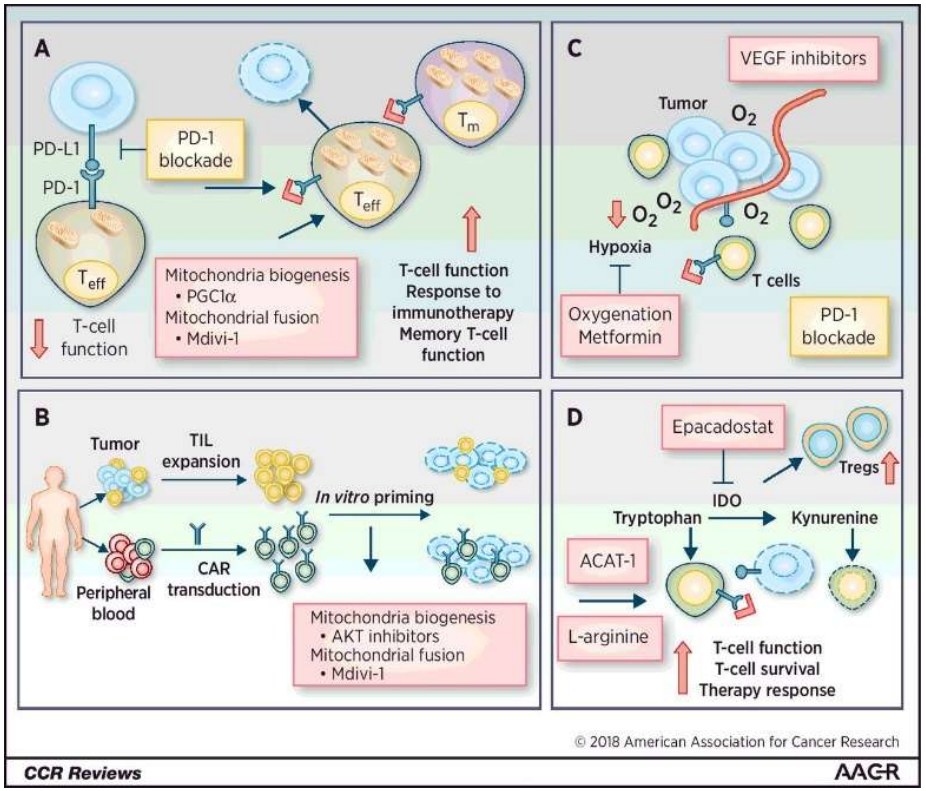






Scharping, Menk et al, *CIR* 2016

Metabolic reprogramming for enhanced cancer immunotherapy



- Immune cells are extremely sensitive to nutrient balance, and encounter with metabolic stress can have persistent effects on fate and function
- Antitumor immunity can encounter a number of metabolic defects
  - Decreased nutrient availability
  - Repressed competition for nutrients
  - Decreased mitochondrial mass
- When considering immune-based strategies for cancer, we should think about how to metabolically support this new immune response