Mitochondrial control of immunity: beyond ATP

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Abstract | Mitochondria are important signalling organelles, and they dictate immunological fate. From T cells to macrophages, mitochondria form the nexus of the various metabolic pathways that define each immune cell subset. In this central position, mitochondria help to control the various metabolic decision points that determine immune cell function. In this Review, we discuss how mitochondrial metabolism varies across different immune cell subsets, how metabolic signalling dictates cell fate and how this signalling could potentially be targeted therapeutically.

The immune system has a crucial role in a variety of disease processes, from protecting the body against infections and cancer to causing pathology in autoimmune diseases. A complex network of immune cells underpins these functions, and the various components of this network have been elucidated within the past few decades. Many initial studies on the metabolism of immune cells were able to characterize the differences between various types of immune cell. However, metabolism was considered to simply support the biosynthetic and bioenergetic needs of a cell that has already committed to a particular immune state, such as being pro-inflammatory or anti-inflammatory. A more intriguing hypothesis is that metabolism can also be an input to the system and act as a signal to control the immune functions of a cell. Indeed, recent studies have begun to demonstrate that mitochondrial metabolism (FIG. 1) has an essential role in controlling the fate of immune cells.

In this Review, we discuss how mitochondrial metabolism dictates the function of immune cells beyond its role in generating ATP or metabolites that support macromolecule synthesis. The subsequent sections of this Review each focus on one particular immune cell type or one aspect of immune function: specifically, T cell activation, $CD4^+$ T cell differentiation, memory $CD8^+$ T cell formation, B cell function, macrophage polarization, inflammasome activation, dendritic cells (DCs) and tumour immunity. We also discuss the therapeutic potential of targeting mitochondrial metabolism. Within each section, we highlight the important metabolic characteristics of each cell type or aspect of immune function and, when known, we also emphasize the signal that metabolism provides to the rest of the cell to dictate immune function, although more research is needed in this area to fill the gaps in our knowledge. Although the main sections of the main text are organized by cell type or aspect of immune function, it is also useful to keep in mind the universal themes of mitochondrial metabolism-mediated signalling that emerge across different immune cell types and immune functions, as these themes exist not only in immunology but also in other fields of biology.

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Adaptive immunity

T cell activation

Naive $CD4^+$ and $CD8^+$ T cells are metabolically quiescent, with low rates of oxygen consumption and glucose consumption. However, stimulation of the T cell receptor (TCR) and co-stimulatory receptors activates signalling pathways and transcription factors that, in turn, increase the flux of metabolites through glycolysis and the mitochondrial tricarboxylic acid (TCA) cycle[1]. In particular, in $CD4^+$ T cells, signalling through the phosphoinositide 3-kinase (PI3K)–AKT, AMP-activated protein kinase (AMPK) and extracellular signal-regulated kinase (ERK)–mitogen-activated protein kinase (MAPK) pathways concomitant with the activation of the MYC and oestrogen-related receptor-α (ERRα) transcriptional networks leads to increased rates of glucose and glutamine uptake; glucose and glutamine can be metabolized by glycolysis and the TCA cycle, respectively[1–5]. Simultaneously, $CD4^+$ T cells greatly expand their ability to utilize these metabolites by increasing their mitochondrial mass and their capacity for oxidative phosphorylation (OXPHOS). In addition, activated $CD4^+$ and $CD8^+$ T cells increase serine-dependent mitochondrial one-carbon metabolism to support their proliferation[6,7]. Pharmacological or genetic inhibition of glycolysis and mitochondrial metabolism limits TCR-dependent $CD4^+$ T cell

proliferation *in vitro* and *in vivo*[8–10]. Collectively, the alterations observed upon T cell activation support the bioenergetic and biosynthetic needs of proliferating T cells.

Aside from inducing these changes, the activation of T cells induces signalling pathways that cause transcriptional changes that lead to an activated phenotype, which is characterized by interleukin-2 (IL-2) production and the upregulation of the IL-2 receptor subunit CD25 (also known as IL-2Rα). IL-2 production is induced by TCR ligation, and requires the activation of the transcription factors nuclear factor of activated T cells (NFAT), nuclear factor-κB (NF-κB) and AP-1. Furthermore, TCR-dependent calcium influx induces the generation of mitochondrial reactive oxygen species (mROS), which have been shown to be essential for $CD4^+$ T cell activation[9,11]. $CD4^+$ T cells with a deficient mitochondrial electron transport chain (ETC) complex III fail to induce the translocation of NFAT to the nucleus, resulting in decreased transcription of IL-2-encoding mRNAs following TCR activation[9]. These changes in complex III-deficient T cells are probably due to the decreased production of mROS. The treatment of complex III-deficient cells with exogenous H_2O_2 is sufficient to recover IL-2 production, whereas treatment with mitochondria-targeted antioxidants abolishes TCR-dependent IL-2 production in wild-type cells[9]. mROS that are generated by other enzymes — such as complex I and glycerol-3-phosphate dehydrogenase — probably also contribute to T cell activation[11].

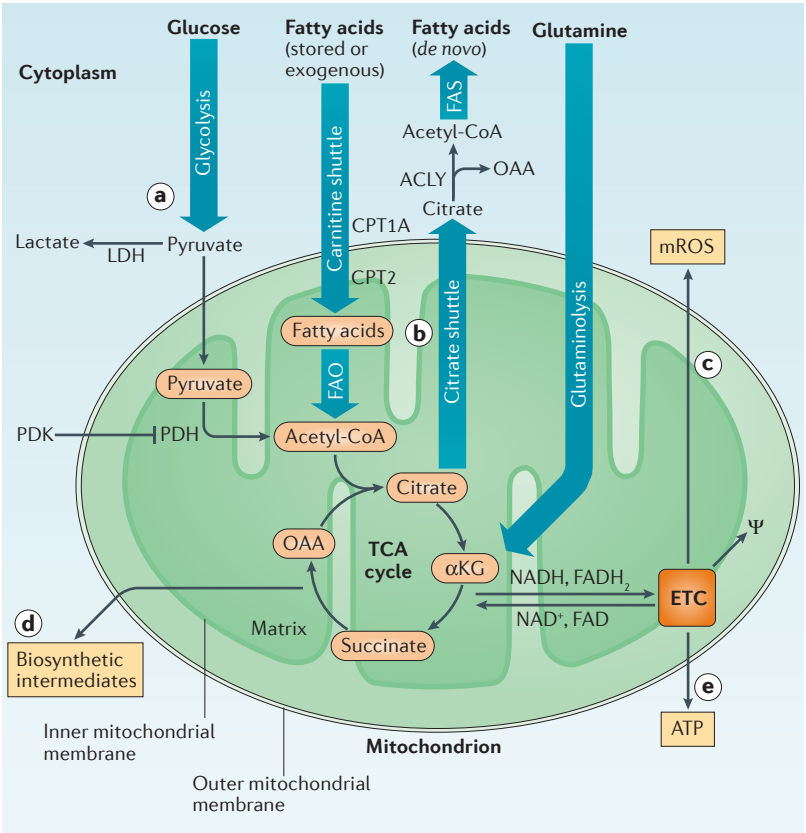


Figure 1 | **Important mitochondrial functions and decision points. a** | Glycolysis produces pyruvate from glucose, and pyruvate can either be secreted as lactate (which is produced in a reaction catalysed by lactate dehydrogenase (LDH)) or oxidized to acetyl-CoA by pyruvate dehydrogenase (PDH). Pyruvate dehydrogenase kinase (PDK) can inhibit PDH to negatively regulate this process. Mitochondrial acetyl-CoA condenses with oxaloacetate (OAA) to enter the tricarboxylic acid (TCA) cycle as citrate. Citrate can be oxidized by the rest of the TCA cycle to generate the reducing equivalents NADH and $FADH_2$, and regenerate OAA. **b** | Alternatively, citrate can be exported to the cytoplasm, where it can be cleaved by ATP-citrate lyase (ACLY) to regenerate acetyl-CoA and OAA in a process known as the citrate shuttle. The regulation of the citrate shuttle therefore controls cytoplasmic acetyl-CoA levels. Fatty acids can be generated from cytosolic acetyl-CoA by fatty acid synthase (FAS). Fatty acid oxidation (FAO) catalyses the opposite reaction and takes place in mitochondria, generating mitochondrial acetyl-CoA from fatty acids. FAO is regulated by fatty acid import into mitochondria through the carnitine shuttle, which is dependent on the enzymes carnitine palmitoyltransferase 1A (CPT1A) and CPT2. The separation of fatty acid catabolism in mitochondria and fatty acid anabolism in the cytoplasm helps to compartmentalize reactions that are typically reciprocally regulated to prevent futile cycles. **c** | Mitochondrial reactive oxygen species (mROS) are produced by the electron transport chain (ETC) through the reduction of O_2 to superoxide. The generation of mROS is controlled by the electron flux through the ETC and other aspects of ETC function. **d** | Various TCA cycle intermediates such as acetyl-CoA and other mitochondrial metabolites serve as important building blocks for macromolecule synthesis, and TCA cycle intermediates can be replenished through glutaminolysis (that is, the conversion of glutamine to α-ketoglutarate (αKG)). **e** | The ETC uses reducing equivalents from the TCA cycle to maintain an electrical potential (Ψ) across the inner mitochondrial membrane, which in turn drives ATP production.

The important role of mROS in T cell activation is further supported by evidence indicating that T cell activation requires the recruitment of mitochondria to the immune synapse[12]. An interesting observation is that a loss of mitochondrial complex III results in impaired antigen-driven T cell responses *in vivo*, but T cell proliferation is not affected following the adoptive transfer of complex III-deficient T cells into lymphopenic mice (that is, homeostatic proliferation)[9]. This indicates that complex III is necessary not for proliferation but to generate mROS in response to T cell activation (FIG. 2). Furthermore, a recent study showed that complex IV-deficient T cells do not have a deficit in T cell activation as measured by IL-2 production[13]. Complex III-deficient T cells and complex IV-deficient T cells would be expected to have similar changes in ATP production but could have differences in mROS signalling, thus providing more evidence that the changes resulting from complex III deficiency are not caused by bioenergetic deficits but rather by metabolic signalling. It is important to note that high levels of mROS can be detrimental to cell viability. A recent study showed that activated T cells inhibit the formation of mitochondrial permeability transition pores to prevent excessive mROS leakage from mitochondria[14]. T cells deficient in their ability to prevent pore formation had an inflammatory phenotype owing to elevated levels of mROS, and they were more susceptible to cell death.

$CD4^+$ T cell subsets

After activation, $CD4^+$ T cells differentiate into different subsets that range from pro-inflammatory effector T (T_{eff}) cells (T helper 1 (T_H1), T_H2 and T_H17 cells) to suppressive regulatory T (T_{reg}) cells. Each lineage is dependent on the expression of specific transcription factors, and displays a unique metabolic phenotype with regards to metabolic substrates and pathways.

Mitochondrial permeability transition pores
High-conductance inner mitochondrial membrane channels. Persistent opening of these pores irreversibly commits cells to death by causing mitochondrial depolarization (which blocks oxidative phosphorylation and reactive oxygen species production), matrix swelling and cristae unfolding, and results in the release of stored calcium^{+} and of apoptogenic proteins.

To date, the data suggest that an increase in glycolysis or a repression of mitochondrial metabolism can boost the differentiation of pro-inflammatory T_{eff} cells, whereas agents or stimuli that promote mitochondrial metabolism boost the differentiation of T_{reg} cells[15,16]. Pro-inflammatory T_{eff} cell subsets, but not T_{reg} cells, display elevated levels of glycolytic enzymes and lactate[15,16]. The importance of glycolysis in pro-inflammatory T_{eff} cells is highlighted by *in vitro* experiments that show that the loss of glucose transporter 1 (GLUT1) diminishes glucose transport and glycolysis, which resulted in decreased absolute numbers of cytokine-producing T_H1, T_H2 and T_H17 cells, but not of T_{reg} cells[10]. The decrease in T_{eff} cell expansion in mice deficient for GLUT1 also reduced the ability to experimentally induce inflammatory diseases such as graft-versus-host disease and colitis. Furthermore, GLUT1 overexpression increases T_{eff} cell numbers and promotes inflammatory disease. Although GLUT1 overexpression also increases the number of T_{reg} cells, it reduced their suppressive capacity[17]. Similarly to GLUT1 loss, pharmacological inhibition of glycolysis using 2-deoxyglucose (2-DG; which inhibits hexokinase, the enzyme that catalyses the first step in glycolysis) leads to decreased T_H17 cell differentiation and increased T_{reg} cell differentiation, both *in vitro* and *in vivo*[18,19].

T_{reg} cells preferentially use glucose for pyruvate oxidation rather than for lactate production, and genetic and pharmacological activation of pyruvate dehydrogenase (PDH) boosts T_{reg} cell differentiation and protects mice against experimental autoimmune encephalomyelitis (EAE)[15]. Furthermore, the inhibition of PDH (which causes increased glycolytic flux; see FIG. 1) results in a shift from T_{reg} cell differentiation to T_H17 cell differentiation. These data are further supported by the observation that genetic ablation of *Hif1a*

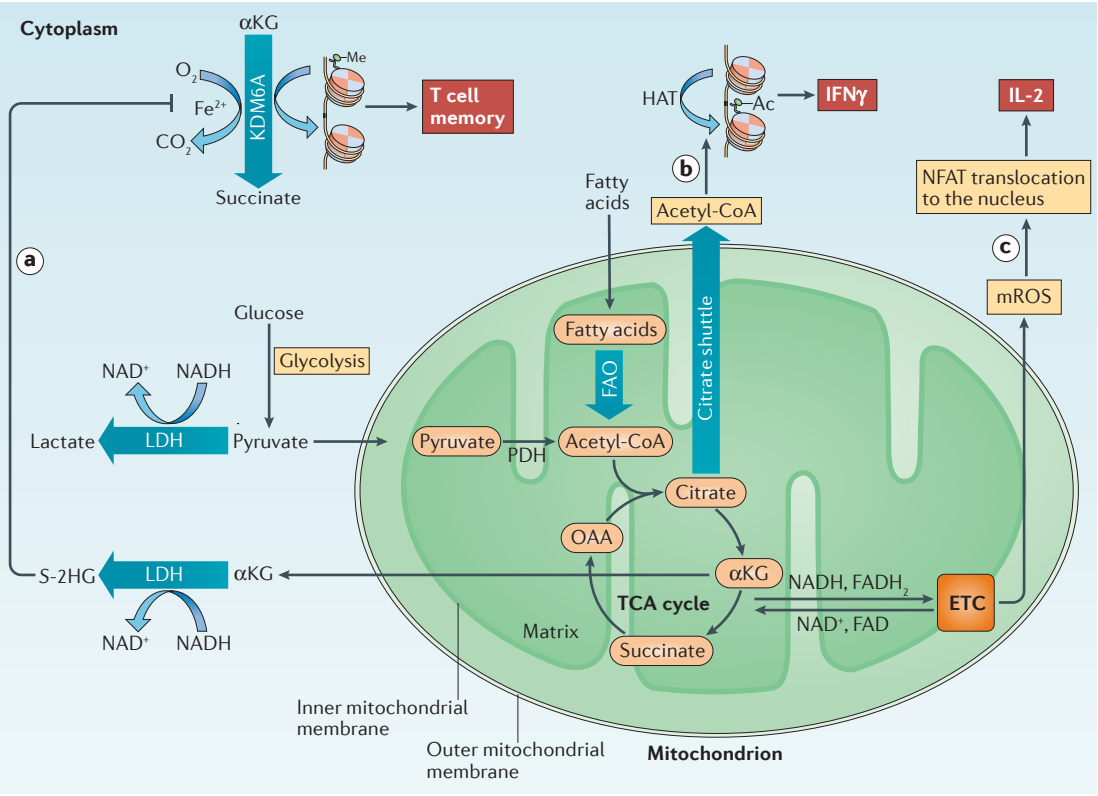


Figure 2 | **T cell metabolism controls immune phenotype.** T cells can derive mitochondrial acetyl-CoA from glucose (via glycolysis and pyruvate oxidation) and fatty acids (via fatty acid oxidation (FAO)). Mitochondrial acetyl-CoA can be transported to the cytoplasm or used to generate reducing equivalents to feed into the electron transport chain (ETC). These metabolic options are differentially important in various aspects of T cell biology. **a** | Metabolic changes in the mitochondria of $CD8^+$ T cells can lead to a high NADH/NAD^+ ratio that favours the production of S-2-hydroxyglutarate (S-2HG) from α-ketoglutarate (αKG); this reaction is catalysed by lactate dehydrogenase (LDH; an enzyme that normally catalyses the conversion of pyruvate to lactate). S-2HG inhibits αKG-dependent dioxygenase reactions such as those mediated by lysine-specific demethylase 6A (KDM6A), which are required for histone demethylation. S-2HG can therefore cause epigenetic changes that correlate with increased memory formation in T cells. **b** | T cells increase citrate transport into the cytoplasm through the citrate shuttle to increase the cytosolic levels of acetyl-CoA; increased cytosolic acetyl-CoA levels are required for histone acetylation and interferon-γ (IFNγ) production. **c** | During T cell activation, mitochondrial metabolism maintains flux through the ETC to generate mitochondrial reactive oxygen species (mROS), which are required for the translocation of nuclear factor of activated T cells (NFAT) to the nucleus and the subsequent transcription of the gene that encodes interleukin-2 (IL-2). Ac, acetylation; HAT, histone acetyltransferase; Me, methylation; OAA, oxaloacetate; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid.

(which encodes hypoxia-inducible factor 1α (HIF1α)) phenocopies the effects of 2-DG[19]. HIF1α is a key regulator that promotes glycolysis in T_{eff} cells. Conversely, mice that have aberrant HIF1α stabilization owing to a loss of von Hippel–Lindau disease tumour suppressor (VHL; a negative regulator of HIF1α) specifically in T_{reg} cells have an increased incidence of inflammatory conditions, and their T_{reg} cells have an increased propensity to secrete interferon-γ (IFNγ)[20]. T_{reg} cells also utilize AMPK-dependent fatty acid oxidation (FAO) to support mitochondrial metabolism[16,21]. The inhibition of FAO using the small molecule etomoxir leads to impaired T_{reg} cell differentiation but, notably, not to the impaired differentiation of pro-inflammatory T cell lineages *in vitro*[22]. It remains to be seen whether this will also be observed *in vivo* in mice with gene knockouts that affect FAO. FAO can be initiated by suppressing *de novo* lipogenesis, which is mediated by fatty acid synthase (FAS). Indeed, pharmacological and genetic inhibition of FAS leads to impaired T_H17 cell differentiation and increased T_{reg} cell differentiation *in vitro* and *in vivo* in the context of EAE[23].

It is not currently clear whether it is the increased glycolytic flux or diminished mitochondrial metabolism that promotes the differentiation of cytokine-producing T_{eff} cells. The challenge has been to decipher the mechanism by which these changes in metabolism cause an increase in T_{eff} cell differentiation. The promotion of T_{reg} cell differentiation and the suppression of T_H17 cell differentiation by activating PDH can be partially rescued by administering the pan-antioxidant *N*-acetylcysteine (NAC), which suggests that increased levels of mROS suppress T_H17 cell differentiation[15]. Diminishing respiration results in decreased mROS production and correlates with an increase in the differentiation of pro-inflammatory T_{eff} cells; conversely, increased mROS production correlates with increased T_{reg} cell differentiation[15]. Other studies have suggested that mROS may modulate the expression of forkhead box protein P3 indirectly through their known effects on TCR signalling and NFAT[24].

The other mechanism by which changes in metabolism can influence cell fate decisions aside from via mROS could be the generation of TCA cycle metabolites that control epigenetic modifications (BOX 1). T_H1 cells require the expression of lactate dehydrogenase (LDH) to shunt pyruvate away from mitochondria and promote lactate production (FIG. 2). Loss of the gene that encodes the A chain of LDH (LDHA) in T cells inhibits T_H1 cytokine production owing to the shunting of pyruvate into mitochondria, lowering the cytosolic levels of acetyl-CoA, which is necessary for histone modifications at loci that are crucial for T_H1 cell differentiation and function, such as the locus that encodes IFNγ[25]. In addition, glucose metabolism may be required for optimal T_H1 cell function through a post-transcriptional mechanism whereby increased glucose flux relieves glyceraldehyde-3-phosphate dehydrogenase-mediated suppression of IFNγ translation[8]. Going forwards, it will be important to develop an understanding of the signalling mechanisms by which changes in mitochondrial metabolism control T_{eff} cells and T_{reg} cells.

CD8⁺ T cell memory

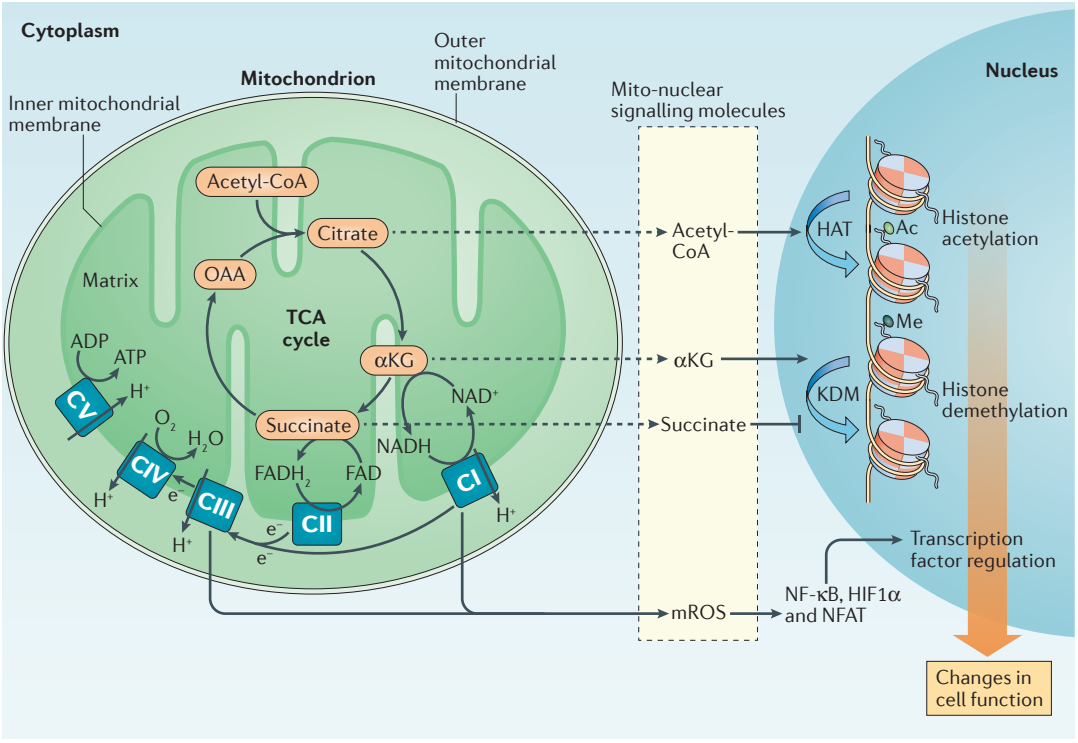
$CD8^+$ T cells are commonly categorized as being T_{eff} cells, central memory cells or effector memory cells, depending on their expression of certain genes and surface markers. However, these populations can also be distinguished on the basis of their metabolic phenotype. $CD8^+$ T_{eff} cells display mitochondria undergoing fission, whereas $CD8^+$ central and effector memory T cells have densely packed fused mitochondria[26]. Early metabolic studies showed that $CD8^+$ central memory T cells tend to be more quiescent than $CD8^+$ T_{eff} cells and have a large capacity for OXPHOS, whereas $CD8^+$ T_{eff} cells tend to be more glycolytic than central memory cells[27–29]. However, one important idea that has emerged from these studies is that increased glycolysis is a signal for effector function, whereas increased OXPHOS is a signal for memory T cell formation. In support of this, $CD8^+$ T cells stimulated *in vitro* and treated with 2-DG show increased differentiation towards memory T cells, whereas $CD8^+$ T cells overexpressing the glycolytic enzyme phosphoglycerate mutase showed a decreased tendency to differentiate into memory T cells[28].

$CD8^+$ central memory T cells have a high spare respiratory capacity that is largely owing to their increased capacity for FAO[29]. However, compared with $CD8^+$ T_{eff} T cells, central memory T cells show decreased uptake of fatty acids, as they have decreased expression of the scavenger receptor CD36 (REF. 30). Instead, the source of fatty acids in central memory T cells is stored intracellular triacylglycerides (TAGs) that are synthesized from glucose[30]. In addition, the synthesis of fatty acids and TAGs has been shown to be necessary and sufficient for IL-7-dependent memory $CD8^+$ T cell longevity[31]. The unexpected conclusion is that memory T cells apparently engage both the FAO and fatty acid synthesis pathways, at least *in vitro*. It is unclear how or why memory T cells utilize this futile cycle.

An important caveat to these findings is that the metabolism of tissue-resident memory cell populations may differ from that of other memory cell populations. A recent study of mice in which the genes that encode fatty acid-binding protein (FABP4; also known as adipocyte fatty acid-binding protein) and FABP5 (also known as epidermal fatty acid-binding protein) — which are a key pair of proteins responsible for intracellular fatty acid transportation — were knocked out showed that FAO was required for tissue-resident memory cell maintenance and function[32]. Importantly, these proteins were not required for central memory T cell maintenance or function. Together, these studies show a link between T_{eff} cells and glycolysis, and between memory T cells and OXPHOS. Going forwards, it will be important to verify the necessity of fatty acid-driven OXPHOS *in vivo* by ablating genes that are essential for FAO, fatty acid synthesis or the ETC in memory T cells. Although some progress has been made in this area, the use of transgenic mouse models with inducible gene deletions would help to dissect the role of metabolism in various phases of the immune response. For example, the inducible deletion of a gene encoding a metabolic enzyme after a

Box 1 | **Mitochondria as signalling organelles**

Mitochondria are well appreciated for their role in generating ATP by oxidative phosphorylation via the electron transport chain (ETC) protein complexes I–V and tricarboxylic acid (TCA) cycle metabolites that are precursors for macromolecule synthesis, such as acetyl-CoA (see the figure, in which the dashed arrows indicate transport or shuttling of metabolites out of the mitochondria). Thus, the bioenergetic and biosynthetic roles of mitochondria can support cell proliferation and growth (also see FIG. 1). However, data have emerged within the past two decades to indicate that mitochondria are necessary to control the signalling pathways and gene expression profiles that ultimately drive biological functions. There are multiple mechanisms by which mitochondria function as signalling organelles (see the figure for examples), including the release of mitochondrial reactive oxygen species (mROS), calcium, metabolites, cytochrome *c* and mitochondrial DNA, as well as the outer mitochondrial membrane serving as a signalling platform. mROS are generated in the mitochondrial intermembrane space and matrix, primarily by complexes I and III of the ETC. The amount of mROS produced is controlled by a variety of factors, including ETC flux, mitochondrial membrane potential and other ETC characteristics. These mROS are able to diffuse into the cytoplasm and cause changes in the activation of transcription factors, specifically hypoxia-inducible factor 1α (HIF1α), nuclear factor of activated T cells (NFAT) and nuclear factor-κB (NF-κB) in immune cells. Transcriptional changes in turn lead to changes in the functions of these cells. Similarly, the TCA cycle metabolites α-ketoglutarate (αKG) and succinate are the substrate and product, respectively, of some histone demethylase enzymes (also see FIG. 3). Consequently, changes in the flux of metabolites through the TCA cycle can alter the availability of these metabolites, and can therefore inhibit or promote histone demethylation. Epigenetic histone modifications can also be initiated by changes in the levels of mito-nuclear signalling molecules such as the mitochondrial metabolite acetyl-CoA, which is required for histone acetylation. Acetyl-CoA is a central metabolite in mitochondrial metabolism as it can be produced by the oxidation of glucose, pyruvate, fatty acids and amino acids (also see FIG. 1). Cytoplasmic acetyl-CoA concentrations are controlled according to mitochondrial acetyl-CoA availability and through the regulation of the citrate shuttle, which transports mitochondrial acetyl-CoA into the cytoplasm (also see FIG. 1). Clearly, perturbing any number of metabolic pathways could alter the levels of cytoplasmic acetyl-CoA, and therefore affect histone acetylation and gene transcription. Together, all of these mito-nuclear signalling molecules can work together to change the function of an immune cell.



Ac, acetylation; C, complex; e⁻, electron; HAT, histone acetyltransferase; KDM, lysine demethylase; Me, methylation; OAA, oxaloacetate.

stable memory population has formed may determine the necessity of a particular metabolic enzyme in maintaining functional memory cells.

Studies that use conditional knockouts of genes that encode proteins that regulate metabolic pathways have demonstrated surprising results. For example, although initial studies suggested that glycolysis is not essential for memory T cell formation, recent *in vivo* studies have provided evidence that these conclusions may not hold true under all circumstances. Mice with VHL-deficient T cells have aberrant increases in glycolysis at the expense of some OXPHOS[33]. Although these cells would

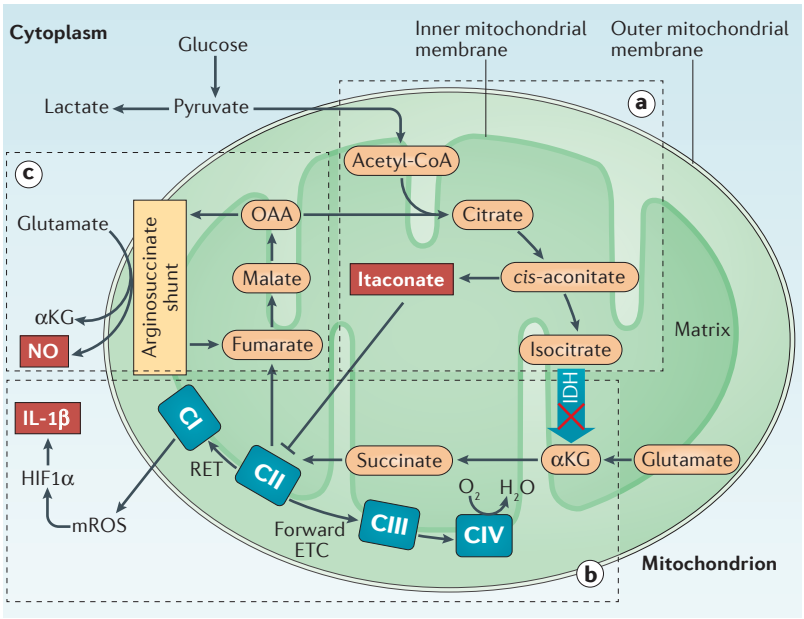


Figure 3 | **M1 macrophage metabolism utilizes a 'broken' tricarboxylic acid cycle to drive inflammation.** M1 macrophages are highly glycolytic and characterized by a high rate of lactate production. However, the unique function of their tricarboxylic acid (TCA) cycle is also crucial for M1 macrophage polarization. M1 macrophages have a 'broken' TCA cycle owing to two breaks that serves three distinct purposes. The TCA cycle is broken at two points: namely, the reactions catalysed by isocitrate dehydrogenase (IDH) and complex II (CII). **a** | A block at the IDH-catalysed step causes a build-up of the upstream metabolite *cis*-aconitate. M1 macrophages upregulate immune-responsive gene 1 protein (IRG1) to drive high itaconate production from *cis*-aconitate. **b** | In most cells, complex II typically receives electrons from succinate and passes them on to complex III for forward electron transport chain (ETC) flux. However, M1 macrophages promote reverse electron transfer (RET)through the ETC by causing electrons to transfer from complex II to complex I, which in turn generates mitochondrial reactive oxygen species (mROS). mROS then drive the stabilization of hypoxia-inducible factor 1α (HIF1α) and the downstream production of interleukin-1β (IL-1β). Itaconate causes a partial block at complex II, thus limiting the amount of mROS generated. Due to the block at the IDH-catalysed step, α-ketoglutarate (αKG) must be replenished by glutamate. **c** | The arginosuccinate shunt and part of the TCA cycle (the conversion of fumarate to oxaloacetate (OAA)) form a cycle that has the net effect of generating inflammatory nitric oxide (NO) from glutamate.

be expected to preferentially become T_{eff} cells owing to increased glycolysis, they do not have an impairment in memory formation[33]. On the contrary, the loss of VHL accelerates memory T cell formation[33]. It is important to note that VHL-deficient T cells preferentially differentiate into effector memory cells rather than central memory cells, and therefore some of the observed discrepancies with previous studies may simply be due to the analysis of different types of memory T cell.

An approach to deciphering how metabolism controls the functions of T cells is to conduct metabolomics experiments. Indeed, such experiments have shown that the most abundant metabolite in $CD8^+$ T cells that are deficient in VHL is *S*-2-hydroxyglutarate (*S*-2HG)[34]. *S*-2HG is generated by LDH or malate dehydrogenase using α-ketoglutarate (αKG) as a promiscuous substrate. This reaction is coupled to the oxidation of NADH to NAD^+ (FIG. 2). *S*-2HG is an antagonist of multiple αKG-dependent dioxygenases, including histone demethylases and the TET family of enzymes that control DNA demethylation. Thus, increases in *S*-2HG levels correlate with increases in methylation of histones and DNA. Specifically, in VHL-deficient $CD8^+$ T cells, the increased levels of *S*-2HG cause epigenetic changes that correlate with memory-like characteristics[34].

VHL deficiency and hypoxia increase the levels of *S*-2HG by elevating the HIF1α-dependent expression of LDHA[35]. How VHL deficiency and physiological hypoxia (defined as <1% O_2) increase the NADH/NAD^+ ratio to drive the conversion of αKG into *S*-2HG is not clear. Hypoxia would increase the NADH/NAD^+ ratio at oxygen levels below 0.5% O_2, at which point respiration is inhibited. It is important to note that the key experiments regarding the necessity of *S*-2HG in controlling the formation of memory T cells have not been performed to date. The levels of *S*-2HG would have to be reduced in VHL-deficient T cells to demonstrate a requirement for *S*-2HG in the control of memory T cell differentiation in this setting. Nevertheless, these findings suggest that controlling *S*-2HG levels might have a key role in the promotion of memory T cell differentiation through increased glycolysis.

Interestingly, *S*-2HG levels also increase when the ETC is inhibited, resulting in an elevated NADH/NAD^+ ratio[36]. At first glance, one would predict on the basis of this finding that the inhibition of respiration should increase the formation of memory $CD8^+$ T cells. However, disrupting the mitochondrial cristae in T cells by knocking out the gene that encodes optic atrophy protein 1 (OPA1; also known as mitochondrial dynamin-like 120 kDa protein; a protein required for mitochondrial fusion) reduces electron flux through the ETC and impairs memory $CD8^+$ T cell formation after infection, but does not impair the T_{eff} cell response[26]. Conversely, an increased electron flux through the ETC due to the loss of methylation-controlled J protein (MCJ; also known as DnaJ homologue subfamily C member 15) increases the secretion, but not the expression, of IFNγ and provides increased protection against influenza virus infection[37]. These results lead us to speculate that *S*-2HG would only promote the formation of memory T cells in conditions in which the ETC is not impaired. However, it is still not fully understood how the ETC controls memory T cell formation. The role of the ETC in this setting might be to sustain FAO-dependent metabolite production as well as the generation of mROS for optimal memory $CD8^+$ T cell formation. Future studies will need to dissect how the ETC controls memory $CD8^+$ T cell formation through metabolite and mROS production.

B cells

B cells also exhibit a number of functions that may be controlled by metabolism, such as clonal expansion, plasma cell differentiation and class switching. During clonal expansion, proliferating B cells increase their uptake of glucose and glutamine, which are required for the hexosamine pathway that produces glucosamine for antibody glycosylation[38–41]. Antibody production is central to B cell function. Long-lived plasma

B cells take up glucose to produce pyruvate, which in turn supports respiration[42]. Loss of the mitochondrial pyruvate carrier MPC1 diminishes the survival of long-lived plasma B cells and the generation of vaccine-specific antibodies *in vivo*[42]. It is unclear whether the survival advantage afforded to plasma cells by pyruvate-dependent respiration is due to an energetic requirement or to a signalling requirement through mROS or metabolite production. Reactive oxygen species in B cells have been shown to be important signals in B cell receptor activation, as they are required for the optimal engagement of the signalling pathways that are downstream of the B cell receptor[43,44]. Although the source of reactive oxygen species was originally believed to be NADPH oxidase 2 (NOX2; also known as cytochrome b245 heavy chain), it has since been shown that sustained mROS production provides the required signal for B cell activation and proliferation[45].

Activated B cells have several possible fates, including class-switch recombination (CSR) and plasma cell differentiation (PCD). CSR and PCD are associated with increased and decreased mitochondrial mass, potential and mROS production, respectively[46]. B cells with a high mitochondrial mass are CSR-committed owing to increased mROS production, which results in diminished haem synthesis. The haem-dependent transcription factor BACH2 is a key positive and negative regulator of CSR and PCD, respectively, and BACH2 function is attenuated when haem levels are high[46]. Reducing mROS levels pharmacologically *in vivo* promotes PCD by increasing haem synthesis[46]. Thus, mROS control fate decisions in activated B cells. Interestingly, part of haem synthesis occurs in mitochondria and requires the TCA cycle metabolite succinyl-CoA and glycine[47]. It is unknown whether TCA cycle metabolites control B cell fate. Future studies will need to examine whether mitochondria also control B cell fate by mechanisms other than the generation of mROS.

Innate immunity

M1 versus M2 macrophage metabolism

Macrophages can have a wide range of pro-inflammatory and anti-inflammatory properties. Macrophages that are characterized by the production of high levels of pro-inflammatory cytokines are referred to as M1 (classically activated) macrophages, and they display potent microbicidal properties as they produce large amounts of reactive oxygen and nitrogen species, and also promote T_H1 cell responses. By contrast, M2 (alternatively activated) macrophages exhibit anti-inflammatory functions; they are involved in controlling tissue remodelling and helminth infections, and can promote the growth of tumours. Although the distinction between M1 and M2 macrophages is an oversimplification of macrophage heterogeneity, it is useful in revealing the different ways in which metabolic pathways can control the broad range of macrophage functions. Initial studies suggested that glycolysis was a central player in shaping M1 macrophage responses, whereas OXPHOS shaped the function of M2 macrophages[48–51]. However, recent data indicate that both glycolysis and OXPHOS have unique roles in controlling the functions of both M1 and M2 macrophages[52].

Lipopolysaccharide (LPS) and IFNγ can activate M1 macrophages by inducing the expression of inducible nitric oxide synthase (which produces nitric oxide (NO) from L-arginine) and cytokines such as IL-1β and tumour necrosis factor (TNF). Initial studies demonstrated that TNF-dependent inflammatory cytokine production required mROS[53]. Further studies elucidated that experimentally decreasing the generation of mROS reduces signalling via multiple Toll-like receptor (TLR)-initiated pathways and thereby reduces the bactericidal activity of macrophages[54,55]. It is not fully understood how TLR signalling proteins control mROS production. Furthermore, patients with TNF receptor-associated periodic syndrome (TRAPS) who display hyper-inflammation have an elevated sensitivity to LPS because of increased mROS production[56].

Recent studies have provided more mechanistic insight into how M1 macrophages reprogramme their metabolism to generate mROS to promote their distinctive immune functions[57–60]. Initial studies indicated that M1 macrophages display an increased glycolytic flux and a decreased ETC flux[48–51]. Increased glycolysis has been shown to be necessary for the pro-inflammatory functions of M1 macrophages *in vitro* and *in vivo*[60–62]. However, although mitochondrial ETC flux is decreased, ETC flux and TCA flux are also necessary beyond ATP production for the pro-inflammatory functions of M1 macrophages. M1 macrophages have a 'broken' TCA cycle owing to two enzymatic blocks that result in three separate TCA cycle sequences[57] (FIG. 3).

In the first sequence, pyruvate enters the TCA cycle to ultimately generate citrate[63]. A decrease in the expression of the TCA cycle enzyme isocitrate dehydrogenase (which converts isocitrate to αKG) leads to increased mitochondrial levels of citrate and the intermediate *cis*-aconitate. M1 macrophages highly express immune-responsive gene 1 protein (IRG1; also known as CAD), which is an enzyme that produces itaconate by decarboxylating *cis*-aconitate[57]. Itaconate is a metabolite known to have antimicrobial properties[64,65]. The increased itaconate levels also cause a second break in the TCA cycle between succinate and fumarate, as itaconate is a natural weak inhibitor of complex II (also known as succinate dehydrogenase)[58]. This leads to the second sequence of the TCA cycle, which consists of αKG and succinate. Glutaminolysis replenishes the TCA cycle with αKG, which goes on to accumulate as succinate owing to the complex II block[57]. Succinate can also be replenished through the γ-aminobutyric acid shunt[60]. It is important to note that complex II is not completely inhibited in M1 macrophages. In fact, succinate oxidation and electron flux through complex II are necessary for IL-1β production, as they promote the activation of HIF1α via mROS generated by reverse electron transport (RET) through complex I[59]. Furthermore, releasing the inhibition of complex II by inducing a loss of the gene that encodes IRG1 decreases succinate levels concomitant with increasing respiration, leading to increased IL-1β production[58]. Pharmacologically preventing RET diminishes the LPS-induced production of IL-1β, whereas diverting electrons away from RET through the

expression of alternative oxidase (AOX; also known as mitochondrial ubiquinol oxidase) increases the expression of the anti-inflammatory cytokine IL-10 (REF. 59). The mechanisms by which LPS induces the generation of mROS by RET is not fully understood, although the increased mitochondrial membrane potential in M1 macrophages is likely to have a role. Genetic confirmation that RET-dependent mROS are necessary for controlling IL-1β production is still needed. The third sequence of the TCA cycle — from fumarate to oxaloacetate — uses the arginosuccinate shunt to produce the inflammatory molecule NO from glutamate-derived amino groups[57].

In stark contrast to M1 macrophages, M2 macrophages display lower levels of glycolytic flux, higher levels of OXPHOS and an intact TCA cycle[57]. Although they have a lower glycolytic flux, this does not imply that glycolysis is not essential for their function. 2-DG markedly prevents the activation of M2 macrophages[52]. M2 macrophages divert carbons from glucose into the hexosamine pathway to generate elevated levels of uridine diphosphate (UDP)-*N*-acetylglucosamine[57]. This ensures the glycosylation of key receptors including the lectin mannose receptors, which sense pathogens (FIG. 4). Importantly, inhibitors of *N*-glycosylation decrease the expression of the M2 macrophage markers CD206 and CD301 (REF. 57). Glycosylation also requires nitrogen from glutamine, and thus M2 macrophage polarization is dependent on increased glutamine metabolism[57].

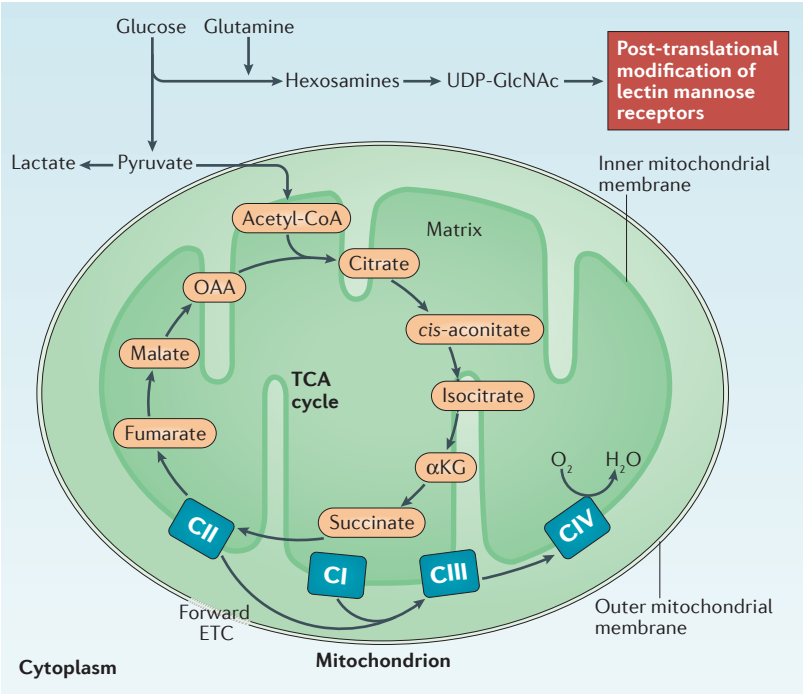


Figure 4 | **M2 macrophages require mitochondrial metabolism and glycolysis.** In contrast to M1 macrophages, M2 macrophages have an intact tricarboxylic acid (TCA) cycle, and they use acetyl-CoA to drive forward flux through the electron transport chain (ETC). They also require glycolysis, in part because of its role in supporting hexosamine biosynthesis. Hexosamine biosynthesis requires glucose and glutamine to generate uridine diphosphate (UDP)-*N*-acetylglucosamine (UDP-GlcNAc) that is necessary for the post-translational modification of lectin mannose receptors, which detect certain pathogens. αKG, α-ketoglutarate; C, complex; OAA, oxaloacetate.

M2 macrophages use AMPK-dependent FAO and mechanistic target of rapamycin complex 2 (mTORC2)-dependent glucose-derived pyruvate oxidation to fuel OXPHOS[52,66–68]. M2 macrophages also display increased mitochondrial biogenesis owing to the expression of peroxisome proliferator-activated receptor-γ (PPARγ) co-activator 1β (PGC1β)[50]. *In vitro* M2 macrophage activation is prevented by etomoxir, which is a widely utilized pharmacological inhibitor of FAO, and by oligomycin, which is an inhibitor of complex V (also known as F_1F_0-ATPase)[50,51]. M2 macrophages acquire lipids via the scavenger receptor CD36, and these lipids undergo intracellular degradation by lysosomal acid lipase to provide the fatty acids that fuel OXPHOS[51]. Genetic inhibition of intracellular lipolysis or CD36 prevents M2 macrophage polarization *in vitro* and *in vivo*[51]. The high mitochondrial mass and increased fatty acid fuel sources of M2 macrophages lead to an increased spare respiratory capacity in these cells. Interestingly, pharmacological experiments targeting ATP-citrate lyase suggest that cytosolic acetyl-CoA generated through OXPHOS is necessary for the acetylation of histones that are associated with genes that are known to be upregulated in M2 macrophages[69]. In this way, OXPHOS and acetyl-CoA may regulate the epigenetic programming of M2 macrophages.

Although the genetic data demonstrate that lipid uptake and subsequent lipolysis are necessary for M2 macrophage polarization[51], it is not clear whether fatty acids are needed for FAO-fuelled OXPHOS. FAO requires the transport of fatty acids via the carnitine shuttle into mitochondria. This is catalysed by the cytosolic enzyme carnitine *O*-palmitoyltransferase 1A (CPT1A; also known as CPT1L) and the mitochondrial enzyme CPT2. Genetic ablation of *Cpt2* in macrophages does not impair M2 macrophage polarization *in vitro* or *in vivo*[70]. These results suggest that etomoxir-mediated inhibition of M2 macrophage polarization is probably due to other off-target effects or that CPT1A has unappreciated roles outside of FAO. These findings provocatively suggest that fatty acid acquisition by M2 macrophages could be used to activate M2 macrophage-specific gene expression profiles in a manner that is independent of mitochondrial metabolism. It is likely that glucose-dependent pyruvate production — rather than FAO-fuelled OXPHOS — is the dominant mechanism by which mitochondrial metabolism controls M2 macrophage-specific gene expression profiles. However, this needs to be genetically confirmed through the use of a conditional knockout of the pyruvate transporter in macrophages. Going forwards, the key question for the field to address is how mitochondrial metabolism controls M2 macrophage-specific gene expression profiles. Again, the obvious candidates would be the release of mROS or metabolites that affect the activation of transcriptional networks and control epigenetic modifications to optimally polarize macrophages to an M2 phenotype.

The NLRP3 inflammasome and ROS

Mitochondria are also involved in the sensing of danger signals by innate immune cells, which can respond

to a wide variety of such signals as they express various pattern recognition receptors (PRRs): for example, RIG-I-like receptors, which can sense viral RNA, signal through the adaptor protein mitochondrial antiviral signalling protein (MAVS); mitochondrial DNA release is an activator of TLR9 and other PRRs; and NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3; a crucial component of the NLRP3 inflammasome) forms an inflammasome in response to mROS. MAVS has been extensively discussed elsewhere[71,72]. In this section, we focus on the various ways in which mROS have been shown to regulate the NLRP3 inflammasome.

Pioneering studies by Tschopp and colleagues have demonstrated that various pathogen-associated molecular patterns and damage-associated molecular patterns trigger the activation of the NLRP3 inflammasome in a mROS-dependent manner[73–75]. Inflammasome activation correlates with damaged mitochondria, which accumulate owing to the caspase-mediated inhibition of mitophagy[76,77]. The inhibition of autophagy, which results in an increase in the number of dysfunctional mitochondria, generates mROS that activate the inflammasome[78]. Furthermore, the excessive mitochondrial fission that is associated with inefficient ETC flux and increased mROS production activates the NLRP3 inflammasome[79]. The ETC is remodelled during inflammasome activation, such that complex I is destabilized and there is a switch to complex II-dependent respiration[80]. How mitochondrial metabolism is fuelled during inflammasome activation to generate mROS is not understood. One possibility might be that cytosolic reactive oxygen species, which are generated by the NADPH oxidase NOX4, increase the expression of the FAO protein CPTA1 to fuel mitochondrial metabolism. Indeed, loss of the gene that encodes NOX4 or CPT1A decreases inflammasome activation[81]. It is important to note that the NLRP3 inflammasome can also be activated by signals such as calcium influx and potassium efflux, and it is not fully understood how these signals integrate with mROS for optimal inflammasome activation[82]. The major challenge for the field is to identify the mechanism that induces mROS generation in the context of NLRP3 inflammasome activation and to identify the direct target of mROS that is necessary for NLPR3 inflammasome activation. Aside from studying mROS, a comprehensive approach to studying metabolomics during the course of NLRP3 inflammasome activation might provide insights into how metabolites control this process.

Dendritic cells

DCs have the ability to differentially promote tolerogenic or immunogenic responses, and they have a crucial role in the immune response. In a manner similar to the subsets of macrophages, different subsets of DCs have different metabolic profiles depending on whether they are tolerogenic or immunogenic. Immunogenic DCs have high glycolytic rates[83–86]. The activation of DCs by several TLR agonists, including LPS and CpG, leads to a rapid increase in glycolysis followed by a delayed decrease in OXPHOS and mitochondrial membrane potential[84,85]. Blocking glycolysis with 2-DG leads to decreased cytokine production and a failure to induce classical activation markers such as CD40 and CD86, indicating that glycolysis is required for DC activation[85]. Glycolysis generates pyruvate, which is transported into mitochondria to generate citrate. DC activation requires mitochondrial pyruvate flux through the TCA cycle in order to generate the citrate that is required to drive fatty acid synthesis[83]. Glycolysis also generates intermediates of the pentose phosphate pathway (PPP), which contributes to DC activation[83]. It is likely that PPP-dependent NADPH generation is necessary for fatty acid synthesis, which then induces expansion of the endoplasmic reticulum and Golgi. This expansion supports the secretion of early activation markers and pro-inflammatory cytokines such as TNF and IL-6 (REF. 83). Interestingly, fuller lipid stores and higher rates of fatty acid synthesis correlate with an increased inflammatory potential in liver DCs[87]. Conversely, it is important to note that the inhibition of fatty acid synthesis has also been reported to increase pro-inflammatory cytokine production[88].

DC function is also regulated by other aspects of mitochondrial metabolism. The initial activation of plasmacytoid DCs induces the production of type I IFNs, which function in an autocrine manner to increase OXPHOS downstream of FAO[89]. This is necessary for the expression of genes that are associated with a fully activated phenotype[89]. Going forwards, a key question to answer is how FAO promotes the full activation of DCs. Further evidence for the role of mitochondria in the regulation of DCs comes from the observation that DCs treated *in vitro* with vitamin D, a known promoter of tolerance, show increased OXPHOS, mitochondrial mass and mROS production[90]. DCs that are deficient in PPARγ (a nuclear receptor that regulates lipid metabolism and mitochondrial biogenesis) have decreased mitochondrial function and display a decreased capacity to induce tolerance[91,92]. However, mitochondrial function is also required for DC differentiation, as the inhibition of ETC flux by rotenone (an inhibitor of complex I) impairs the upregulation of CD1a (a marker of DC differentiation), and DC differentiation is associated with increased mitochondrial mass[93,94]. Although the mechanistic details of how glycolysis and mitochondria control DC function are currently unknown, it is important to note that these pathways are likely to have distinct roles in controlling DC function.

Tumour immunity

A growing field of immunology focuses on the role of the immune system in cancer and how the immune system may be harnessed for cancer therapy. Accordingly, recent studies have shown that various aspects of tumour immunity can be controlled by the metabolism of immune cells. T cells are essential to the host immune response to cancer. Activated $CD8^+$ T cells exert cytotoxic effects on tumour cells.

Mitophagy
A special form of autophagy, in which mitochondria (in a damaged or depolarized state) are engulfed by autophagosomes and degraded.

Autophagy
A cellular process, by which cytoplasmic organelles and macromolecular complexes are engulfed by double membrane-bound vesicles for delivery to lysosomes and subsequent degradation. This process is involved in the constitutive turnover of proteins and organelles, and is central to cellular activities that maintain a balance between the synthesis and breakdown of various proteins.

Pentose phosphate pathway
(PPP). A pathway that uses glucose to generate NADPH and pentose sugars (such as ribose). The first (oxidative) phase converts glucose-6-phosphate to ribulose-5-phosphate and generates NADPH. The second (non-oxidative) phase synthesizes other sugars from ribulose-5-phosphate.

However, the tumour microenvironment becomes immunosuppressive during cancer progression, and this reduces T cell-mediated cytotoxicity. Tumour-infiltrating T cells (TILs) display a loss of mitochondrial function owing to a defect in the programming of mitochondrial biogenesis by PGC1α. This is independent of signalling via the checkpoint molecule programmed cell death protein 1 (PD1) or the suppression of TILs via T_{reg} cells[95]. Ectopic PGC1α expression in T cells rescues mitochondrial function and induces more effective antitumour responses[95]. These data are supported by the observation that pharmacologically increasing mitochondrial fusion, which promotes mitochondrial metabolism in memory T cells, also augments antitumour responses[26]. In another study, the adoptive transfer of melanoma-specific $CD8^+$ T cells led to an improved antitumour response when cells that had a low mitochondrial membrane potential were selectively transferred[96]. This effect was attributed to an enrichment of cells engaging in OXPHOS and FAO, and an enrichment of long-lived memory cells[96].

How the tumour microenvironment represses mitochondrial metabolism is not fully understood. The tumour microenvironment is limited in nutrients, and this could repress T cell activation. Indeed, low glucose levels in the tumour microenvironment cause TIL dysfunction. Recent studies suggest that cancer cells that consume high levels of glucose restrict the amount of glucose that is available to TILs, thus inhibiting their cytotoxic function[97,98]. Restoring the glycolytic intermediate phosphoenolpyruvate (PEP) in T cells *in vivo* through the overexpression of the enzyme phosphoenolpyruvate carboxykinase 1 (which generates PEP from the TCA intermediate oxaloacetate) is sufficient for optimal calcium-dependent NFAT activation in the context of glucose deprivation and boosts the antitumour responses of TILs[98]. Furthermore, prolyl hydroxylase 2-deficient T cells, which have increased glycolysis owing to HIF1α stabilization, are also more tumoricidal compared with their wild-type counterparts[99]. Oxygen can also be limited in the tumour microenvironment owing to high consumption by proliferative tumour cells. Using a hyperoxic chamber to provide supplemental oxygen to tumour-bearing mice as a strategy to decrease hypoxia in the tumour microenvironment has been shown to increase T cell infiltration and antitumour responses[100].

Aside from displaying tumour-induced glucose and oxygen depletion, the tumour microenvironment has an altered metabolite composition that promotes immunosuppression. Tumour cells express high levels of the tryptophan-metabolizing enzyme indoleamine 2,3-dioxygenase, which generates the immunosuppressive metabolite kynurenine[101]. Thus, tryptophan levels are low in the tumour microenvironment. The levels of arginine, which is required for T cell proliferation, are low in the tumour microenvironment owing to arginine consumption by tumour cells[102,103]. Finally, lactic acid produced by tumour cells can impair T cell motility and function, and can induce the polarization of macrophages into cancer-promoting tumour-associated M2-like macrophages[104,105]. Together, these studies provide proof-of-concept evidence that metabolic competition for nutrients in the tumour microenvironment is involved in establishing and maintaining an immunosuppressive tumour microenvironment.

Cells and metabolites in the tumour microenvironment can alter T cell metabolism by upregulating co-inhibitory molecules such as the checkpoint molecules cytotoxic T lymphocyte antigen 4 (CTLA4) and PD1, and immunotherapeutic approaches based on targeting these molecules (referred to as checkpoint blockade therapy) may work in part by reversing changes in T cell metabolism. Interestingly, the activation of PD1 on T cells has been shown to decrease their uptake of glucose and glutamine, causing an increased reliance on FAO and an immunosuppressed phenotype[97]. Disrupting this co-inhibitory signalling increases glucose utilization in TILs, implying that metabolism has a role in restoring their tumoricidal potential[97]. An exciting idea is to combine current therapies that target cellular metabolism and checkpoint blockade therapy. A recent study demonstrated that metformin (a widely utilized type 2 diabetes treatment and known mitochondrial inhibitor) had little therapeutic benefit in mouse models of highly aggressive tumours, but compared to either treatment alone, the combination of metformin and PD1 checkpoint blockers resulted in increased TIL function and tumour clearance[106]. However, it is unclear whether these findings reflect the effects of metformin on the tumour or on TILs, or a combination of both. In other experimental models, metformin has been shown to directly inhibit tumour cell mitochondrial complex I to inhibit tumorigenesis[107]. Thus, multiple mechanisms could underlie the anticancer properties of metformin.

Another anticancer immunotherapeutic approach is based on adoptively transferred T cells such as chimeric antigen receptor (CAR)-expressing T cells (hereafter referred to as CAR T cells). These cells are genetically engineered to recognize cancer cells, and have been shown to lead to robust, durable responses in patients with certain malignancies[108]. One avenue of research has focused on how to improve anticancer responses by modulating the metabolism of CAR T cells. For example, the choice of intracellular signalling domain in the CAR construct affects the metabolism of CAR T cells and also their effectiveness; CARs engineered to include the intracellular signalling domain of the co-stimulatory molecule 41BB (also known as TNFRSF9), instead of that of the co-stimulatory molecule CD28, have an increased mitochondrial mass and increased expression of genes that are involved in the ETC, leading to increased levels of FAO[109]. These changes correlate with increased CAR T cell survival and memory, thus improving the response to adoptive transfer therapy[109]. Another strategy uses $CD8^+$ T cells that are cultured in the presence of cell-permeable *S*-2HG. The subsequent transfer of these cells into tumour-bearing hosts leads to remarkably increased antitumour activity compared with the transfer of untreated T cells[34]. Treatment with *S*-2HG might be efficacious in determining the cell fate of T cells before adoptive transfer for immunotherapy.

Looking to the future, there is much excitement about the interconnection between the fields of cancer and immunometabolism.

Therapeutic targeting of metabolism

If metabolism controls the fate of immune cells then it is logical to assume that targeting immunometabolism can also alter the course of diseases that are mediated by dysfunctional immune cells. For example, metformin displays anti-inflammatory properties (FIG. 5), and treatment with metformin decreases the rates of LPS-induced sepsis in mice[110]; it also changes the composition of the microbiome[111,112] and prevents age-related pathologies in mice[113,114]. Paradoxically, it can also augment the immune response, as metformin treatment in humans has been shown to improve the control of *Mycobacterium tuberculosis* infection and reduce disease severity[115]. Metformin is a safe and inexpensive drug, and these features have led investigators to consider evaluating metformin in the first human anti-ageing trial[116]. We speculate that many of the beneficial effects attributed to metformin might be due to its anti-inflammatory properties. Although the intracellular target or targets) of metformin have not been fully identified, its inhibition of mitochondrial complex I has emerged as a leading mechanism underlying its anti-inflammatory effects[117].

One target of metformin may be T cell metabolism. In a mouse model of the autoimmune disease systemic lupus erythematosus, T cells were shown to have an overly inflammatory phenotype owing to increased levels of OXPHOS and glycolysis[118]. Furthermore, the treatment of mice with a combination of metformin and 2-DG reversed these metabolic changes and alleviated disease[118]. Of note, antibody production in response to foreign antigen was not impaired, indicating that the treatment spares at least some aspects of normal immune function. This aspect of the treatment is particularly important, as a disease-modifying therapy for lupus that spares normal immune function would also have the potential to prevent deaths that are due to the side effects of current immunosuppressive treatment options. Interestingly, the treatment of a mouse model of lupus with pioglitazone (a PPARγ agonist known to alter glucose and fatty acid metabolism) also decreases lupus pathology, as does treatment with Bz-423, which is a novel inhibitor of complex V[119,120].

Similarly, allograft rejection is an immune-dependent process that is driven by T_{eff} cells, and a combination of inhibitors of glycolysis, glutaminolysis and OXPHOS (2-DG, 6-diazo-5-oxo-L-norleucine and metformin, respectively) has been shown to delay the rejection of grafted tissue[121]. Similarly, treatment with Bz-423 inhibited allograft rejection in mouse models[122]. Furthermore, in the autoinflammatory disorder TRAPS, immune cells have been shown to produce excessive amounts of mROS, leading to excessive inflammasome activation and cytokine production[56]. Peripheral blood monocytes from patients with TRAPS who were treated with MitoQ — which is a mitochondria-targeted antioxidant — show reduced cytokine production, indicating that such agents may offer a potential therapeutic benefit. Other promising mROS suppressors are also being developed, such as suppressors of site I_Q electron leak (S1QELs) and suppressors of site III_Q electron leak (S3QELs), which are novel compounds that selectively suppress complex I-dependent and complex III-dependent superoxide production, respectively[123,124]. Clearly, the study of immunometabolism has highlighted a wide variety of molecules as potential targets for the treatment of diseases that involve aberrant inflammation.

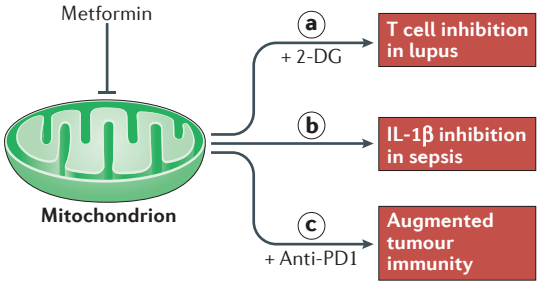


Figure 5 | **Targeting mitochondrial metabolism as a therapeutic strategy.** Mitochondrial inhibitors such as metformin have been successfully used in mouse models to target inflammatory T cells in lupus in combination with glycolytic inhibition (part **a**); to inhibit interleukin-1β (IL-1β) production in lipopolysaccharide (LPS)-induced sepsis (part **b**); and to increase tumour immunity synergistically with programmed cell death protein 1 (PD1) blockade (part **c**). 2-DG, 2-deoxyglucose.

Conclusion

Mitochondrial metabolism has a crucial role in controlling a wide range of immunological functions, such as T cell differentiation, macrophage polarization and tumour immunity. As our knowledge of the different metabolic profiles of various immune cell subsets increases, recent studies of immunometabolism have begun to elucidate the mechanisms by which changes in metabolism cause changes in immune cell function. Central to this idea is the question of how mROS and mitochondria-generated metabolites control immunity. Many mechanistic details still remain to be elucidated, such as how these mROS and metabolites are generated, and what their targets are. Future studies may help to elucidate the relevant targets of mROS that act to control immune cell fate. Reactive oxygen species-induced signal transduction is dependent on the oxidation of thiol groups on target proteins, either directly or through an intermediate step. Discovering which thiol groups are oxidized on relevant proteins is fundamental to understanding the mechanism of action of reactive oxygen species. For example, it was recently shown that the oxidation of a particular cysteine residue (Cys253) of uncoupling protein 1 (UCP1) allows reactive oxygen species to increase UCP1 activity[125]. Similarly, discovering other reactive oxygen species-sensitive target proteins may lead to insights into how mROS mediate changes in immune cells. A deeper understanding of this process has the potential to reveal novel ways in which immune cells may be manipulated for therapeutic benefit.

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