Metabolism of inflammation limited by AMPK and pseudo-starvation

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Metabolic changes in cells that participate in inflammation, such as activated macrophages and T-helper 17 cells, include a shift towards enhanced glucose uptake, glycolysis and increased activity of the pentose phosphate pathway. Opposing roles in these changes for hypoxia-inducible factor 1α and AMP-activated protein kinase have been proposed. By contrast, anti-inflammatory cells, such as M2 macrophages, regulatory T cells and quiescent memory T cells, have lower glycolytic rates and higher levels of oxidative metabolism. Some anti-inflammatory agents might act by inducing, through activation of AMP-activated protein kinase, a state akin to pseudo-starvation. Altered metabolism may thus participate in the signal-directed programs that promote or inhibit inflammation.

To many biologists, metabolism is viewed simply as a means to generate a store of energy by catabolism, and to generate macromolecules for cell maintenance and growth through anabolic pathways. The elucidation of metabolic pathways in the twentieth century gave insight into disorders in which there are obvious dysfunctions in metabolism, such as diabetes and atherosclerosis. However, in the twenty-first century, alterations in metabolic regulation are now seen to be just as important in other diseases, such as cancer and inflammatory conditions. As long ago as 1923, the German biochemist Otto Warburg proposed that metabolic dysregulation was a feature of tumour cells[1]. Normal cells metabolize glucose through glycolysis, and under normoxic conditions, the pyruvate that is generated is then further oxidized in mitochondria. When oxygen becomes limiting, however, mitochondrial oxidative metabolism is restricted, and the pyruvate is converted to lactate instead. Warburg discovered that this latter process predominates in tumour cells, even when oxygen is plentiful, a process that became known as the 'Warburg effect' or aerobic glycolysis.

Studies on the metabolism of activated macrophages began in the 1960s. Monocytes from peritoneal exudates were shown to depend mainly on glycolysis as a source of metabolic energy[2], whereas *in vitro* culture of monocytes caused a significant increase in glycolysis[3]. In 1982, a study[4] showed that in an *in vivo* model (graft versus host) of immune activation, lymphocytes exhibited increased glycolysis — which was shown to be important for proliferation — together with a marked increase in glutamine use[4]. Activated macrophages have been shown to have a high hexokinase activity, the first enzyme involved in glycolysis and in the pentose phosphate pathway. Glycolysis and glutamine metabolism are also markedly increased during phagocytosis[5].

More recent studies have confirmed and extended these earlier findings. A shift towards aerobic glycolysis occurs in macrophages and dendritic cells activated by the bacterial product lipopolysaccharide, acting through Toll-like receptor 4 (TLR4)[6], in M1 inflammatory macrophages[7], and in T-helper (T_H) 17 lymphocytes that produce the pro-inflammatory cytokine interleukin-17 (IL-17)[8,9]. On the other hand, cells that limit inflammation, such as regulatory T cells[8,9], M2 anti-inflammatory macrophages[7] and quiescent memory T cells that carry the CD8 antigen[10], exhibit oxidative metabolism with more limited rates of glycolysis. Mechanistic insight into these changes has lagged behind tumour cells, but they are likely to share several features, such as the involvement of the transcription factor hypoxia-inducible factor 1α (HIF-1α) and AMP-activated protein kinase (AMPK). Here we discuss these recent developments in the case of inflammation, compare them with findings obtained with tumour cells and speculate on what this new information might mean for our understanding of the pathogenesis of inflammatory diseases, and for possible new directions for therapeutics.

Inflammation and metabolism

Inflammation is triggered in response to tissue injury or infection, and mounting an inflammatory response is an energy-intensive process. In the presence of pathogens or the products of inflamed tissues that provoke inflammation, macrophages rapidly switch from a resting state to a highly active state, exhibiting a pronounced increase in production of host defence factors, enhanced phagocytosis and antigen presentation. It is perhaps no surprise that such highly active cells undergo metabolic changes similar to those seen in tumour cells. The similarity between the metabolism that occurs in tumour cells and activated T cells has already been pointed out, with particular roles for the phosphatidylinositol-3-OH kinase (PI(3)K)–Akt and the mechanistic target of rapamycin (mTOR) pathways, as well as the transcription factor c-myc[11]. One difference between metabolic pathways in tumour cells and inflammatory cells (Fig. 1) is that the changes in tumour cells are driven largely by mutations, whereas those in inflammatory cells are driven by extracellular signals.

Macrophage and dendritic-cell activation

Resting dendritic cells predominantly use oxidative phosphorylation to generate ATP, but on activation of TLRs — which are the primary sensors of microbial products that trigger inflammation by pathogen-associated molecular patterns (PAMPs) — they undergo a switch to glycolysis[6] (Fig. 2). Such activated dendritic cells have elevated expression of the glucose transporter GLUT1 and increased lactate production, with a decrease in oxygen consumption by mitochondria — effects that are dependent on both PI(3)K and Akt1 (ref. 6). As discussed later, these effects are opposed by AMPK activation. Also of note is the effect of the immunomodulatory cytokine IL-10, which limits the switch to glycolysis and blocks TLR action in dendritic cells, again suggesting that a switch to mitochondrial metabolism has a net anti-inflammatory effect[6]. Depression of oxidative metabolism by TLR4 involves upregulation of the inducible form of nitric oxide synthase (iNOS)[12]; NO competes with

oxygen to inhibit the terminal electron acceptor of the respiratory chain, cytochrome *c* oxidase. By preventing reoxidation of NADH, this limits flux through the tricarboxylic acid (TCA) cycle, leading to an increase in pyruvate that is diverted to generate lactate, an alternative route for reoxidizing the NADH produced by glycolysis. The increase in glycolysis therefore occurs in concert with the attenuation of oxidative phosphorylation, and serves as an alternative pathway to maintain ATP levels. ATP is required not only by many ATP-dependent enzymes, but also to maintain mitochondrial membrane potential through reversal of adenine nucleotide translocase and the F_OF_1-ATP synthase when NO inhibits the respiratory chain[13]. Without this, a drop in the mitochondrial membrane potential would cause recruitment of the apoptosis regulator BAX to the mitochondria, release of cytochrome *c* into the cytosol, and consequent activation of caspases and apoptotic cell death. Enhanced ATP levels could, therefore, prolong the lifespan of dendritic cells and promote adaptive immunity.

Apart from the attenuation in oxidative phosphorylation in macrophages in response to TLR stimulation, the production of reactive oxygen species (ROS) from mitochondria increases. Mitochondria are recruited to phagosomes that contain bacteria, and the ROS that they produce augment the bactericidal activity of macrophages[14]. This provides an alternative source of ROS to the NADPH oxidase system, with current evidence indicating that mitochondrial ROS are required for macrophage bactericidal activity[14,15]. The TLR-signalling protein TRAF6 is recruited to mitochondria on activation of TLR2 or TLR4, but not TLR3 or TLR9, and engages with the adaptor protein ECSIT on the mitochondrial surface. Both TRAF6 and ECSIT are required for the generation of ROS[14]. The different effects of specific TLRs on this process can be rationalized. The TLR1–TLR2 dimer senses bacterial lipopeptides and TLR4 senses lipopolysaccharide, whereas TLR3 and TLR9 sense double-stranded RNA and CpG-rich DNA, respectively, both of which are common in viruses. TLR1–TLR2 and TLR4 also signal from the plasma membrane, whereas TLR3 and TLR9 signal from endosomes. In TLR1–TLR2 and TLR4 signalling, induction of ROS is required to kill bacteria in phagosomes, whereas for TLR3 and TLR9 this is not required for antiviral effects.

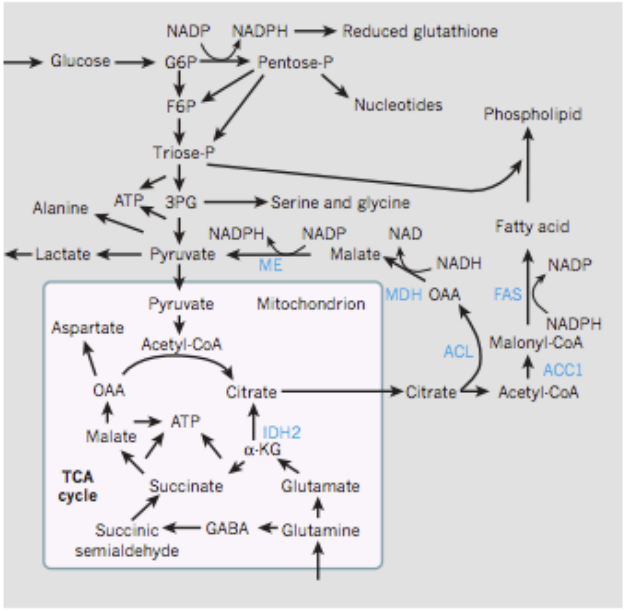

Figure 1 | Central metabolic pathways involved in the Warburg effect in tumour cells and inflammatory cells. Glucose is taken up by the cell and converted to pyruvate by glycolysis; the pyruvate then enters the energy-generating TCA cycle. The pentose phosphate pathway generates reduced glutathione and nucleotides. Citrate produced by the TCA cycle is converted into fatty acids and phospholipids. Glutamine is taken up by the cell and converted, by glutaminolysis, to glutamate or GABA (γ-aminobutyric acid) — produced by the GABA shunt — for entry into the TCA cycle. Enzymes involved in the pathways are shown in blue. ACC1, acetyl-coenzyme A (CoA) carboxylase 1; ACL, ATP citrate lyase; FAS, fatty-acid synthase; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; IDH2, isocitrate dehydrogenase 2; α-KG, α-ketoglutarate; MDH, malate dehydrogenase; ME, malic enzyme; OAA, oxaloacetate; P, phosphate; 3PG, 3-phosphoglycerate.

Mechanisms of metabolic changes in macrophages

If increased glycolysis is required because of a decrease in oxidative phosphorylation in dendritic cells and macrophages, how is it switched on? Here the similarity to tumour cells becomes clear. Macrophages activated with lipopolysaccharide and interferon-γ (IFN-γ) acquire an innate or inflammatory phenotype, and are often termed M1 macrophages[7]. They have a high microbicidal activity, as well as enhanced production of pro-inflammatory cytokines and ROS. There is also a marked switch from expression of the liver isoform of 6-phosphofructo-2-kinase (encoded by *PFKFB1*) to the PFKFB3 isoform, the type also commonly found in tumour cells[7]. This leads to accumulation of fructose-2,6-bisphosphate and, thus, increased glycolytic flux. The opposite is true when macrophages are activated by IL-4 and IL-13, which promote the alternative phenotype, often termed M2 macrophages. These macrophages are associated with tissue repair and humoral immunity, as well as anti-inflammatory cytokine production and reduced expression of major histocompatibility complex (MHC) class II and therefore antigen presentation. They exhibit enhanced oxidative phosphorylation and much lower rates of glycolysis[7], and have no detectable PFKFB3, expressing PFKFB1 instead. M2 macrophages still produce cytokines, but these are largely anti-inflammatory in action. Why oxidative phosphorylation is associated with production of anti-inflammatory cytokines, whereas glycolysis is associated with production of pro-inflammatory cytokines, remains unclear. However, peroxisome-proliferator-activated receptor-γ co-activator-1β (PGC-1β) has a key role in the mechanism[16]. PGC-1β is a transcriptional co-activator that promotes oxidative metabolism, notably by upregulating expression of genes involved in fatty-acid oxidation[17]. In macrophages activated with IL-4 to generate the M2 phenotype, there is a profound increase in the entire program for fatty-acid metabolism, including uptake and oxidation of fatty acids and mitochondrial biogenesis (Fig. 3a). These changes are dependent on activation by IL-4 of transcription factor STAT6, which in turn induces PGC-1β (ref. 16). Over-expression of PGC-1β promotes the M2 phenotype and attenuates macrophage-mediated inflammation *in vivo*. Conversely, knockdown of the gene that encodes PGC-1β limits the shift to oxidative phosphorylation and the function of M2 macrophages, and greatly boosts the activating effects of lipopolysaccharide[16]. STAT6 and PGC-1β have been shown to interact directly at the promoters of genes expressed specifically in M2 macrophages, such as ARG1 (ref. 16). A mechanism has therefore been defined whereby cytokines such as IL-4, acting through STAT6, promote PGC-1β function, leading to increased fatty-acid oxidation, mitochondrial biogenesis and oxidative phosphorylation (Fig. 3a).

In another study, IFN-γ, acting through STAT1, was shown to induce ROS through the nuclear receptor oestrogen-related receptor-α (ERR-α), which in turn was dependent on PGC-1β. ERR-α deficient mice were shown to be more susceptible to infection with *Listeria monocytogenes*[15]. This study further emphasizes the importance of mitochondrial ROS in killing bacteria.

Function of sirtuins in macrophages

Sirtuins are a family of NAD^+-dependent lysine deacetylases that act on a wide range of protein substrates. They are thought to act as NAD^+ sensors that connect nutrition and metabolism to chromatin structure[18]. Sirtuin activation has been shown to be anti-inflammatory, and knockdown experiments in macrophages have shown that two sirtuins, Sirt1 and Sirt6, promote a switch from glycolysis to fatty-acid oxidation[19], which requires the enzyme nicotinamide phosphoribosyltransferase

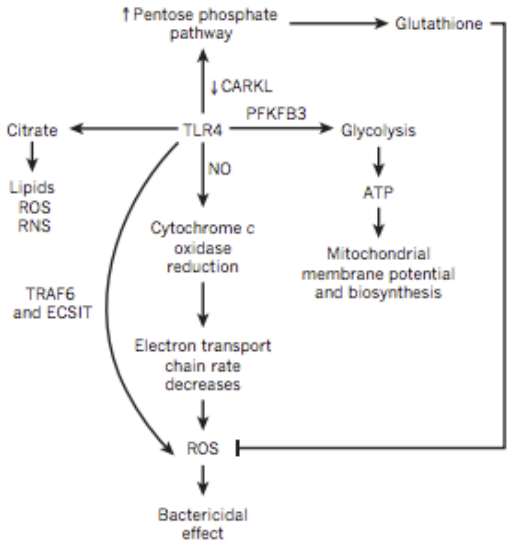

Figure 2 | Metabolic changes in TLR4-activated M1 macrophages. The lipopolysaccharide receptor TLR4 promotes a major shift in the metabolism of macrophages and dendritic cells, which is a determining factor in their activation. TLR4 induces the enzymes involved in glycolysis, including the key controlling enzyme PFKFB3; the ATP produced is required for biosynthesis and to maintain mitochondrial membrane potential. TLR4 activation also results in oxidative metabolism attenuation in mitochondria, mediated in part by nitric oxide (NO), causing the displacement of oxygen from cytochrome *c* oxidase. This leads to a limitation in the rate of transfer in the electron transport chain, generating reactive oxygen species (ROS), which have a bactericidal effect. An increase in ROS is promoted by the TLR4 signals TRAF6 and ECSIT. The activity of the pentose phosphate pathway increases owing to a decrease in the enzyme carbohydrate kinase-like protein (CARKL). The pentose phosphate pathway generates NADPH, which is needed for glutathione function — a key antioxidant that limits ROS. TLR4 also generates citrate, which is used in lipid biosynthesis, as well as to generate further ROS and reactive nitrogen species (RNS).

(NAMPT) — involved in the synthesis of NAD^+. Sirtuins are known to be involved in the effects of dietary restriction, and may partly explain why this manipulation is beneficial (because it would limit inflammation). Sirt1 deacetylates and inactivates the p65 component of the nuclear factor-κB (NF-κB) pathway[20], thus limiting the expression of NF-κB-dependent genes. Sirt1 also deacetylates and activates PGC-1β[21] (Fig. 3a), thus promoting a shift towards fatty-acid oxidation[22]. Activation of TLR4 will actually induce NAMPT late in the time course of macrophage activation, and thereby cause a negative feedback effect on macrophage activation[23]. Similarly, the anti-inflammatory effects of dietary restriction, or pharmacological activation of sirtuins, might occur through the reprogramming of macrophages to an anti-inflammatory state. Sirtuins were originally identified as having a role in prolonging lifespan, but the beneficial effects of sirtuin activation in mammals may owe more to their anti-inflammatory effects.

The role of carbohydrate kinase-like protein

In a screen for new regulators of macrophage activation, carbohydrate kinase-like protein (CARKL) was found to be downregulated in macrophages[24]. When CARKL is overexpressed, the induction of pro-inflammatory cytokines by lipopolysaccharide is repressed, whereas knocking down its gene had the opposite effect. This indicates that CARKL is a key driver of the M2 phenotype, with lipopolysaccharide suppressing its expression to promote the M1 phenotype. Intriguingly, CARKL is a sedoheptulose kinase that catalyses the formation of sedoheptulose 7-phosphate (S7P) (an intermediate in the pentose phosphate pathway), which seems to limit flux into the pentose phosphate pathway. This leads to a reduction in the formation of NADPH and hence reduced glutathione, perhaps leading to a pro-oxidant state that limits M1 macrophage function. When CARKL levels are low, as is triggered by TLR4, a more antioxidant environment prevails in the macrophage. IL-4 induces CARKL expression (Fig. 3a). The requirement for this antioxidant state in M1 macrophages may involve NF-κB, a key transcription factor for pro-inflammatory genes such as tumour necrosis factor-α (TNF-α), because NF-κB subunits such as RelA must remain in a reduced state to function properly[25].

This study on CARKL is important because it integrates several concepts. Firstly, a key feature of M1 macrophages may be their ability to produce ROS, which are needed to kill phagocytosed bacteria, from mitochondria. However, these ROS must not cause intracellular damage. Glycolysis can provide ATP to maintain mitochondrial membrane potential in the absence of full function of the respiratory chain, and to drive reactions involved in the biosynthesis of pro-inflammatory proteins. Increased glucose uptake also promotes the pentose phosphate pathway, which is needed, in part, to generate the NADPH required for maintenance of reduced glutathione, limiting the cellular damage that ROS might otherwise cause. Activated macrophages have increased expression of glucose-6-phosphate 1-dehydrogenase and 6-phosphogluconate dehydrogenase, two key enzymes in the pentose phosphate pathway. In addition, glutamine metabolism can generate NADPH, and a key enzyme in this process is glutamate dehydrogenase, which is also elevated in lipopolysaccharide-activated macrophages[26].

The roles of M1 and M2 macrophages

ROS production is a key functional feature of the acute inflammatory, antibacterial M1 macrophage, and the metabolic alterations that occur are integral to this process. The anti-inflammatory, tissue-repair-promoting M2 macrophage, however, has a quite different metabolism. It is likely that the metabolic switching is needed for the distinct functions of these macrophages. M1 macrophages need to have a rapid, vigorous response to bacteria, involving the generation of ROS and pro-inflammatory cytokines such as IL-1β and TNF-α, which are needed to deal with infection and danger from damaged tissue. The increase in ROS occurs partly because of attenuation in the respiratory chain, whereas increased glycolytic flux is needed to maintain ATP levels for biosynthesis, as well as to maintain mitochondrial membrane potential and prevent apoptosis. Equally, increased glucose uptake and glycolysis are needed to allow the induction of expression of inflammatory genes (many of which require HIF-1α, as discussed later), and the production of NADPH by the pentose phosphate pathway to limit the damage caused by ROS. By contrast, M2 macrophages are more involved in long-term tissue repair, and provide defence against extracellular parasites. STAT6 controls the genetic program for long-term macrophage activation. Oxidative metabolism could be better suited to these longer-term roles of M2 macrophages. This oxidative metabolism requires the STAT6 target gene encoding PGC-1β, whereas the M1 phenotype requires HIF-1α and glycolysis. Although M2 macrophages are highly active and can proliferate, they rely mainly on oxidative metabolism for their energetic and biosynthetic demands.

A role for citrate in macrophage activation

A final aspect of macrophage metabolism and inflammation concerns the role of citrate. Altered metabolism of citrate is an important feature of the metabolic switch that occurs in proliferating tumour cells, when the TCA cycle changes from being a purely catabolic pathway generating ATP to become, at least in part, an anabolic pathway. Citrate is withdrawn from the cycle (Fig. 1) to provide, through the reaction catalysed by ATP citrate lyase, a source of cytoplasmic acetyl-coenzyme A (CoA) for the synthesis of large amounts of fatty acids that are required for the synthesis of new membrane lipids — including phospholipids, which are the source of arachidonic acid for prostaglandin production. Another product of citrate metabolism is oxaloacetate, which produces NADPH through cytosolic malate dehydrogenase and malic enzyme (Fig. 1). NADPH is required to

generate ROS through NADPH oxidase, and NO through NO synthase. Interestingly, knockdown of the mitochondrial citrate carrier gene limits the export of citrate and blocks the production of prostaglandins, ROS and NO in response to lipopolysaccharide[27]. Citrate derived from mitochondria is therefore required for certain responses to lipopolysaccharide.

T_H17 lymphocytes versus regulatory T cells

There are intriguing similarities between the metabolic profile of T lymphocytes involved in the inflammatory process, such as T_H17 lymphocytes, and those exhibited by inflammatory macrophages and dendritic cells. Equally, anti-inflammatory T lymphocytes such as regulatory T (T_{reg}) cells have a metabolism that is characterized by mitochondrial oxidative metabolism, analogous to M2 macrophages. We can postulate, therefore, that a shift towards high glycolysis is a hallmark of inflammatory cells, whereas oxidative phosphorylation is a hallmark of anti-inflammatory cells. Increased glycolysis in lymphocytes grown in culture was shown as early as 1966 (ref. 28). This has also been shown in cells that carry the CD4 antigen, in which CD28 signalling increases glycolysis through activation of PI(3)K and Akt[29]. Glucose uptake and glycolysis are increased in T_H17 cells (as well as T_H2 and T_H1 cells) compared with T_{reg} cells[30], which in turn have increased membrane potential and oxidize lipids at a higher rate than other subsets of cells that carry the CD4 antigen. There is, however, likely to be a gradation towards either glycolysis or oxidative metabolism because T_H17 cells still exhibit some lipid oxidation.

Shi and colleagues have reported that in conditions favouring T_H17-cell development from naive T cells, glycolysis is strongly upregulated and glycolytic enzymes are induced; however, this did not occur under conditions in which T_{reg} cells were induced[8]. Blocking glycolysis with 2-deoxyglucose led to a switch in lineage, with T_H17 cells becoming T_{reg} cells; this striking result suggests that the plasticity of T cells, a key theme in immunology, might be controlled by metabolic cues and activated by polarizing signals such as IL-17 or transforming growth factor-β (TGF-β). HIF-1α expression was also induced in T_H17 cells, and this required mTOR complex-1 (mTORC1), with rapamycin limiting these changes and promoting development of T_{reg} cells. A link between mTORC1 and HIF-1α is that mTORC1 enhances translation of the messenger RNA encoding HIF-1α, which contains 5′-terminal oligopyrimidine (5′-TOP) sequences[31]; translation of mRNAs containing such sequences is known to be upregulated following mTORC1 activation. The HIF-1α-dependent glycolytic pathway therefore provides a stimulus for the differentiation of T_H17 cells, and limits T_{reg} cell development[8].

The PI(3)K–mTOR pathway has been studied extensively in T-cell function. It has an essential role in promoting glucose use in effector T-cell development, including T_H17 cells, and at the same time inhibits T_{reg} cell generation[32,33]. mTORC1 acts downstream of Akt, while mTORC2 promotes Akt activation. T cells with a T-cell-specific mTOR knockout do not differentiate into effector T cells, including T_H17 cells, and default to the T_{reg} phenotype[34], which is consistent with results showing that rapamycin promotes fatty-acid oxidation and generates T_{reg} cells[35]. mTORC1 and mTORC2 also have specific effects on effector T-cell subsets. T cells deficient in the GTP-binding protein Rheb, which lack mTORC1 function, can only differentiate into T_H2 cells, whereas mTORC2-deficient T cells differentiate into T_H1 and T_H17, but not T_H2 cells[36]. The molecular basis of these differences and the role of metabolism in the effects of mTORC1 and mTORC2 deficiency are not known.

In a study similar to that of Shi *et al.*[8], Dang and colleagues demonstrated that HIF-1α enhances T_H17 cell development through the direct transcriptional activation of the key T_H17 transcription factor retinoic-acid-related orphan receptor-γt (RORγt), which then forms a complex with p300 on the IL-17 promoter[9] (Fig. 3b). One role of HIF-1α is therefore to promote the function of RORγt. HIF-1α also binds to the T_{reg} transcription factor Foxp3 and targets it for proteosomal degradation through recruitment of the E3 ligase VHL, which occurs under normoxic conditions. Crucially, therefore, HIF-1α is a molecular switch in T-cell differentiation, because it directly promotes RORγt, but limits Foxp3. The mechanism of HIF-1α induction has been shown to involve activation of STAT3 by IL-6, with STAT3 then binding to the HIF-1α promoter and increasing its transcription[7].

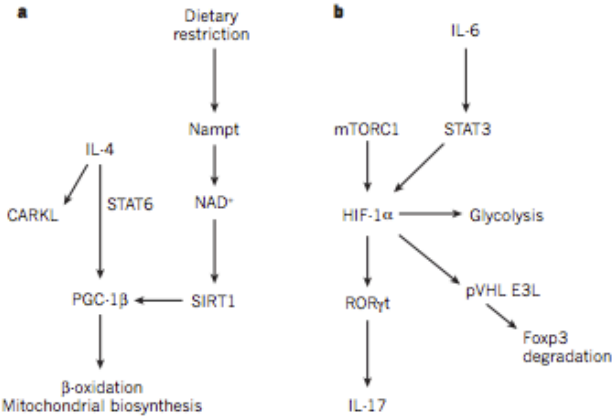

Figure 3 | Metabolic regulation of M2 macrophages and T_H17 cells. **a**, In M2 macrophages, the cytokine IL-4 acts through STAT6 to induce PGC-1β, a key transcription factor for β-oxidation in mitochondria and mitochondrial biogenesis. M2 macrophages are also activated by dietary restriction, through promotion of NAMPT which generates a key cofactor for SIRT1, NAD^+. This enzyme deactylates and activates PGC-1β. The M2 macrophage, therefore, generates mainly ATP from oxidative metabolism in mitochondria, which can use multiple carbon sources and produces less ROS compared with M1 macrophages. This reflects the role of M2 macrophages in longer term processes, such as tissue repair and antiparasitic effects. CARKL is a key driver of the M2 phenotype, and its expression is induced by IL-4. **b**, In T_H17 cell differentiation, IL-6 activates STAT3, which induces HIF-1α. mTORC1 is also involved in this process, as it stabilizes HIF-1α-encoding mRNA. HIF-1α induces RORγt, which is the key transcription factor for IL-17. HIF-1α also promotes degradation of the T_{reg} transcription factor Foxp3 through the von Hippel-Lindau E3 ligase (pVHL E3L), strengthening the phenotype of the T_H17 cell. It may also promote glycolysis, but there is currently no evidence for this.

Importantly, HIF-1α is not required to promote the glycolysis that occurs in initial T-cell activation, but influences instead the proportion of T_H17 cells relative to T_{reg} cells[8,9]. The transcription factor c-myc has been shown to be more important for glycolysis and glutamine metabolism in the initial phase of T-cell activation[11]. It is not clear why there are temporal differences in the regulation of glycolysis by these two important transcription factors.

From these studies, along with those in macrophages, we can conclude that HIF-1α promotes glycolysis and the pentose phosphate pathway through induction of the appropriate enzymes and transporters, and the function of this is perhaps to generate sufficient ATP in the face of decreased mitochondrial metabolism while promoting biosynthesis and antioxidant defence. This could be why HIF-1α plays a part in the later stages of T-cell activation, as it is activated as a result of decreased mitochondrial metabolism. In the case of macrophages, HIF-1α is also required to induce a set of pro-inflammatory genes. In the T cell, HIF-1α promotes the action of RORγt on the IL-17 promoter, but limits Foxp3 and prevents T_{reg} development. HIF-1α, therefore, has a pivotal role in both cases. However, similar to macrophages, the question arises why the more anti-inflammatory cell has a predominance of mitochondrial oxidative metabolism. Again the answer may lie partly in timing: inflammation must be activated rapidly to counter infection or deal with injury, so T_H17 cells emerge rapidly with a high glycolytic rate and the capacity to release the important pro-inflammatory cytokine IL-17. T_{reg} cells, however, will also emerge, become activated and proliferate, limiting inflammation

Table 1 | Metabolic characteristics of tumour and inflammatory cells

	Tumour cell	M1 macrophage	T_H17 cell	M2 macrophage	T_{reg} cell
Hallmark metabolic processes	Glycolysis and pentose phosphase pathway	Glycolysis and pentose phosphate pathway	Glycolysis	Oxidative phosphorylation	Oxidative phosphorylation
Key regulatory enzymes	PFKFB3 and mTORC1	PFKFB3 and mTORC1	mTORC1	PFKFB1, AMPK and sirtuins	NA
Transcription factors	HIF-1α	HIF-1α	STAT3, HIF-1α and RORγt	STAT6 and PGC-1β	Foxp3
CARKL status	Not described	Low	Not described	High	Not described
Use of citrate	Lipid biosynthesis, ROS and RNS	Lipid biosynthesis, ROS and RNS	NA	NA	NA
Overall phenotype	Tumour	Pro-inflammatory and bactericidal	Pro-inflammatory	Anti-inflammatory, tissue repair and antiparasitic	Anti-inflammatory and tissue repair

NA, not applicable; RNS, reactive nitrogen species; ROS, reactive oxygen species.

once the danger has passed, as well as promoting repair and recovery. This might require the sustained production of ATP from multiple carbon sources through oxidative phosphorylation, while limiting ROS production.

An important role for HIF-1α in inflammation can also be inferred from studies in myeloid cells. HIF-1α-deficient macrophages are attenuated in their capacity to kill bacteria, and HIF-1α is important for the production of antimicrobial peptides and granule proteases[37,38]. The transcription factor has also been shown to be crucial for controlling the inflammatory response of macrophages in skin and joints, and promoting their phagocytic activity[39]. HIF-1α-deficient mice are resistant to septic shock induced by lipopolysaccharide, and macrophages from HIF-1α-deficient mice make fewer pro-inflammatory cytokines[37] than those of wild-type mice. This is also observed in macrophages from HIF-2α-deficient mice suggesting that both isoforms of HIF are required for lipopolysaccharide responses *in vivo*, possibly acting in different cell types[40]. The overall role of HIF-1α can therefore be summarized as being required not only for glycolysis, but also in the induction of key pro-inflammatory proteins.

$CD8^+$ memory T cells

Another example of the importance of mitochondrial respiration is in T cells that carry the CD8 antigen. The establishment of a memory population of T cells is a key feature of adaptive immunity. TRAF6 is known to be required for the transition from effector to memory T cells, with TRAF6-deficient T cells being unable to switch from glycolysis to fatty-acid oxidation[41], a result that suggests that this metabolic shift is required for the formation of memory T cells. In addition, rapamycin promotes the generation of memory T cells in mice that have been infected with a virus, again probably by promoting mitochondrial metabolism[42]. Van der Windt and colleagues studied mitochondrial metabolism in effector T cells that carry the CD8 antigen and compared this to memory cells[10]. They found that the memory population had substantial spare mitochondrial capacity, which was accounted for by the cytokine IL-15, a key to memory T-cell function. IL-15 caused mitochondrial biogenesis and expression of carnitine palmitoyl transferase-1 (CPT1), a regulatory enzyme involved in mitochondrial fatty-acid oxidation. The authors propose that mitochondrial respiratory capacity regulates T-cell survival after infection. Effector T cells, such as those that carry the CD8 antigen or T_H17 cells, use mainly glycolysis to maintain growth and function. However, the attenuated mitochondrial metabolism in these cells causes them to become unstable because they are unable to generate energy from diverse substrates through oxidative phosphorylation, and thus cannot maintain viability when infection clears. However, a small number of these T cells survive because IL-15, or similar cytokines such as IL-7, promote a switch to mitochondrial biogenesis, fatty-acid oxidation and survival, with these cells then persisting as memory T cells. The cells enter a state of quiescence using mitochondrial metabolism to generate ATP until a similar infection appears, when they rapidly undergo differentiation into effector T cells to deal with the infection. The main metabolic features of tumour cells, M1 and M2 macrophages, T_H17 and T_{reg} cells are shown in Table 1.

AMPK as a key signalling pathway

Insight into the role of metabolism in inflammation is supported by recent findings concerning the role of AMPK in inflammation and immunity; these findings also provide the enticing prospect of new therapeutic approaches for inflammatory diseases. AMPK is a highly conserved protein kinase that exists in essentially all eukaryotic cells in the form of heterotrimeric complexes comprising catalytic α-subunits and regulatory β- and γ-subunits. The α-subunits contain a conserved threonine residue (Thr 172 in rat α2) for which phosphorylation by upstream kinases causes more than 100-fold activation. The primary upstream kinase that phosphorylates Thr 172 is the protein kinase LKB1 (refs 43–45). This was an exciting finding because LKB1 was previously identified as a tumour suppressor that was mutated in the inherited cancer-susceptibility disorder Peutz–Jeghers syndrome, and in many sporadic cancers[46]. LKB1 has a high basal activity and, thus, phosphorylates Thr 172 on AMPK constitutively[47]. However, binding of adenine nucleotides to the regulatory γ-subunit of AMPK causes conformational changes that modulate the phosphorylation state of Thr 172, thereby adjusting the kinase activity of AMPK according to the energy status of the cell. Binding of ATP (which signifies adequate energy status) reduces net Thr 172 phosphorylation, whereas binding of ADP or AMP (which signifies impaired energy status) enhances net Thr 172 phosphorylation, increasing the kinase activity[48–50].

Evolutionary conservation of the role of AMPK

Genes encoding the three subunits of the AMPK heterotrimer can be easily recognized in almost all eukaryotic genomes. The orthologue in the yeast *Saccharomyces cerevisiae*, the SNF1 complex, is activated by glucose starvation and is required for growth at low glucose concentrations[51,52]. Interestingly, yeast exhibits a phenomenon that is closely related to the Warburg effect, although it generates ethanol rather than lactate. When grown at high glucose concentrations, it uses glycolysis to generate ethanol (through fermentation) to make ATP, and the expression of enzymes of oxidative metabolism is repressed[53]. Conversely, when yeast runs short of glucose, growth slows and it switches from fermentation to the more energy-efficient oxidative metabolism. This adaptation involves upregulation of genes that are required for oxidative metabolism and downregulation of genes that are required for glycolysis, and a functional SNF1 complex is required for most of these changes[54]. Thus, it can be argued that the SNF1 complex exerts an anti-Warburg effect, implying that the mammalian orthologue might do the same. The overall effects of AMPK activation on mammalian metabolism are, in general, compatible with the idea that it is a tumour suppressor that promotes the oxidative metabolism that is typical of quiescent or anti-inflammatory cells, rather than the glycolytic metabolism typical of tumour cells or inflammatory cells. Once switched on, AMPK attempts to restore energy homeostasis by activating catabolic pathways that generate ATP efficiently, while switching off energy-consuming processes such as biosynthesis and cell-cycle progression[55,56] (Fig. 4). A parallel can therefore be drawn between starving yeast

cells and M2 macrophages, T_{reg} cells and memory T cells that carry the CD8 antigen, all of which are in a state characterized by oxidative metabolism, perhaps mediated partly by AMPK activation. If this is correct, AMPK-activating drugs will be anti-inflammatory, inducing what could be called a state of pseudo-starvation.

Catabolic processes that are switched on by AMPK include expression of genes involved in mitochondrial oxidative metabolism, as well as mitochondrial biogenesis itself — an effect mediated by covalent modification of PGC-1α, which is closely related to PGC-1β (ref. 57). AMPK triggers both phosphorylation[58] and deacetylation of PGC-1α, the latter catalysed by Sirt1, with the effects of AMPK on Sirt1 possibly being mediated by increases in NAD^+. AMPK activation also switches off almost all anabolic pathways, including the synthesis of lipids, polysaccharides, ribosomal RNA and proteins (Fig. 4). Finally, turning to regulation of protein synthesis, mTORC1 (which is activated by amino acid availability, as well as by the PI(3)K–Akt pathway) phosphorylates several downstream targets that activate protein synthesis. As already discussed, mTORC1 activation particularly enhances translation of 5′-TOP mRNAs[31] (which include mRNAs encoding ribosomal proteins and translation elongation factors) as well as HIF-1α. AMPK inactivates mTORC1 by two complementary mechanisms[59,60]. Thus, not only does AMPK switch off translation of ribosomal proteins (complementing its effect to switch off ribosomal RNA transcription), but it also downregulates expression of HIF-1α. Supporting the latter, HIF-1α protein expression is markedly upregulated in LKB1-deficient or AMPK-deficient mouse embryo fibroblasts, an effect sensitive to rapamycin[61]. The important role of AMPK in regulating mTORC1 in T cells was also revealed by studies of AMPK-null T cells, which displayed increased glycolysis and expression of glycolytic enzymes, elevated phosphorylation of mTORC1 targets, and release of IFN-γ in response to stimulation of the antigen receptor, with the last two effects being reversed by rapamycin[62].

AMPK as a suppressor of tumorigenesis and inflammation

AMPK activation not only causes cell-cycle arrest but also opposes most of the metabolic changes that occur in rapidly proliferating cells. It therefore seems likely that tumour cells would be under selection pressure in favour of changes that downregulate AMPK. In fact, several such changes have been reported. For example, although loss of — or mutations in — the AMPK genes themselves seem to be relatively rare in tumour cells, genetic loss in *LKB1* is frequent. The *LKB1* gene undergoes homozygous loss-of-function mutations, which are known to abolish activation of AMPK in response to energy stress[43], in around 30% of non-small-cell lung carcinomas[63] and in 20% of cervical cancers.

AMPK is activated by many existing drugs or xenobiotics, including 2-deoxyglucose; AICA riboside (also known as acadesine)[64]; metformin[65], used for treatment of type 2 diabetes; berberine[66], a natural product used in traditional Chinese medicine; and A-769662 (ref. 67), derived from a high-throughput screen for AMPK activators. These have differing mechanisms of action: AICA riboside is metabolized to the ribotide ZMP that mimics the effects of AMP on AMPK[64], A-769662 binds directly to a site on AMPK distinct from those used by adenine nucleotides, whereas the others — being

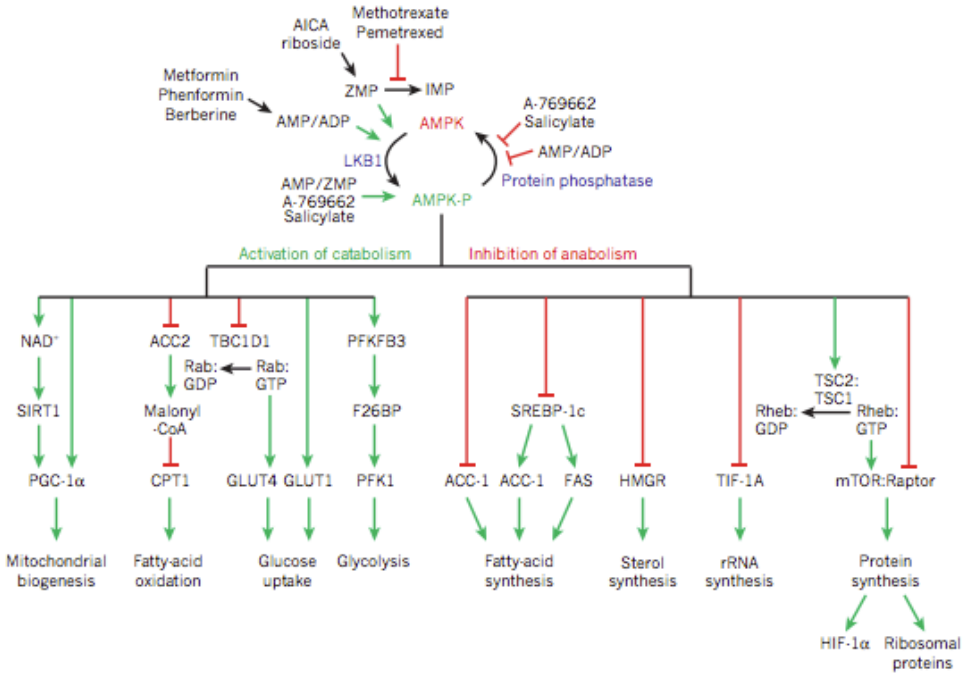

Figure 4 | Regulation of AMPK by drugs, including anti-inflammatory drugs, and the principal metabolic pathways it regulates. AMPK is activated by phosphorylation by LKB1, and inactivated by dephosphorylation by protein phosphatases. Binding of AMP or ADP (which increase in response to drugs inhibiting mitochondrial function; for example, metformin, phenformin and berberine) or ZMP (which increases in response to drugs modulating purine biosynthesis; for example, AICA riboside, methotrexate and pemetrexed) activates AMPK by promoting phosphorylation, by inhibiting dephosphorylation and by direct allosteric activation. A-769662 and the anti-inflammatory agent salicylate bind to AMPK at a site distinct from the nucleotides, causing allosteric activation and inhibition of dephosphorylation. Once activated, AMPK activates catabolic pathways (left) and inhibits anabolic pathways (right). One mechanism to explain how AMPK activation has an anti-inflammatory effects would be its promotion of mitochondrial biogenesis and fatty-acid oxidation (left), while down-regulating glycolysis by inhibiting mTORC1 (shown here as mTOR:Raptor) and hence expression of HIF-1α (right). ACC-1, acetyl-coenzyme A carboxylase-1; HMGR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; FAS, fatty acid synthase; F26BP, fructose 2,6-bisphosphate; PFK1, phosphofructokinase-1; rRNA, ribosomal RNA; SREBP-1, sterol regulatory element binding protein-1; TIF-1A, transcription intermediary factor-1A; TSC, tuberous sclerosis complex protein.

inhibitors of glycolysis (2-deoxyglucose) or the respiratory chain (metformin and berberine) — activate AMPK indirectly by increasing cellular AMP and ADP[68–71]. Intriguingly, metformin use for the treatment of diabetes is associated with a significantly reduced risk of developing cancer[72].

All of the AMPK activators mentioned have been reported to inhibit inflammatory responses in various model systems. These include inhibition by AICA riboside of iNOS induction by TNF-α, IFN-γ and lipopolysaccharide in myocytes, adipocytes or macrophages grown in culture, or by AICA riboside or metformin in muscle and adipose tissue of lipopolysaccharide-treated rats[73]; prophylactic and therapeutic effects of AICA riboside[74] and metformin[75] in experimental autoimmune encephalomyelitis — a mouse model of multiple sclerosis; inhibition by AICA riboside of lipopolysaccharide-induced NF-κB phosphorylation and TNF-α production in macrophages, differentiation of T_H1 and T_H17 cells *in vitro*, and expression of inflammatory markers in mouse models of acute and chronic colitis[76,77]; inhibition by AICA riboside or metformin production of inflammatory cytokines by primary bronchial epithelial cells from patients with or without cystic fibrosis[78]; inhibition by AICA riboside or berberine of lipopolysaccharide-induced inflammatory responses in neutrophils *in vitro*, and amelioration by AICA riboside of lipopolysaccharide-induced lung injury in mice[79]; reversal of reduced AMPK activation and fatty-acid oxidation in CD8 T cells, and of memory T-cell survival *in vivo*, in mice with defective TRAF6 (ref. 41); and inhibition by 2-deoxyglucose or AICA riboside activation, and the switch to aerobic glycolysis, of mouse dendritic cells induced by lipopolysaccharide; downregulation of AMPK using short hairpin RNA had the opposite effect[6].

It should be emphasized that AMPK activators can have 'off-target' effects, and there is evidence that AICA riboside can have anti-inflammatory effects in primary rat microglial cells that are AMPK-independent[80]. It is therefore important to back up pharmacological studies using genetic approaches. Several such studies do support the anti-inflammatory actions of AMPK: experimental autoimmune encephalomyelitis was more severe in *Ampk-α1*$^{-/-}$ mice than in controls[81]; cultured T cells isolated from spleen of *Ampk-α1*$^{-/-}$ mice secreted more IFN-γ and less IL-17 in response to antigen challenge than those from controls, suggesting that AMPK loss favours a pro-inflammatory state[81]; knockdown of the gene that encodes AMPK in macrophages, or expression of a dominant negative mutant, promoted a pro-inflammatory state[82]; and AMPK-null T cells were pro-inflammatory, hyperactivated and produced more IFN-γ than controls[62].

Relatively few studies have been carried out on the role of AMPK in the metabolism of cells of the immune system. However, many studies have suggested that AMPK downregulates NF-κB signalling through multiple mechanisms, although in most cases this was not in cells of the immune system (reviewed in ref. 83). In mouse bone-marrow dendritic cells, lipopolysaccharide caused a marked reduction of Thr 172 phosphorylation on AMPK, whereas knockdown of AMPK enhanced lipopolysaccharide-induced activation, assessed by expression of interleukin-12 p40 subunit (IL-12p40) and CD86. Administration of AICA riboside reduced expression of IL-12p40 and decreased the switch to aerobic glycolysis, whereas IL-10, an inhibitor of dendritic cell activation, reduced the effect of lipopolysaccharide on AMPK phosphorylation[6]. Glycolysis, and expression of glycolytic enzymes, is also elevated in T cells from *Ampk-α1*$^{-/-}$ mice[62], consistent with the idea that AMPK represses aerobic glycolysis. However, importantly, although the T cells from *Ampk-α1*$^{-/-}$ mice exhibited elevated glycolysis, they did not display a bias towards pro-inflammatory CD4$^+$ lineages. Enhanced glycolysis is therefore necessary, but not sufficient, for such a bias.

Interestingly, there is one situation in which AMPK activation may have a pro-Warburg effect. After induction by lipopolysaccharide, monocytes express the PFKFB3 variant of 6-phosphofructo-2-kinase. AMPK phosphorylates this protein and increases its kinase activity, thus increasing the cellular concentration of the glycolytic activator fructose-2,6-bisphosphate[84]. This mechanism may allow activated macrophages to continue to generate ATP even in hypoxic areas of infection or tissue damage, and should perhaps be viewed as an aspect of the catabolic, ATP-generating function of AMPK.

Activation of AMPK by anti-inflammatory drugs

As already described, several AMPK-activating drugs do have anti-inflammatory actions in animal models, although they are not yet used clinically for this purpose. In addition, the classic anti-inflammatory drug salicylate has recently been found to be an AMPK activator[85]. Salicylate or its derivatives, such as salicin and methyl salicylate, are produced by plants as hormones that are released in response to infection by fungal or bacterial pathogens, and trigger defence responses in uninfected tissues (they can therefore be regarded as equivalent to cytokines). Plant tissues rich in salicylates, such as willow bark, were described as medicines in the third millennium BC, and aspirin (acetylsalicylic acid) is a modern synthetic derivative. Aspirin is rapidly hydrolysed to salicylate following its absorption, with the peak plasma concentration and half-life of salicylate being much higher and longer than those of aspirin. The classic targets for aspirin are the cyclooxygenases involved in prostanoid biosynthesis, which are inhibited potently and irreversibly by aspirin, but less potently (and reversibly) by salicylate[86]. Although aspirin may be responsible for rapid antithrombotic effects owing to the inhibition of thromboxane synthesis in platelets, its more long-term anti-inflammatory effects may instead be mediated by salicylate[86]. It is also not certain that cyclooxygenases are the sole targets for the anti-inflammatory actions of aspirin.

In recent studies, salicylate has been found to activate AMPK by direct binding to the same site as the synthetic activator A-769662, causing conformational changes that both inhibited Thr 172 dephosphorylation and caused allosteric activation[85]. Only AMPK-β1 complexes are sensitive to salicylate and A-769662, and using mice with the gene encoding the β1 complex knocked out it was shown that at least one effect of these drugs *in vivo* — activation of fatty-acid oxidation — was mediated by AMPK[85]. Although the extent to which the anti-inflammatory effects of salicylate are mediated by AMPK remains unclear, the fact that increased fatty-acid oxidation is a common feature of anti-inflammatory cells suggests that this is worth investigating.

Aspirin and salicylate also inhibit the NF-κB pathway, and it has been proposed that this is due to inhibition of the upstream kinase IKK-β (ref. 87). However, inhibition of IKK-β — being competitive with ATP — is much less potent at physiological ATP concentrations[87]. This raises the possibility that inhibition of the NF-κB pathway may be mediated by AMPK activation, rather than by direct inhibition of IKK-β.

Intriguingly, methotrexate — another drug used to treat inflammatory conditions — can also activate AMPK. ZMP, which is synthesized intracellularly from AICA riboside, is an intermediate in purine nucleotide biosynthesis. ZMP is metabolized in two steps to inosine monophosphate (IMP), the first step being catalysed by an enzyme that transfers a formyl group from N10-formyltetrahydrofolate to ZMP. Methotrexate is a folate analogue that inhibits this enzyme, along with other enzymes that utilize tetrahydrofolates. Thus, methotrexate has been found to cause increased accumulation of ZMP and potentiates AMPK activation when breast cancer cells are incubated with AICA riboside[88]. In a human leukaemia cell line, another antifolate drug, pemetrexed, causes AMPK activation even in the absence of AICA riboside[89]. This raises the possibility that the anti-inflammatory effects of antifolate drugs may also be partly mediated by AMPK.

Anti-inflammatory agents may create pseudo-starvation

The ancestral role of AMPK — based on the function of the yeast orthologue — seems to have been in the response to glucose starvation; AMPK is still activated by glucose deprivation in mammalian cells, including cytotoxic T lymphocytes[90]. We have argued in this Review that activation of AMPK in cells of the immune system would promote the

switch from a pro-inflammatory to an anti-inflammatory phenotype, partly by causing a switch away from rapid glucose uptake and glycolysis towards mitochondrial oxidative metabolism, including fatty-acid oxidation. Intriguingly, the AMPK-activating drug metformin imposes this switch on cells that carry the CD4 antigen[30]. In cells that are growing or proliferating rapidly, including activated T lymphocytes, aerobic glycolysis allows the use of the TCA cycle to provide precursors for biosynthesis, rather than purely for ATP production. A reduced reliance on the TCA cycle and the respiratory chain for ATP production also allows mitochondria to generate ROS, which are used in antibacterial defence, and to accumulate TCA-cycle intermediates such as citrate that may have a signalling role in inflammation. However, the downside of increased use of glycolysis and the pentose phosphate pathway by inflammatory cells is that they will have a greatly increased demand for glucose. Accelerated glucose use by immune cells is a luxury that may not be possible when the individual is in a state of starvation, helping to explain why starvation depresses the immune system, although glucocorticoids undoubtedly also have a role here.

In an over-nourished state, such as during obesity, there may be a shift towards pro-inflammatory states of immune cells, whereas during dietary restriction the balance may shift towards anti-inflammatory phenotypes instead. Interestingly, in the nematode *Caenorhabditis elegans*, the orthologues of Sirt1 and AMPK, both of which seem to promote anti-inflammatory states in mammals, are required for extension of lifespan in response to dietary restriction[91,92]. The plant polyphenol resveratrol, which extends lifespan in *C. elegans* and in mice that are fed a high-fat diet, has also been reported to activate both Sirt1 and AMPK, although which of the two is the primary target remains controversial[93]. As already discussed, at least two other existing drugs used as anti-inflammatory agents (salicylate and methotrexate) also activate AMPK. Finally, rapamycin can also create a pseudo-starvation state, promoting fatty-acid oxidation and generating T_{reg} cells by inhibiting mTORC1, and inhibiting dendritic cell activation by lipopolysaccharide. It is therefore possible that many anti-inflammatory agents work, in part, by hijacking a system that decreases inflammation in the face of nutrient deprivation. Given the similarities in the metabolism of tumour cells and pro-inflammatory cells, it is not surprising that the same agents — many of which are AMPK activators — should have both antitumour and anti-inflammatory effects. In this context, there is epidemiological evidence that patients who take aspirin long term seem to have some protection against cancer[94]. It is also conceivable that some of the protection against cancer in people with type 2 diabetes provided by the AMPK-activating drug metformin[95] could be due to systemic effects on inflammation.

In addition to glucocorticoids, fasting and starvation in humans cause changes in the circulating levels of many other hormones or adipokines. Interestingly, many of the hormones associated with an overfed state (such as insulin and leptin) inhibit AMPK[96], whereas those associated with an under-fed state (such as ghrelin and adiponectin) activate AMPK[97]. Thus, it seems appropriate that this ancient starvation signalling pathway should have a role in the depression of inflammation, a bodily response that is very expensive in terms of glucose consumption.

Final perspectives

Metabolism has recently returned with a vengeance to become a hot topic in biomedical research. Insight into the changes in metabolism that occur in metabolic diseases, cancer and inflammation may allow us to understand more about disease pathogenesis and point the way to new therapies. Multiple pathways and processes have been uncovered that might lend themselves to therapeutic manipulation. The mechanistic overlap in the metabolic changes that are evident in cancer and inflammation could indicate that some of the targets they have in common (for example, AMPK, mTORC1 or HIF-1α) could be useful in treatment of both disorders. Finally, a reconfiguring of our understanding of metabolism is needed to provide new approaches. Both tumour cells and inflammatory cells are often viewed as acting in a cell-autonomous manner that ignores the needs of the whole organism. However, a key feature that emerged from studies during the heyday of metabolism research was that metabolism is a closely integrated system, involving many hormones that coordinate functions between different tissues. Perhaps the insulin resistance that occurs during inflammatory conditions reflects one aspect of this, as it would reduce glucose uptake by tissues such as muscle, liver and adipocytes, conserving glucose for use by macrophages and T_H17 cells instead. On the other hand, chronic, uncontrolled inflammation or cancer may deplete other cells and tissues of nutrients, promoting cachexia. Perhaps this is why chronic inflammation is so damaging, and shortens lifespan in the same way as cancer. It is also revealing that inflamed tissues, such as the rheumatoid synovium, can be visualized with [^{18}F]-fluorodeoxyglucose[98], reflecting their increased glucose uptake in the same way as tumours. This might help to explain why inflammation lies at the heart of so many diseases — it puts the whole body under metabolic stress, driving symptoms and causing morbidity. The prospect of targeting the altered metabolism in inflammation, as well as in cancer, holds substantial therapeutic promise. ■

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