Minireview

Endogenous drivers of altered immune cell metabolism

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Impact Statement

Metabolic reprogramming of immune cells has emerged as a central mechanism governing the control of cell fate and function. Furthermore, the finding that dysregulated immunometabolism is a feature of many inflammatory and autoimmune disorders has opened up new therapeutic possibilities. Our knowledge of this area is continuing to grow, and in this review, we focus on damage or disease–associated molecular patterns (DAMPs), which are now coming to the fore as drivers of altered immune cell metabolism. This adds to a growing body of evidence highlighting the dynamic cross-talk between metabolic process and immune effector mechanisms. Furthermore, it underscores a new facet of DAMPs and expands our knowledge of how they contribute to sterile inflammatory disease.

Abstract

Dysregulated metabolism has long been recognized as a feature of many metabolic disorders. However, recent studies demonstrating that metabolic reprogramming occurs in immune cells have led to a growing interest in the relationship between metabolic rewiring and immune-mediated disease pathogeneses. It is clear now that immune cell subsets engage in different metabolic pathways depending on their activation and/or maturation state. As a result, it may be possible to modulate metabolic reprogramming for clinical benefit. In this review, we provide an overview of immune cell metabolism with focus on endogenous drivers of metabolic reprogramming given their link to a number of immune-mediated disorders.

Keywords: Immunometabolism, cellular respiration, metabolic reprogramming, damage or disease-associated molecular patterns

Experimental Biology and Medicine **2022; 247: 2192–2200. DOI: 10.1177/15353702221134093**

Introduction

Cellular respiration and fatty acid metabolism

Cellular respiration is a multistep, catabolic process, typically utilizing glucose as an energy source to drive the generation of adenosine triphosphate (ATP). Glucose first passes through glycolysis, which takes place in the cytosol of the cell. The first step in this pathway is catalyzed by the rate-limiting enzyme hexokinase 2 (HK2), which irreversibly phosphorylates glucose, resulting in the generation of glucose-6-phosphate. Glucose-6-phosphate is isomerized to fructose-6-phosphate, which is then converted to fructose-1,6-bisphosphate by the enzyme phosphofructokinase, another key regulatory enzyme catalyzing an irreversible step in this pathway. Fructose-1,6-biphosphate is unstable, and is split into two sugars: glyceraldehyde-3-phosphate, which continues through the remaining steps in the glycolytic pathway, and dihydroxyacetone phosphate, which cannot continue through glycolysis unless it is first converted to glyceraldehyde-3-phosphate. The remaining steps in glycolysis are reversible, until the final step, which is catalyzed by pyruvate kinase. Under normoxic conditions, pyruvate is generated which passes further through the respiratory chain. However, glycolysis can also occur in the absence of oxygen (anaerobic glycolysis), resulting in the generation of lactate. Although glycolysis is a relatively fast process, it is relatively inefficient in terms of energy production, resulting in the generation of only two ATP molecules. Despite this low energy yield, it is the primary pathway for the generation of many intermediates that are utilized to produce nucleotides, fatty acids, and amino acids.

In order to yield additional energy, pyruvate is oxidized to acetyl-CoA, which then enters the tricarboxylic acid (TCA) cycle inside the mitochondria. The TCA cycle (also

known as the Krebs cycle) is more efficient in terms of ATP production compared to glycolysis, and occurs in conjunction with oxidative phosphorylation in cells that are in a quiescent or non-proliferative state. Acetyl-CoA is first oxidized to CO_2, leading to the production of the intermediate metabolites, citrate and isocitrate. As isocitrate converts to α-ketoglutarate, CO_2 is produced and nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH. α-ketoglutarate is converted to succinyl CoA by α-ketoglutarate dehydrogenase, and ATP is generated upon conversion of succinyl-CoA to succinate, which is then further converted to fumarate via the action of succinate dehydrogenase. Flavin adenine dinucleotide (FAD) is reduced to $FADH_2$ during this reaction, and fumarate is subsequently converted to malate. Finally, malate is converted to oxaloacetate (which is again accompanied by the reduction of NAD^+ to $NADH_2$), with oxaloacetate condensing with acetyl-CoA to form citrate, hence the term TCA "cycle." When intact, this fuels the process of oxidative phosphorylation (OXPHOS), whereby NADH and FADH function as sources of electrons that contribute to energy generation via a proton gradient set up by the electron transport chain that, in turn, powers ATP synthase. The electron transport chain is located at the inner mitochondrial membrane and consists of a series of complexes (I–V) and two electron carriers (coenzyme Q and cytochrome c). NADH donates electrons to complex I while $FADH_2$ donates electrons to complex II. This process powers the pumping of protons from the matrix into the intermembrane space and an electrochemical gradient is established, termed the "proton motive force." This then powers the final complex, ATP synthase, which operates by combining adenosine di-phosphate (ADP) and inorganic phosphate (Pi), resulting in the generation of ATP. This process is generally slower than glycolysis; however, the yield of ATP (32 molecules) is much higher.

Fatty acid metabolism consists of two pathways: fatty acid synthesis (FAS) and fatty acid oxidation (FAO). FAS, an anabolic process, generates fatty acids which act as precursors for other compounds. FAS takes place in the cytosol of the cell, and begins when acetyl-CoA exits the TCA cycle in the mitochondria and enters the cytosol, where it is carboxylated by acetyl-CoA carboxylase (ACC) to form malonyl-CoA. This is the first committed step in this pathway. Malonyl-CoA then continues through the remaining steps in FAS, which are catalyzed by enzymes known as fatty acid synthases (FASN). This results in the production of a 16-carbon palmitic acid, which can act as a building block for other compounds including phospholipids and hormones. However, in the catabolic process of FAO, or beta-oxidation as it is also known, fatty acids are broken down to generate energy. Fatty acids enter the cell and are first converted to acyl-CoA in order to be able to enter the mitochondria. Acyl-CoA enters the mitochondria through the carnitine shuttle, the first step of which is catalyzed by carnitine palmitoyltransferase 1 (CPT1), where acyl-CoA is temporarily transferred to carnitine. This acyl-carnitine is shuttled into the mitochondria while carnitine is shuttled out, before being converted back to acyl-CoA once inside. The acyl-CoA then continues through the four steps of FAO, resulting in the generation of acetyl-CoA, which can then enter the TCA cycle.

Regulation of cellular respiration

Engagement of cellular respiration is controlled in a number of ways, including regulation through feedback mechanisms by the enzymes and products involved in this process. Mammalian target of rapamycin (mTOR), which forms part of the mTORC1 complex, functions as a cellular energy and nutrient sensor and controls protein synthesis and other cellular processes including anabolic pathways, such as glycolysis. Activation of mTORC1 results in the induction of a number of glycolytic genes, including the glucose transporter, GLUT1, which transports glucose into the cell, and HK2, the enzyme crucial for the phosphorylation of glucose in the first step of glycolysis.[1] These glycolytic genes are upregulated by the mTORC1 pathway upon activation of the transcription factor hypoxia-inducible factor 1 (HIF1α).[1] mTOR also promotes FAS through activation of sterol regulatory element binding protein (SREBP), which activates the enzyme ACC. This results in the carboxylation of acetyl-CoA to malonyl-CoA. When malonyl-CoA becomes committed to FAS, FAO is subsequently inhibited, occurring as a result of ACC-mediated inhibition of the FAO-associated enzyme, CPT. The block on FAO can be lifted following inhibition of FAS by 5′ adenosine monophosphate–activated protein kinase (AMPK). This enzyme inhibits FAS in two ways: first by directly inhibiting mTOR activation, and second by phosphorylating and inactivating the FAS-associated enzyme, ACC (Figure 1).[2]

Immune cell metabolism

In 1956, Otto Warburg first observed that some cancer cells increase glucose uptake and glycolysis, despite the presence of oxygen.[3] Referred to as the "Warburg effect," this phenomenon has now been observed in other cell types, including activated immune cells where energy demands are increased. For example, despite the TCA cycle and oxidative phosphorylation generally remaining functional in activated dendritic cells (DCs) and macrophages, glycolytic rate is increased concomitant with a decrease in mitochondrial energy generation.[4,5] Many effector T cell subsets – including $CD8^+$ T cells, Th1, Th2, and Th17 cells – alter their metabolism in a similar manner to the innate immune cells specified above.[6] In the case of pro-inflammatory classically activated (M1) macrophages and activated DC, the TCA cycle has been shown to be broken in two places, once after isocitrate dehydrogenase which can be inhibited by a build-up of NADH, while a second break occurs upon inhibition of succinate dehydrogenase following accumulation of intermediate metabolites such as succinate and itaconate. Notably, the accumulation of these metabolites can also lead to stabilization of HIF1α, which can drive the production of pro-inflammatory cytokines, including interleukin (IL)-1β.[7–9] An impaired TCA cycle also results in the accumulation of citrate, which can feed into the process of FAS. Increased FAS is crucial for the activation of M1 macrophages and DC, and is elevated upon stimulation of pattern recognition receptors (PRRs) in both cell types.[9,10] However, FAO is more prominent in anti-inflammatory, alternatively activated (M2) macrophages, and contributes to reduced production of inflammatory cytokines by inflammatory macrophages. (It should be noted that a spectrum of intermediary macrophage subtypes exist

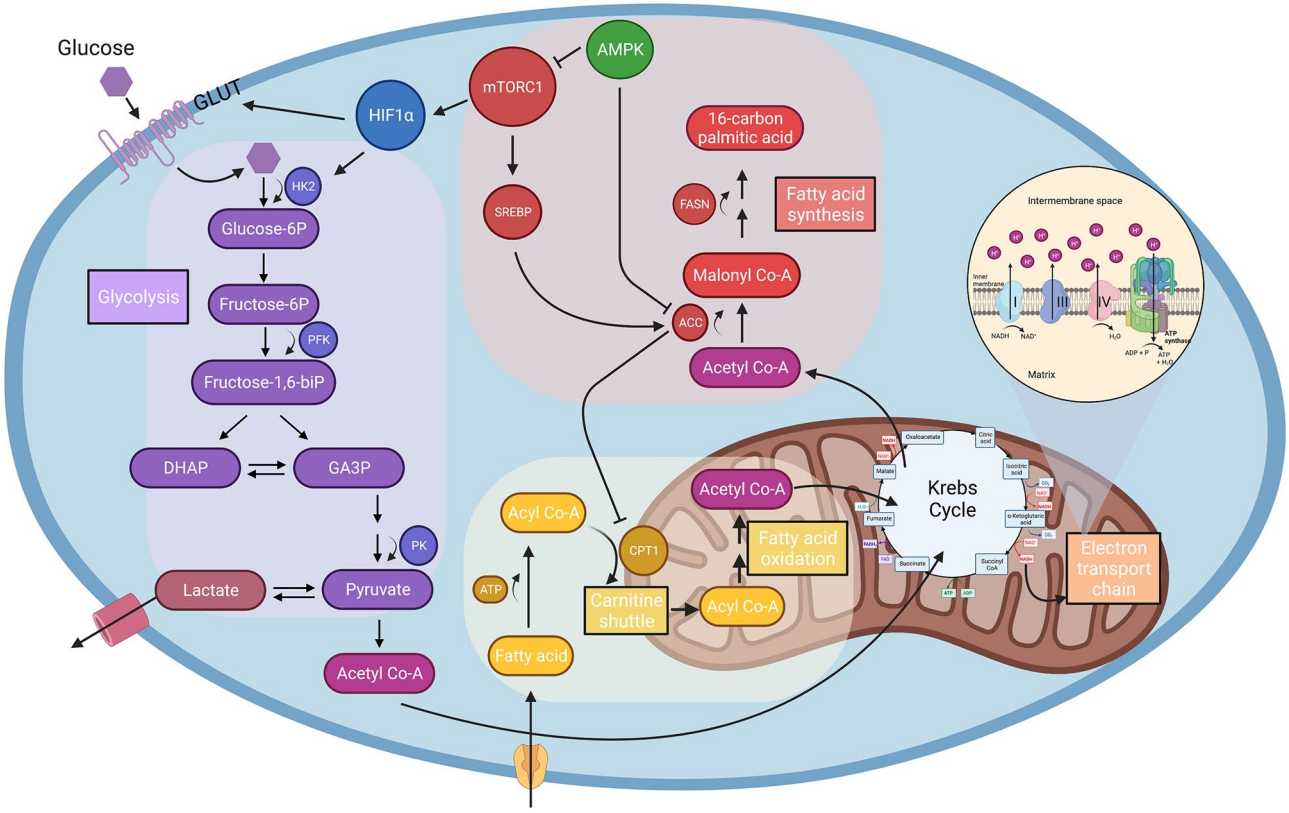

Figure 1. Cellular respiration and fatty acid metabolism. Cells engage a range of metabolic pathways depending on their needs. During glycolysis, glucose is first phosphorylated by hexokinase 2 (HK2) to generate glucose-6-phosphate (glucose-6P). Glucose-6P is isomerized to fructose-6-phosphate (fructose-6P), which is converted to fructose-1,6-biphosphate (fructose-1,6-biP) by phosphofructokinase (PFK). This is split into two: dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GA3P). GA3P continues through the remaining steps of glycolysis and is finally converted to pyruvate by pyruvate kinase (PK). Pyruvate can be exported from the cell as lactate under anaerobic conditions. In the presence of oxygen, pyruvate is converted to acetyl-CoA which enters the TCA cycle. NADH from the TCA cycle passes into the electron transport chain, completing oxidative phosphorylation and generating energy through a proton gradient. Acetyl-CoA can then enter the fatty acid synthesis pathway, where it is carboxylated by acetyl-CoA carboxylase (ACC) to form malonyl-CoA. Malonyl-CoA then passes through the remaining steps of this pathway, which are catalyzed by fatty acid synthases (FASN) resulting in 16-carbon palmitic acid. The fatty acid synthesis pathway is activated by mTORC1. mTORC1 is also responsible for the activation of HIF1α, which in turn can activate glycolysis by upregulating GLUT1 and HK2. Inhibition of fatty acid synthesis by AMPK results in activation of the fatty acid oxidation pathway, by lifting the inhibition placed on the enzyme carnitine palmitoyltransferase 1 (CPT1). Fatty acids first enter the cell and are converted to acyl-CoA, which then enters the carnitine shuttle to translocate into the mitochondria. Acyl-CoA then continues through the fatty acid oxidation pathway, generating acetyl-CoA, which can then enter the TCA cycle. (A color version of this figure is available in the online journal.)

in vivo and extensive metabolic profiling of these individual subtypes has not yet been carried out.).[11,12] The balance of fatty acid metabolism is also of particular importance for T cell responses and their effector function. Tregs exhibit increased levels of FAO and increased expression of the genes involved in this process, including CPT1A, while effector T cells downregulate FAO during activation.[13,14] Furthermore, effector T cells, and in particular Th17 cells, exhibit increased FAS (Figure 2).[15] This difference in T cell metabolism could be of particular importance for diseases in which the Th17/Treg balance is skewed.

While less well established, *in vitro* studies have revealed that B cells also undergo changes in their metabolic state upon activation. For example, B-cell receptor (BCR)–activated B cells upregulate GLUT1, increase glycolysis, and activate mTOR in a protein kinase Cβ (PKCβ)–dependent manner.[16] When B cells are fully activated upon engagement of co-stimulatory molecules such as CD40, glucose and glutamine uptake is further enhanced to support proliferation.[17] A link has also been established between antibody production by plasma cells (PCs) and energy metabolism, whereby long-lived PCs have been shown to exhibit enhanced glucose uptake compared to their shorter-lived counterparts. This is primarily utilized to glycosylate antibodies, although this can be diverted to glycolysis if energy demands are low.[18] Less is known regarding energy metabolism in memory B cells, but a recent study has demonstrated that, compared to naïve B cells, human memory B cells of the IgD isotype exhibit enhanced glycolysis and mTORC activation upon stimulation with interferon alpha (IFN-α) and Toll-like receptor (TLR) ligands.[19] They also differentiate into PCs to a greater extent, and it has been suggested that longevity and antibody secretion are regulated by metabolic pathways. In light of these findings, altered B cell metabolism should also be considered in the context of immune-mediated disease pathogenesis, as is currently the case for dysregulated T cell, DC, and macrophage metabolism.

Endogenous drivers of metabolic reprogramming

Cytokines

Cytokines can differentially alter immune cell metabolism.[20] For example, macrophages exhibit an increase in glycolysis in response to recombinant IL-1 treatment.[21] Tumor necrosis

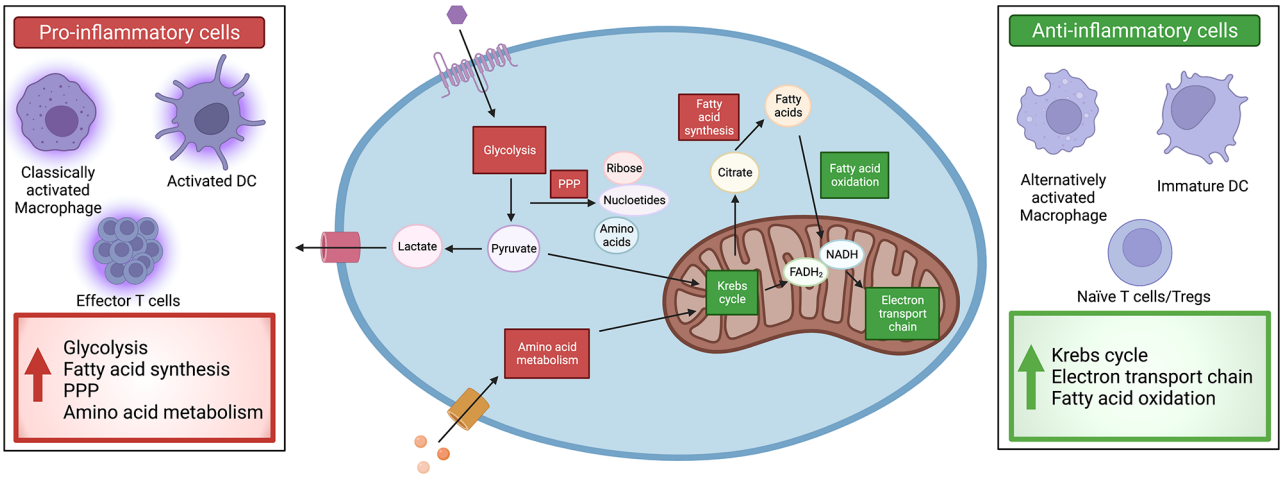

Figure 2. Major metabolic pathways engaged in immune cells. The metabolic pathways engaged by an immune cell depend on its activation state, maturation state, or specific effector subset. The pathways shown in red: glycolysis, fatty acid synthesis, amino acid metabolism, and the pentose phosphate pathway (PPP) are generally associated with activated and effector immune cells. Some examples of these cells include pro-inflammatory macrophages, activated DC, and specific effector T cell subsets. The pathways shown in green: TCA cycle and the electron transport chain (together comprising oxidative phosphorylation) and fatty acid oxidation are generally associated with naive, immature, or regulatory immune cells. Some examples of these cells include alternatively activated macrophages, immature DC, and naïve or regulatory T cells (Tregs). (A color version of this figure is available in the online journal.)

factor (TNF) has also has been linked to metabolic reprogramming via upregulation of the mitochondrial citrate carrier (CIC), leading to accumulation of citrate in the cytosol of inflammatory M1 macrophages, and is capable of upregulating ATP-citrate lyase (ACLY) which catalyses the conversion of glucose-derived citrate to acetyl-CoA.[22,23] TNF has also been shown to promote a potential glycolytic shift in human $CD4^+$ T cells, as well as human fibroblast–like synoviocytes.[24,25] In the case of the latter, this was accompanied by increased expression of inflammatory rheumatoid arthritis (RA) biomarkers, while in a clinical setting, treatment with anti-TNF significantly reduces glycolytic activity in the RA synovium.[26] This is coupled with a downregulation of the glucose transporter, GLUT1, and the glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH),[26] further bolstering the evidence for TNF-α as driver of metabolic reprogramming in disease pathogenesis.

Blockade of IL-6 signaling appears to have a similar effect in RA, particularly in leukocytes, where inhibition of the IL-6 receptor results in reduced oxidative stress and inflammation.[27] A further example of IL-6-mediated metabolic reprogramming has been observed in $CD8^+$ T cells, which undergo IL-6-dependent metabolic rewiring to allow for pro-inflammatory cytokine production and proliferation in response to lipopolysaccharide (LPS).[28] The downstream modulation of metabolism by pro-inflammatory cytokines may be attributed to activation of the nuclear factor-κB (NF-κB) pathway, which is now understood to hold a critical role in shaping the inflammatory response via metabolic rewiring.[29] In the presence of varying stimuli, including TNF or IL-1, the IkappaB kinase (IKK) complex becomes activated, resulting in the release of NF-κB, which subsequently translocates to the nucleus and promotes transcription of HIF1α. As mentioned above, HIF1α promotes glycolysis via upregulation of glycolytic genes such and HK2 and GLUT1. NF-κB is also capable of activating mTOR, which further stabilizes HIF1α, sustaining its transcriptional activity.

However, IL-10, which is considered an anti-inflammatory cytokine, limits glucose uptake and glycolysis in LPS-treated macrophages.[30] Furthermore, IL-10 has been shown to protect mitochondrial function and eliminate dysfunctional mitochondria through the upregulation of mitophagy in the cell. This response appears to be regulated through inhibition of mTOR activity, as mice lacking the IL-10 receptor demonstrate sustained mTOR activation, and consequential impaired mitochondrial activity. IL-4 signaling has also been linked to mTOR activity, via its interactions with the mTOR complexes, mTORC1 and mTORC2, which in turn modulate glycolytic activity and FAO in IL-4-treated macrophages.[31]

Nitric oxide

Nitric oxide (NO) plays a role in a plethora of biological functions, and is most notably generated by activated myeloid cells in response to inflammatory stimuli. NO synthases (NOSs) convert L-arginine and oxygen (O_2) to NO and L-citrulline, and NO is well documented as being produced at nanomolar concentrations under normal physiological conditions.[32] In the context of the immune response, however, NO functions at higher concentrations over time to kill infectious agents and plays a role in immunoregulation. There is a growing body of evidence to show that NO can do this through modulation of cellular metabolism, and furthermore, it is known that NO and NO-derivatives can act to reprogram macrophage cell function.[33]

NO has been shown to exert effects through interaction with metal ions found in proteins, notably associating with heme centers such as those found in cytochrome c oxidase, that is, complex IV of the mitochondrial electron transport chain. NO works in concert with inversely correlated O_2 levels to exert an inhibitory effect on complex IV, and therefore inhibit oxidative phosphorylation. Indeed, it has been shown that inhibition or ablation of inducible NO synthase (iNOS) in myeloid cells can rescue ATP-linked mitochondrial

respiration.[34,35] Early evidence also demonstrated that when NO levels are high, O_2 cannot be sufficiently utilized by the cell in a state termed "metabolic hypoxia." In the case of pro-inflammatory macrophage polarization, macrophages increase their utilization of the pentose phosphate pathway and consequent generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Generation of the latter is, in part, mediated through iNOS to exert antimicrobial effects.[36,37] One notable study investigated the effect of NO on inflammatory macrophage metabolic reprogramming using NO-deficient macrophages, and demonstrated that NO modulates expression of the subunits forming complex I of the mitochondrial electron transport chain. Furthermore, it was shown that NO-deficient macrophages differentially modulate levels of important TCA cycle intermediates including succinate, citrate, and itaconate.[34] Interestingly, NO depletion resulted in enhanced IL-1β levels which may be attributed to the fact that NO can act to inhibit formation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome through nitrosation.[38] It is worth noting that these studies were carried out in murine cells which differ from human cells, given that human macrophages do not exhibit such a potent NO response; hence, it will be of interest to further explore how NO-induced metabolic reprogramming impacts on human disease.

DAMPs as drivers of altered immune cell metabolism

There is no doubt that pathogen-associated molecules (PAMPs) can alter immune cell metabolism, and this has been reviewed in detail elsewhere.[33,39] As outlined above, cytokines themselves and the hypoxic environment associated with diseased tissues can lead to metabolic reprogramming of immune cells; however, there is emerging evidence that disease or damage–associated molecular patterns (DAMPs) – also referred to as alarmins – can also contribute to alterations in immune cell metabolism. This is perhaps not so surprising, given that many DAMPs bind to the same receptors as PAMPs and stimulate immune effector responses akin to those generated during microbial infection. For example, many particulates including beta amyloid (Aβ) fibrils and cholesterol crystals activate the NLRP3 inflammasome, leading to the production of mature IL-1β, while other DAMPs such as HMGB1, S100 proteins, and oxLDL have been shown to activate members of the TLR family to drive inflammatory cytokine and chemokine production.[40–43] Hence, DAMPs themselves, and the pathways they activate, have become attractive therapeutic targets. Research into DAMP-induced metabolic changes is very much in its infancy; however, there have been recent studies highlighting the impact of certain DAMPs on macrophage and microglia metabolic phenotype as outlined below.

Aβ alters microglia metabolism

Although the brain primarily utilizes glucose to meet its energy demands, in disease states, it has been shown to shift toward consumption of other substances. In the aging brain, there is evidence of impaired glucose metabolism that is exacerbated under conditions of neuroinflammatory or neurodegenerative disease.[44,45] In the case of Alzheimer's disease (AD), primary focus has been on neurons and astrocytes as contributors to disease progression; however, metabolic reprogramming of microglia is receiving considerable attention, given the chronic inflammation and altered glucose metabolism that serve as hallmarks of this disease.[46] In a non-activated state, microglia rely primarily on OXPHOS and switch to favor glycolysis upon activation.[47] In early AD, this shift in metabolism toward aerobic glycolysis may facilitate Aβ clearance.[48] However, chronically activated or dysfunctional microglia can contribute to disease progression, with direct links shown between activated microglia and synapse loss.[49] Indeed, recent work has implicated Aβ itself as a driver of microglial metabolic reprogramming, and there is evidence of metabolic rewiring from OXPHOS to aerobic glycolysis following Aβ stimulation of primary microglia.[48] Furthermore, it has been demonstrated that inhibition of glycolysis using the glucose analogue, 2-deoxy-D-glucose (2-DG), leads to decreased oxidative stress and a reduction in Aβ oligomers in the brains of transgenic AD mice.[50] On a functional level, metabolic reprogramming of microglia has been directly linked to Aβ-induced-phosphorylation of mTOR and consequential activation of the mTOR-HIF1α-pathway, which facilitates the switch from OXPHOS to glycolysis.[48] In support of this, inhibition of the mTOR pathway was shown to reduce Aβ-induced microglial inflammation in primary microglia, as demonstrated by reduced expression of pro-inflammatory cytokines IL-1β and TNF-α.[48] A reduction in activation markers has also been observed in LPS-activated microglia following treatment with 2-DG.[51]

It is worth noting also that Aβ can activate the NLRP3 inflammasome, and recent studies have highlighted a link between glycolysis and inflammasome activation, with inhibition of glycolysis using aminooxyacetic acid (an inhibitor of aspartate aminotransferase) or 2-DG resulting in attenuation of NLRP3 inflammasome activation in activated murine macrophages, and in a murine model of acute lung injury, respectively.[52,53] Furthermore, HK activity was shown to be essential for NLRP3 inflammasome activation in macrophages, as demonstrated by short hairpin RNA (shRNA) knockdown of HK1 in murine peritoneal macrophages, which resulted in decreased inflammasome-dependent IL-1β and IL-18 secretion.[54] HK2 has been shown to be upregulated in microglia favoring glycolysis under inflammatory conditions;[55] it will therefore be of interest to determine if Aβ-induced inflammasome activation is also dependent on HK activity in this cell type. The ketone body metabolite, β-hydroxybutyrate (BHB), was also shown to reduce macrophage activation and inhibit NLRP3 inflammasome activation in murine models of NLRP3-mediated disease.[56] Moreover, BHB reduced Aβ-induced mitochondrial dysfunction and ROS formation in primary cortical neurons, and further restored mitochondrial complex I activity when administered peripherally in a murine AD model. This effect was accompanied by a reduction in Aβ levels.[57] It might therefore be worth considering modulation of ketogenic metabolism, in addition to glucose metabolism, as a potential therapeutic avenue to attenuate Aβ- and other inflammasome-associated diseases.

Crystalline particulates alter immune cell metabolism

Dysregulated metabolism has been observed in several forms of joint disease including RA, osteoarthritis (OA), and gout.[26,58,59] The hypoxic environment of the RA joint promotes such alternations, where metabolites such as lactate and citrate can act on synovial fibroblasts and macrophages to enhance glycolysis, FAS, and inflammatory mediator production.[60] In OA and gout, crystalline particulates have been shown to directly impact on monocyte/macrophage metabolism. For example, in the case of gouty arthritis, insoluble monosodium urate (MSU) crystals were shown to enhance glucose uptake by driving increased GLUT1 expression in human macrophages and neutrophils, while treatment with either a GLUT1 inhibitor or 2-DG suppressed crystal-induced NLRP3 activation and IL-1β production in an *in vivo* model of MSU-induced inflammation. Peripheral blood mononuclear cell (PBMC) and monocytes stimulated with MSU crystals also exhibited enhanced mTOR activity, while mTOR pathway gene expression was found to be elevated in monocytes from gout patients versus healthy controls.[61] Furthermore, MSU crystals were recently shown to induce a metabolic-inflammatory transcriptional profile that is distinct from that promoted by the well-studied PAMP, LPS, and is dependent on activation of the mitogen-activated protein (MAP) kinase, c-Jun N-terminal kinase (JNK). Interestingly, despite MSU crystals being considered potent activators of the NLRP3 inflammasome, this study found no evidence that the inflammasome is required for the upregulation of metabolic genes by these particulates.[62] The ketone body, BHB, was, however, found to inhibit MSU-induced NLRP3 inflammasome formation, as has been shown for Aβ-dependent inflammasome activation.[63]

OA-associated basic calcium phosphate (BCP) crystals were also recently shown to directly promote M1 polarization and metabolic reprogramming in primary human macrophages, providing further evidence that targeting altered metabolism may serve to limit the inflammation associated with certain crystal arthropathies.[64] In this case, the BCP-mediated polarization of macrophages toward an inflammatory phenotype was prevented upon inhibition of glycolysis, as was the production of the key OA-associated DAMP, S100A8.[64] Indeed, it is worth noting that such findings extend beyond the joint, and we recently demonstrated that atherosclerosis-associated cholesterol crystals can alter macrophage bioenergetics by increasing the glycolytic profile of the cell.[65] This *in vitro* effect was dependent on the glycolytic enzyme, pyruvate kinase isoenzyme type M2 (PKM2), and supports a recent *in vivo* study demonstrating that inhibition of PKM2 nuclear translocation reduces glycolytic rate while also suppressing atherosclerosis and inflammation in $Ldlr^{-/-}$ mice.[66] Taken together, these studies reveal potential new targets to limit inflammation and macrophage polarization by modulating the metabolic state of pathogenic immune cells.

Immunometabolism as a therapeutic target

Given that the metabolism of immune cells plays an important role in their activation status and/or effector function, manipulation of metabolic pathways hold great potential as a therapeutic approach to treat many diseases characterized by dysregulated inflammation. Indeed, some autoimmune diseases show specific alterations to the metabolism of the immune cells involved. For example, in systemic lupus erythematosus (SLE), T cells exhibit persistent mitochondrial hyperpolarization (an early step in T cell activation), as well as ATP depletion,[67] while in RA, $CD4^+$ T cell metabolism is characterized by a reduced utilization of glycolysis and an increase in the amount of glucose passing through the pentose phosphate pathway.[68] Similarly, T cells from patients with MS exhibit dampened glycolysis and mitochondrial respiration, which is restored to levels comparable to healthy subjects upon treatment with IFN-β, a first-line treatment for MS.[69]

Comparable effects were also observed in T cells from patients with relapsing-remitting multiple sclerosis (RRMS) upon treatment with dimethyl fumarate (DMF), an oral, first-line RRMS treatment.[70] DMF is a citric acid cycle intermediate and is known to exhibit immunomodulatory effects through activation of the redox-sensitive transcription factor, nuclear factor erythroid 2–related factor 2 (NRF2), and further anti-inflammatory effects via inhibition of NF-κB.[70] The mechanism of action of DMF is complex; however, a recent study involving peripheral cells from RRMS patients demonstrated that the DMF increases monocyte ROS generation and epigenetic methylation changes in monocytes prior to exerting epigenetic changes in $CD4^+$ T cells.[71] While ROS production is generally associated with oxidative damage, there is evidence to show that myeloid-derived ROS can limit T cell activity *in vitro*.[71] Further study is required to uncover the complexity of DMF's mechanism of action, and effects are likely to be dependent on multiple factors including disease stage and immune cell phenotype.

In terms of targeting specific metabolites, effector T cells convert pyruvate to lactate, while Tregs utilize the enzyme pyruvate dehydrogenase (PDH) to convert pyruvate to acetyl-CoA to continue further through the TCA cycle. This is controlled by the enzyme pyruvate dehydrogenase kinase (PDHK), which inhibits PDH in effector T cells, thereby preventing them from converting pyruvate to acetyl-CoA. Inhibition of PDHK overcomes this effect and could therefore be used as a potential therapy to skew the metabolic pathways engaged in T cells by enhancing processes associated with regulatory T cells. This has shown promise in a number of animal models of disease, including experimental autoimmune encephalomyelitis (EAE).[14] Similarly, blockade of mTOR activity in the EAE model results in increased Tregs and an amelioration of disease progression.[72] Inhibition of ACC, a key enzyme involved in FAS downstream of mTOR, also increased Treg numbers while decreasing IL-17 and IFN-γ producing T cells in the EAE model. This was accompanied by reduced disease pathology.[15] Inhibition of mTORC1 with rapamycin also inhibits Th17 cells and promotes Tregs in patients with SLE.[73] Furthermore, metformin, a drug traditionally used to treat type II diabetes, is now showing potential as a treatment for autoimmune diseases by altering the metabolic state of immune cells. One of the established mechanisms of action for metformin is through

activation of AMPK, which in turn inhibits mTOR.[74,75] This likely accounts for some of the metabolism-altering functions it carries out, although multiple mechanisms cannot be ruled out. In the EAE model, metformin treatment was shown to decrease pro-inflammatory cytokines, increase Treg numbers, and slow disease progression.[76] Th17-mediated inflammation was also reduced by metformin treatment in animal models of RA and colitis, and again, effects were dependent on AMPK and mTOR signaling.[77,78] These studies provide further evidence that modulating metabolic pathways engaged by immune cells may have therapeutic potential for the treatment of autoimmune/inflammatory conditions. Further detailed evaluation of immunometabolism-based therapies can be found elsewhere.[79,80]

Conclusions

There a now wide ranging studies confirming that when immune cells are activated, they ramp up glycolysis as a requirement for many of their effector functions, including upregulation of co-stimulatory markers and cytokine production.[4,81,82] When glycolysis is blocked, these processes are impaired, leading to an inability to activate adaptive immune cells.[4,81,82] Further exploration of the metabolic switches, proteins, and metabolites that govern these changes will undoubtedly provide further insight into disease mechanisms, and how these pathways can be manipulated to halt the destructive processes associated with deleterious immune cell activation. Already, there is encouraging evidence from *in vivo* studies to support metabolic targeting in immune-mediated disease such as RA, SLE, and MS. However, direct inhibition of immune cell activation or promotion of regulatory immune cell activity via metabolic manipulation requires careful consideration, given the potential for "off target" effects. Nonetheless, dissecting the links between immune cell activation and metabolic status could have major implications for health and disease, where current strategies have thus far failed to make an impact.

AUTHORS' CONTRIBUTIONS

All authors participated in the conceptualization and writing of this manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: LCS and AD are supported by the Science Foundation Ireland (SFI), Ireland, through the Advanced Materials and Bioengineering Research (AMBER) Centre (SFI/12/RC/2278_P2, partly supported by the European Regional Development Fund). HKF and AD are also supported by Enterprise Ireland (CF-2020-1340). SAO is funded by an IntraFaculty PhD Fellowship and the Schools of Engineering and Biochemistry and Immunology at Trinity College Dublin.

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REFERENCES

1. Düvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, Triantafellow E, Ma Q, Gorski R, Cleaver S, Vander Heiden MG, MacKeigan JP, Finan PM, Clish CB, Murphy LO, Manning BD. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell* 2010;**39**:171–83
2. Ma EH, Poffenberger MC, Wong AH, Jones RG. The role of AMPK in T cell metabolism and function. *Curr Opin Immunol* 2017;**46**:45–52
3. Warburg O. On the origin of cancer cells. *Science* 1956;**123**:309–14
4. O'Neill LA, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J Exp Med* 2016;**213**:15–23
5. Pearce EJ, Everts B. Dendritic cell metabolism. *Nat Rev Immunol* 2015;**15**:18–29
6. Almeida L, Lochner M, Berod L, Sparwasser T. Metabolic pathways in T cell activation and lineage differentiation. *Semin Immunol* 2016;**28**:514–24
7. Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, Chmielewski K, Stewart KM, Ashall J, Everts B, Pearce EJ, Driggers EM, Artyomov MN. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* 2015;**42**:419–30
8. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH, Zheng L, Gardet A, Tong Z, Jany SS, Corr SC, Haneklaus M, Caffrey BE, Pierce K, Walmsley S, Beasley FC, Cummins E, Nizet V, Whyte M, Taylor CT, Lin H, Masters SL, Gottlieb E, Kelly VP, Clish C, Auron PE, Xavier RJ, O'Neill LA. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* 2013;**496**:238–42
9. Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, Redmann V, Freitas TC, Blagih J, van der Windt GJ, Artyomov MN, Jones RG, Pearce EL, Pearce EJ. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nat Immunol* 2014;**15**:323–32
10. Ecker J, Liebisch G, Englmaier M, Grandl M, Robenek H, Schmitz G. Induction of fatty acid synthesis is a key requirement for phagocytic differentiation of human monocytes. *Proc Natl Acad Sci USA* 2010;**107**:7817–22
11. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, Wagner RA, Greaves DR, Murray PJ, Chawla A. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* 2006;**4**:13–24
12. Malandrino MI, Fucho R, Weber M, Calderon-Dominguez M, Mir JF, Valcarcel L, Escoté X, Gómez-Serrano M, Peral B, Salvadó L, Fernández-Veledo S, Casals N, Vázquez-Carrera M, Villarroya F, Vendrell JJ, Serra D, Herrero L. Enhanced fatty acid oxidation in adipocytes and macrophages reduces lipid-induced triglyceride accumulation and inflammation. *Am J Physiol Endocrinol Metab* 2015;**308**:E756–169
13. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, McCormick LL, Fitzgerald P, Chi H, Munger J, Green DR. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* 2011;**35**:871–82
14. Gerriets VA, Kishton RJ, Nichols AG, Macintyre AN, Inoue M, Ilkayeva O, Winter PS, Liu X, Priyadharshini B, Slawinska ME, Haeberli L, Huck C, Turka LA, Wood KC, Hale LP, Smith PA, Schneider MA, MacIver NJ, Locasale JW, Newgard CB, Shinohara ML, Rathmell JC. Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. *J Clin Invest* 2015;**125**:194–207
15. Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, Sandouk A, Hesse C, Castro CN, Bähre H, Tschirner SK, Gorinski N, Gohmert M, Mayer CT, Huehn J, Ponimaskin E, Abraham WR, Müller R, Lochner M, Sparwasser T. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med* 2014;**20**:1327–33
16. Blair D, Dufort FJ, Chiles TC. Protein kinase Cβ is critical for the metabolic switch to glycolysis following B-cell antigen receptor engagement. *Biochem J* 2012;**448**:165–9

17. Egawa T, Bhattacharya D. Regulation of metabolic supply and demand during B cell activation and subsequent differentiation. *Curr Opin Immunol* 2019;**57**:8–14
18. Lam WY, Becker AM, Kennerly KM, Wong R, Curtis JD, Llufrio EM, McCommis KS, Fahrmann J, Pizzato HA, Nunley RM, Lee J, Wolfgang MJ, Patti GJ, Finck BN, Pearce EL, Bhattacharya D. Mitochondrial pyruvate import promotes long-term survival of antibody-secreting plasma cells. *Immunity* 2016;**45**:60–73
19. Torigoe M, Iwata S, Nakayamada S, Sakata K, Zhang M, Hajime M, Miyazaki Y, Narisawa M, Ishii K, Shibata H, Tanaka Y. Metabolic reprogramming commits differentiation of human CD27. *J Immunol* 2017;**199**:425–34
20. Zhang S, Carriere J, Lin X, Xie N, Feng P. Interplay between cellular metabolism and cytokine responses during viral infection. *Viruses* 2018;**10**:521
21. Finucane OM, Sugrue J, Rubio-Araiz A, Guillot-Sestier MV, Lynch MA. The NLRP3 inflammasome modulates glycolysis by increasing PFKFB3 in an IL-1β-dependent manner in macrophages. *Sci Rep* 2019; **9**:4034
22. Infantino V, Iacobazzi V, Palmieri F, Menga A. ATP-citrate lyase is essential for macrophage inflammatory response. *Biochem Biophys Res Commun* 2013;**440**:105–11
23. Infantino V, Iacobazzi V, Menga A, Avantaggiati ML, Palmieri F. A key role of the mitochondrial citrate carrier (SLC25A1) in TNFα- and IFNγ-triggered inflammation. *Biochim Biophys Acta* 2014;**1839**:1217–25
24. Chimenti MS, Tucci P, Candi E, Perricone R, Melino G, Willis AE. Metabolic profiling of human CD4+ cells following treatment with methotrexate and anti-TNF-α infliximab. *Cell Cycle* 2013;**12**:3025–36
25. Koedderitzsch K, Zezina E, Li L, Herrmann M, Biesemann N. TNF induces glycolytic shift in fibroblast like synoviocytes via GLUT1 and HIF1A. *Sci Rep* 2021;**11**:19385
26. Biniecka M, Canavan M, McGarry T, Gao W, McCormick J, Cregan S, Gallagher L, Smith T, Phelan JJ, Ryan J, O'Sullivan J, Ng CT, Veale DJ, Fearon U. Dysregulated bioenergetics: a key regulator of joint inflammation. *Ann Rheum Dis* 2016;**75**:2192–200
27. Ruiz-Limón P, Ortega R, Arias de la Rosa I, Abalos-Aguilera MDC, Perez-Sanchez C, Jimenez-Gomez Y, Peralbo-Santaella E, Font P, Ruiz-Vilches D, Ferrin G, Collantes-Estevez E, Escudero-Contreras A, López-Pedrera C, Barbarroja N. Tocilizumab improves the proatherothrombotic profile of rheumatoid arthritis patients modulating endothelial dysfunction, NETosis, and inflammation. *Transl Res* 2017; **183**:87–103
28. Dudek M, Lohr K, Donakonda S, Baumann T, Lüdemann M, Hegenbarth S, Dübbel L, Eberhagen C, Michailidou S, Yassin A, Prinz M, Popper B, Rose-John S, Zischka H, Knolle PA. IL-6-induced FOXO1 activity determines the dynamics of metabolism in CD8 T cells cross-primed by liver sinusoidal endothelial cells. *Cell Rep* 2022;**38**:110389
29. Kracht M, Müller-Ladner U, Schmitz ML. Mutual regulation of metabolic processes and proinflammatory NF-κB signaling. *J Allergy Clin Immunol* 2020;**146**:694–705
30. Ip WKE, Hoshi N, Shouval DS, Snapper S, Medzhitov R. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. *Science* 2017;**356**:513–9
31. Huang SC, Smith AM, Everts B, Colonna M, Pearce EL, Schilling JD, Pearce EJ. Metabolic reprogramming mediated by the mTORC2-IRF4 signaling axis is essential for macrophage alternative activation. *Immunity* 2016;**45**:817–30
32. Markiewicz R, Litowczenko J, Gapiński J, Woźniak A, Jurga S, Patkowski A. Nanomolar nitric oxide concentrations in living cells measured by means of fluorescence correlation spectroscopy. *Molecules* 2022;**27**:1010
33. Kelly B, O'Neill LA. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res* 2015;**25**:771–84
34. Bailey JD, Diotallevi M, Nicol T, McNeill E, Shaw A, Chuaiphichai S, Hale A, Starr A, Nandi M, Stylianou E, McShane H, Davis S, Fischer R, Kessler BM, McCullagh J, Channon KM, Crabtree MJ. Nitric oxide modulates metabolic remodeling in inflammatory macrophages through TCA cycle regulation and itaconate accumulation. *Cell Rep* 2019;**28**:218–307
35. Everts B, Amiel E, van der Windt GJ, Freitas TC, Chott R, Yarasheski KE, Pearce EL, Pearce EJ. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. *Blood* 2012;**120**:1422–31
36. MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. *Annu Rev Immunol* 1997;**15**:323–50
37. Palmieri EM, McGinity C, Wink DA, McVicar DW. Nitric oxide in macrophage immunometabolism: hiding in plain sight. *Metabolites* 2020;**10**:429
38. Mao K, Chen S, Chen M, Ma Y, Wang Y, Huang B, He Z, Zeng Y, Hu Y, Sun S, Li J, Wu X, Wang X, Strober W, Chen C, Meng G, Sun B. Nitric oxide suppresses NLRP3 inflammasome activation and protects against LPS-induced septic shock. *Cell Res* 2013;**23**:201–12
39. Ganeshan K, Chawla A. Metabolic regulation of immune responses. *Annu Rev Immunol* 2014;**32**:609–34
40. Piccinini AM, Midwood KS. DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm* 2010;**2010**:672395
41. Foell D, Wittkowski H, Vogl T, Roth J. S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. *J Leukoc Biol* 2007;**81**:28–37
42. Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in inflammation and cancer. *Annu Rev Immunol* 2010;**28**:367–88
43. Rocha DM, Caldas AP, Oliveira LL, Bressan J, Hermsdorff HH. Saturated fatty acids trigger TLR4-mediated inflammatory response. *Atherosclerosis* 2016;**244**:211–5
44. Weise CM, Chen K, Chen Y, Kuang X, Savage CR, Reiman EM, Alzheimer's Disease Neuroimaging Initiative. Left lateralized cerebral glucose metabolism declines in amyloid-β positive persons with mild cognitive impairment. *Neuroimage Clin* 2018;**20**:286–96
45. Johnson KA, Fox NC, Sperling RA, Klunk WE. Brain imaging in Alzheimer disease. *Cold Spring Harb Perspect Med* 2012;**2**:a006213
46. Lu Y, Ren J, Cui S, Chen J, Huang Y, Tang C, Shan B, Nie B, Xinsheng L. Cerebral glucose metabolism assessment in rat models of Alzheimer's disease: an 18F-FDG-PET study. *Am J Alzheimers Dis Other Demen* 2016; **31**:333–40
47. Shippy DC, Ulland TK. Microglial immunometabolism in Alzheimer's disease. *Front Cell Neurosci* 2020;**14**:563446
48. Baik SH, Kang S, Lee W, Choi H, Chung S, Kim JI, Mook-Jung I. A breakdown in metabolic reprogramming causes microglia dysfunction in Alzheimer's disease. *Cell Metab* 2019;**30**:493.e–507.e6
49. Spangenberg EE, Green KN. Inflammation in Alzheimer's disease: lessons learned from microglia-depletion models. *Brain Behav Immun* 2017;**61**:1–11
50. Yao J, Chen S, Mao Z, Cadenas E, Brinton RD. 2-Deoxy-D-glucose treatment induces ketogenesis, sustains mitochondrial function, and reduces pathology in female mouse model of Alzheimer's disease. *PLoS ONE* 2011;**6**:e21788
51. Ghosh S, Castillo E, Frias ES, Swanson RA. Bioenergetic regulation of microglia. *Glia* 2018;**66**:1200–12
52. Zhao P, Zhou W, Zhang Y, Li J, Zhao Y, Pan L, Shen Z, Chen W, Hui J. Aminooxyacetic acid attenuates post-infarct cardiac dysfunction by balancing macrophage polarization through modulating macrophage metabolism in mice. *J Cell Mol Med* 2020;**24**:2593–609
53. Zhong WJ, Yang HH, Guan XX, Xiong JB, Sun CC, Zhang CY, Luo XQ, Zhang YF, Zhang J, Duan JX, Zhou Y, Guan CX. Inhibition of glycolysis alleviates lipopolysaccharide-induced acute lung injury in a mouse model. *J Cell Physiol* 2019;**234**:4641–54
54. Moon JS, Hisata S, Park MA, DeNicola GM, Ryter SW, Nakahira K, Choi AMK. mTORC1-induced HK1-dependent glycolysis regulates NLRP3 inflammasome activation. *Cell Rep* 2015;**12**:102–15
55. Lauro C, Limatola C. Metabolic reprograming of microglia in the regulation of the innate inflammatory response. *Front Immunol* 2020; **11**:493
56. Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, D'Agostino D, Planavsky N, Lupfer C, Kanneganti TD, Kang S, Horvath TL, Fahmy TM, Crawford PA, Biragyn A, Alnemri E, Dixit VD. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med* 2015;**21**:263–9
57. Yin JX, Maalouf M, Han P, Zhao M, Gao M, Dharshaun T, Ryan C, Whitelegge J, Wu J, Eisenberg D, Reiman EM, Schweizer FE, Shi J.

Ketones block amyloid entry and improve cognition in an Alzheimer's model. *Neurobiol Aging* 2016;**39**:25–37
58. Mobasheri A, Rayman MP, Gualillo O, Sellam J, van der Kraan P, Fearon U. The role of metabolism in the pathogenesis of osteoarthritis. *Nat Rev Rheumatol* 2017;**13**:302–11
59. Fearon U, Hanlon MM, Wade SM, Fletcher JM. Altered metabolic pathways regulate synovial inflammation in rheumatoid arthritis. *Clin Exp Immunol* 2019;**197**:170–80
60. Hanlon MM, Canavan M, Barker BE, Fearon U. Metabolites as drivers and targets in rheumatoid arthritis. *Clin Exp Immunol* 2022;**208**:167–80
61. Renaudin F, Orliaguet L, Castelli F, Fenaille F, Prignon A, Alzaid F, Combes C, Delvaux A, Adimy Y, Cohen-Solal M, Richette P, Bardin T, Riveline JP, Venteclef N, Lioté F, Campillo-Gimenez L, Ea HK. Gout and pseudo-gout-related crystals promote GLUT1-mediated glycolysis that governs NLRP3 and interleukin-1β activation on macrophages. *Ann Rheum Dis* 2020;**79**:1506–14
62. Cobo I, Cheng A, Murillo-Saich J, Coras R, Torres A, Abe Y, Lana AJ, Schlachetzki J, Liu-Bryan R, Terkeltaub R, Sanchez-Lopez E, Glass CK, Guma M. Monosodium urate crystals regulate a unique JNK-dependent macrophage metabolic and inflammatory response. *Cell Rep* 2022;**38**:110489
63. Goldberg EL, Asher JL, Molony RD, Shaw AC, Zeiss CJ, Wang C, Morozova-Roche LA, Herzog RI, Iwasaki A, Dixit VD. β-hydroxybutyrate deactivates neutrophil NLRP3 inflammasome to relieve gout flares. *Cell Rep* 2017;**18**:2077–87
64. Mahon OR, Kelly DJ, McCarthy GM, Dunne A. Osteoarthritis-associated basic calcium phosphate crystals alter immune cell metabolism and promote M1 macrophage polarization. *Osteoarth Cart* 2020;**28**:603–12
65. O'Rourke SA, Neto NGB, Devilly E, Shanley LC, Fitzgerald HK, Monaghan MG, Dunne A. Cholesterol crystals drive metabolic reprogramming and M1 macrophage polarisation in primary human macrophages. *Atherosclerosis* 2022;**352**:35–45
66. Doddapattar P, Dev R, Ghatge M, Patel RB, Jain M, Dhanesha N, Lentz SR, Chauhan AK. Myeloid cell PKM2 deletion enhances efferocytosis and reduces atherosclerosis. *Circ Res* 2022;**130**:1289–305
67. Gergely P Jr, Grossman C, Niland B, Puskas F, Neupane H, Allam F, Banki K, Phillips PE, Perl A. Mitochondrial hyperpolarization and ATP depletion in patients with systemic lupus erythematosus. *Arthritis Rheum* 2002;**46**:175–90
68. Weyand CM, Goronzy JJ. Immunometabolism in early and late stages of rheumatoid arthritis. *Nat Rev Rheumatol* 2017;**13**:291–301
69. La Rocca C, Carbone F, De Rosa V, Colamatteo A, Galgani M, Perna F, Lanzillo R, Brescia Morra V, Orefice G, Cerillo I, Florio C, Maniscalco GT, Salvetti M, Centonze D, Uccelli A, Longobardi S, Visconti A, Matarese G. Immunometabolic profiling of T cells from patients with relapsing-remitting multiple sclerosis reveals an impairment in glycolysis and mitochondrial respiration. *Metabolism* 2017;**77**:39–46
70. Montes Diaz G, Hupperts R, Fraussen J, Somers V. Dimethyl fumarate treatment in multiple sclerosis: recent advances in clinical and immunological studies. *Autoimmun Rev* 2018;**17**:1240–50
71. Carlström KE, Ewing E, Granqvist M, Gyllenberg A, Aeinehband S, Enoksson SL, Checa A, Badam TVS, Huang J, Gomez-Cabrero D, Gustafsson M, Al Nimer F, Wheelock CE, Kockum I, Olsson T, Jagodic M, Piehl F. Therapeutic efficacy of dimethyl fumarate in relapsing-remitting multiple sclerosis associates with ROS pathway in monocytes. *Nat Commun* 2019;**10**:3081
72. Procaccini C, De Rosa V, Galgani M, Abanni L, Calì G, Porcellini A, Carbone F, Fontana S, Horvath TL, La Cava A, Matarese G. An oscillatory switch in mTOR kinase activity sets regulatory T cell responsiveness. *Immunity* 2010;**33**:929–41
73. Lai ZW, Borsuk R, Shadakshari A, Yu J, Dawood M, Garcia R, Francis L, Tily H, Bartos A, Faraone SV, Phillips P, Perl A. Mechanistic target of rapamycin activation triggers IL-4 production and necrotic death of double-negative T cells in patients with systemic lupus erythematosus. *J Immunol* 2013;**191**:2236–46
74. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. *Diabetologia* 2017;**60**:1577–85
75. Dowling RJ, Zakikhani M, Fantus IG, Pollak M, Sonenberg N. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. *Cancer Res* 2007;**67**:10804–12
76. Nath N, Khan M, Paintlia MK, Singh I, Hoda MN, Giri S. Metformin attenuated the autoimmune disease of the central nervous system in animal models of multiple sclerosis. *J Immunol* 2009;**182**:8005–14
77. Lee SY, Lee SH, Yang EJ, Kim EK, Kim JK, Shin DY, Cho ML. Metformin ameliorates inflammatory bowel disease by suppression of the STAT3 signaling pathway and regulation of the between Th17/Treg balance. *PLoS ONE* 2015;**10**:e0135858
78. Son HJ, Lee J, Lee SY, Kim EK, Park MJ, Kim KW, Park SH, Cho ML. Metformin attenuates experimental autoimmune arthritis through reciprocal regulation of Th17/Treg balance and osteoclastogenesis. *Mediators Inflamm* 2014;**2014**:973986
79. Pålsson-McDermott EM, O'Neill LAJ. Targeting immunometabolism as an anti-inflammatory strategy. *Cell Res* 2020;**30**:300–14
80. Piranavan P, Bhamra M, Perl A. Metabolic targets for treatment of autoimmune diseases. *Immunometabolism* 2020;**2**:e200012
81. Wculek SK, Khouili SC, Priego E, Heras-Murillo I, Sancho D. Metabolic control of dendritic cell functions: digesting information. *Front Immunol* 2019;**10**:775
82. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol* 2016;**16**:553–65