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STATE-OF-THE-ART REVIEW

Glycolysis – a key player in the inflammatory response

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The inflammatory response involves the activation of several cell types to fight insults caused by a plethora of agents, and to maintain the tissue homoeostasis. On the one hand, cells involved in the pro-inflammatory response, such as inflammatory M1 macrophages, Th1 and Th17 lymphocytes or activated microglia, must rapidly provide energy to fuel inflammation, which is essentially accomplished by glycolysis and high lactate production. On the other hand, regulatory T cells or M2 macrophages, which are involved in immune regulation and resolution of inflammation, preferentially use fatty acid oxidation through the TCA cycle as a main source for energy production. Here, we discuss the impact of glycolytic metabolism at the different steps of the inflammatory response. Finally, we review a wide variety of molecular mechanisms which could explain the relationship between glycolytic metabolites and the pro-inflammatory phenotype, including signalling events, epigenetic remodelling, post-transcriptional regulation and post-translational modifications. Inflammatory processes are a common feature of many age-associated diseases, such as cardiovascular and neurodegenerative disorders. The finding that immunometabolism could be a master regulator of inflammation broadens the avenue for treating inflammation-related pathologies through the manipulation of the vascular and immune cell metabolism.

Introduction

Inflammation is a protective response that occurs to defend and repair our tissues in response to an infection, tissue damage or stress [1]. An acute inflammatory response involves the coordinated delivery of blood components (plasma and leucocytes) to the target site. The recruitment of inflammatory cells from the blood, together with tissue-resident immune cells, ensures a relatively rapid way to eliminate pathogens or contribute to healing. Importantly, the duration and the intensity of the inflammatory response is

Abbreviations

2-DG, 2-deoxyglucose; ACLY, ATP-citrate lyase; AD, Alzheimer's disease; AGEs, advanced glycation end products; Aβ, amyloid-β; BMDMs, bone marrow-derived macrophages; CIC, citrate carrier; Citr, citrate; DCs, dendritic cells; ECs, endothelial cells; ER, endoplasmic reticulum; F2,6BP, fructose-6-phosphate to fructose-2,6-bisP; FAO, fatty acid oxidation; Fum, fumarate; GAP, glyceraldehyde 3-phosphate; Itac, itaconate; Lact, lactate; LDHA, lactate dehydrogenase A; LPS, lipopolysaccharide; MS, Multiple Sclerosis; NADH, nicotinamide adenine dinucleotide; NETs, neutrophil extracellular traps; NFAT, nuclear factor of activated T cells; NO, nitric oxide; OXPHOS, oxidative phosphorylation; PD, Parkinson's disease; PEP, phosphoenolpyruvate; PFK-1, 6-phosphofructokinase-1; PFK-2, phosphofructo-2-kinase; PPP, pentose phosphate pathway; Pyr, pyruvate; ROS, reactive oxygen species; SAM, S-adenosylmethionine; SDH, succinate dehydrogenase; Succ, succinate; TCA, tricarboxylic acid; TET, ten-eleven translocation; Tregs, regulatory T cells; α-KG, α-ketoglutarate.

controlled by the secretion of certain extracellular factors like cytokines or lipid mediators and chemokines that attract innate and adaptive immune cells to the site of damage. A successful inflammatory response is followed by the resolution phase where the inflammatory response is gradually shut down once the danger signal or the injury has been eliminated, and this resolution phase is critical for restoring homoeostasis [2]. Under certain circumstances, the immune cells fail to switch from inflammatory to anti-inflammatory states, and the inflammation persists over time, giving rise to chronic inflammation. Since chronic inflammation is associated with many human age-associated pathologies [3], such as neurodegenerative, cardiovascular, bone and muscular diseases, controlling the resolution of the inflammatory response and preventing chronic inflammation must be a priority. Several complex molecular mechanisms control the switch from pro-inflammatory to anti-inflammatory states and, recently, the metabolism of immune cells has been pointed to be essential in this control between pro-inflammatory and anti-inflammatory profile.

Metabolism is the core process underlying all biological phenomena, providing energy and building blocks for macromolecules. One of the main metabolic pathways that provides energy for cellular processes is glycolysis, which comprises multiple enzymes that transform glucose into pyruvate. This pyruvate can access the tricarboxylic acid (TCA) cycle to be fully metabolized to CO_2, generating NADH and reduced $FADH_2$ to perform the oxidative phosphorylation (OXPHOS). On the other hand, pyruvate, even in the presence of oxygen, can be fermented to lactate without producing ATP but regenerating NAD^+, which is commonly known as aerobic glycolysis. Despite glycolysis is much less efficient than OXPHOS, it is the metabolic pathway preferred by highly proliferating cells like cancer cells. Immune cells also undergo this metabolic reprogramming when they become activated to mount an inflammatory response. During the peak of inflammation, immune cells preferentially use glycolysis as a source of energy, whereas during the resolution phase they rely mainly on OXPHOS metabolism, acquiring a pro-resolving phenotype [4] (Fig. 1). Thus, in recent years it has become evident that different metabolic routes determine the fate of immune cells and impact the inflammatory response.

The metabolic state of activated immune cells has been deeply characterized [5,6]. During the glycolytic switch, glycolysis supplies metabolic intermediates for other biosynthetic pathways necessary for cellular growth and differentiation. In addition to glycolysis, the pentose phosphate pathway (PPP), the hexosamine pathway and the glutaminolysis are increased upon activation [7]. The cellular levels of metabolites resulting from this metabolic state determine the activation/repression of signalling pathways, the epigenetic and post-transcriptional regulation of inflammatory genes and the post-translational modification of proteins.

In this review, we summarize recent knowledge on how glycolysis orchestrates inflammation and how metabolic pathways control different steps of the inflammatory response, from vascular activation, leucocyte migration and expansion to the resolution phase. We finally discuss the mechanisms by which metabolism is associated with inflammation, explaining the role of different metabolites accumulated during glycolysis and the broken TCA cycle.

The glycolytic players of the inflammatory response

The inflammatory response involves the coordination of multiple players, including innate immune cells, such as neutrophils and macrophages; adaptive immune cells, mainly T lymphocytes; and vascular cells, including endothelial cells (ECs) and vascular smooth muscle cells. The classical inflammatory response progresses through two main clearly distinguished phases: initiation and resolution. The initiation phase is characterized by the sensing of the damaging agent by resident immune cells, the activation of the endothelial cells, and the clonal expansion, the proliferation and the recruitment of additional immune cells to the site of injury. The resolution phase consists in the clearance of activated immune cells, the generation of memory immune cells and the recovery of homoeostasis.

One of the first decisive steps involved in the onset of inflammation is the activation of the endothelium, which is characterized by an increase of the expression of cellular adhesion molecules such as E-selectin, ICAM-1 and VCAM-1. These adhesion receptors facilitate the rolling, tethering and firm adhesion of immune cells to the vascular wall, increasing vascular permeability, promoting oedema and blushing. The activation of ECs is accomplished by the production of nitric oxide (NO), a well-established inflammatory agent that induces an increase in vessel dilation and permeability [8]. In the steady state, ECs rely almost exclusively on glycolysis for energy generation. Their mitochondria mainly function as signalling organelles and metabolize fatty acids and glutamine for the production of TCA cycle intermediates [9,10] and for the synthesis of dNTPs [11]. However, forcing ECs to be even more glycolytic by the overexpression of HIF-1α,
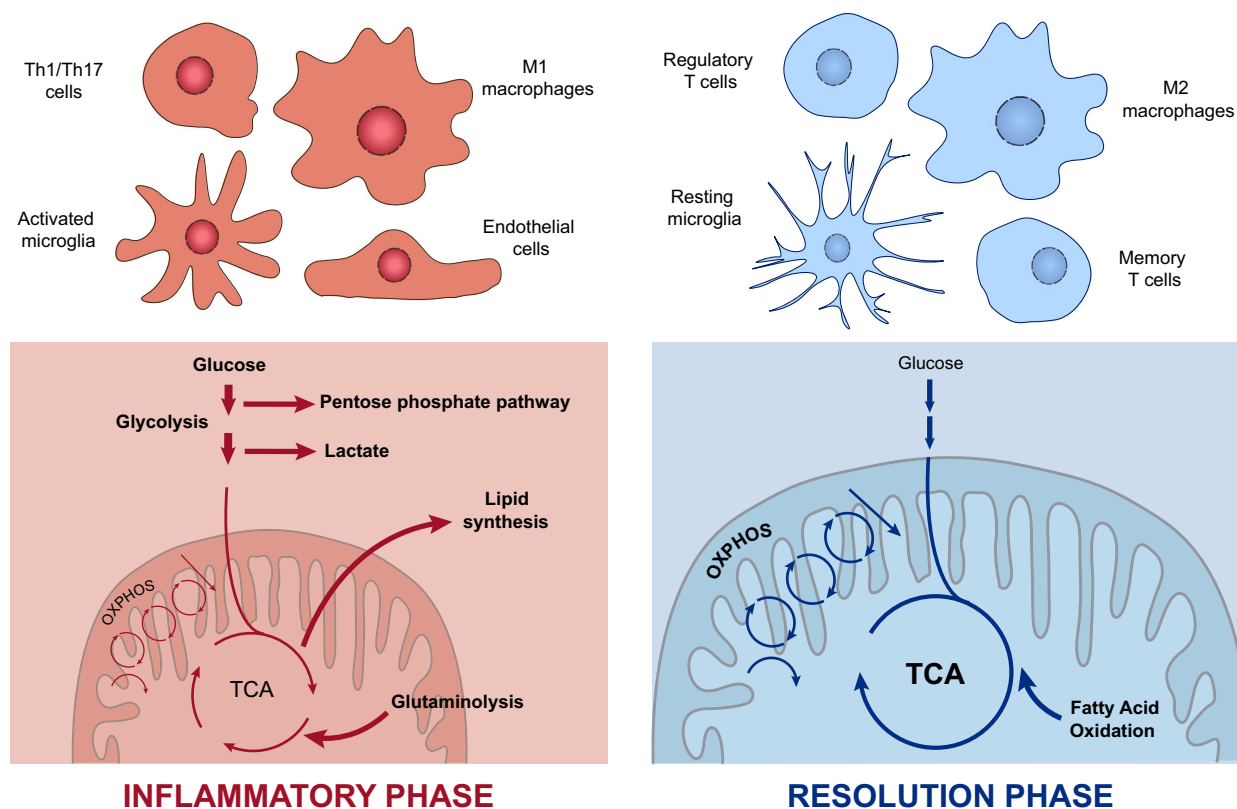
Fig. 1. Distinctive metabolic profile of cells during the inflammatory response. Inflammatory cells such as Th1 and Th17 cells, activated microglia, endothelial cells or M1-type macrophages are known to rely mainly on glycolysis and glutaminolysis upon inflammation, displaying a dysregulated TCA cycle and increased rate of lipid synthesis and the PPP to meet the highly biosynthetic and bioenergetic demand. Once the inflammatory response is completed, regulatory and memory T cells, resting microglia and M2-type macrophages, which depend mostly on OXPHOS and fatty acid oxidation, are involved in the maintenance of the tissue homoeostasis to dampen the response.

a transcription factor that regulates many genes associated with glycolysis, increases the expression of activation markers such as VCAM-1, pro-inflammatory cytokines such as IL-8 and chemokines such as CCL2 [12]. The glycolytic rate in ECs is tightly controlled by 6-phosphofructo-2-kinase (PFK-2), which converts fructose-6-phosphate to fructose-2,6-bisP (F2,6BP). F2,6BP is an allosteric activator of 6-phosphofructokinase-1 (PFK-1) that enhances glycolysis. Inflammatory cytokines such as VEGF, TNF-α and IL-6 stimulate PFK-2 expression and activity, thus stimulating glycolysis in ECs [13]. Moreover, PFK-2 partial inhibition reduces NF-κB activation, resulting in repressed expression of adhesion molecules [14], pro-inflammatory cytokines and chemokines [15]. Hence, the modulation of glycolysis in vascular cells might be a potential target to balance chronic inflammatory diseases.

The next step in the inflammatory cascade is the production and influx of neutrophils into the damaged tissue. It has been observed that granulopoiesis depends on the maintenance of mitochondrial fitness by autophagy [16]. Consequently, the disruption of autophagy by *Atg7* deletion promotes a metabolic rewiring towards glycolysis in neutrophils, leading to defective differentiation [17]. Although mature neutrophils are mainly glycolytic, some examples have shown that mitochondrial respiration is also important for neutrophil transmigration. Blocking mitochondrial ATP production or mTOR activation impaired neutrophil chemotaxis [18,19]. This suggests that both glycolysis and mitochondrial respiration could be mediating neutrophil transmigration. After transmigration, neutrophils reach the injured tissue and activate a number of antipathogen responses that include phagocytosis, degranulation, production of high levels of extracellular ROS and release of neutrophil extracellular traps (NETs). The production of extracellular ROS is mediated by the action of the membrane-bound enzyme NADPH oxidase. Dampening glycolysis led to impaired NADPH oxidase function and subsequent deficient pathogen clearance [20,21]. This confirms that glycolysis is essential for the microbial clearance of

neutrophils. The production of NETs is also dependent on metabolism [22]. During this process, neutrophil DNA is released into the extracellular media alongside with proteases that possess antibacterial activity. The DNA nets trap the pathogen, immobilizing it and allowing the proteases to achieve their function [23]. Upon neutrophil activation, mTOR positively regulates the translation of HIF-1α, inducing glycolytic metabolism that is essential for NETs production and bacterial killing [24,25].

After the neutrophil influx, innate and adaptive immune cells are recruited to the injured or infected tissue. Again, the metabolic reprogramming drives both the migration and the fate of macrophages and T cells. The link between glycolysis and immune cell migration is revealed by the dual role of the mTORC1 and HIF-1α pathways in controlling glycolytic metabolism and T-cell trafficking. The mTORC1/HIF-1α axis controls the expression of glucose transporters and rate-limiting glycolytic enzymes, but also chemokine and adhesion receptors that regulate T-cell trafficking. The mTORC1/HIF-1α axis inhibits the expression of the adhesion molecules CD62L and CCR7, allowing the release of T cells to the blood [26,27]. Interestingly, T-cell migration and their metabolism are bidirectionally regulated since migratory signals may change the metabolic state of cells. Pro-migratory stimuli upregulate glucokinase expression which provokes actin cytoskeleton rearrangements in regulatory T cells (Tregs) [28].

Dendritic cells (DCs) analyse their environment to identify pathogens through different pattern recognition receptors. Apart from antigen presentation, DCs produce cytokines, chemokines and costimulatory signals for instructing appropriate T-cell responses. DCs, like other immune cells, undergo a metabolic switch accompanying their differentiation and activation [29-31]. The differentiation of DCs occurs with an increase of mitochondrial biogenesis and remodelling, supporting an important role of mitochondria for the development of DCs [32,33]. DCs become active upon binding TLR agonists, which causes a marked increase in glucose consumption and lactic acid production [34,35]. Activated DCs then increase the expression of Nos2 and, consequently, produce NO, which restricts the metabolism to glycolysis by blocking OXPHOS [36]. Inhibition of glycolysis with 2-deoxyglucose (2-DG) blocks both activation and differentiation of DCs [35,37]. In contrast, glucose deprived DCs enhance T-cell responses [38].

Similar to DCs, macrophages and T cells present different metabolism based on their activation state. Based on surface markers, genes expressed and cytokines produced, macrophages are classically classified into pro-inflammatory or M1 macrophages [39,40] and anti-inflammatory or M2 macrophages [41,42]. M1 differentiation is induced by pathogens or Th1 T cells, and their function is to kill pathogens and present antigens to T lymphocytes. In contrast, M2 macrophages are induced by anti-inflammatory innate responses and Th2 T cells, and they play an important role in the resolution phase, producing anti-inflammatory cytokines such as IL-10 and dampening the production of TNF-α and NO [42].

M1 macrophages change their metabolism upon activation, enhancing glycolysis, PPP and displaying a broken TCA cycle [43-45]. Glycolysis inhibition in M1 macrophages impairs their function by interfering with phagocytosis, ROS production and cytokine secretion [44,46,47]. Two of the most important signalling pathways for M1 macrophage activation are NF-κB and Akt/mTOR [48-53]. Akt regulates the polarization of macrophages since Akt1 inhibition promotes a M1 phenotype, whereas Akt2 deletion enhances M2 differentiation [50]. On the other hand, NF-κB is activated in response to inflammatory signals and coordinates all macrophage pro-inflammatory responses [48,54]. Both NF-κB and Akt pathways converge in the stabilization of HIF-1α, which increases the expression of glucose transporters [55] and glycolytic enzymes to maintain the glycolytic flux [56-58]. HIF-1α also promotes the expression of inflammatory mediators such as IL-1β by directly binding the IL-1β promoter [59,60], by activating NF-κB [54] or by activating the inflammasome through the expression of the glycolytic enzymes hexokinase-1 [61] and PKM2 [62] in macrophages. Additionally, the metabolic switch of macrophages is controlled by the glycerol 3-phosphate dehydrogenase (GPD2), a key component of the glycerol-phosphate shuttle, that mediates the transport of electrons to the mitochondria. Upon lipopolysaccharide (LPS)-induced activation, the expression and activity of the GPD2 is increased, boosting the oxidation of glucose. This enzyme is critical for macrophage function as the loss of GPD2 impairs IFN-γ and IL-6 secretion upon LPS activation [63].

The metabolic response in T cells follows a similar pattern to macrophages. Naïve T cells, which have a low energy demand, obtain their ATP from OXPHOS fuelled by glucose and FAO. Upon activation, effector T-cell lymphocytes change completely their metabolism to fulfil higher energetic demand. Effector CD4+ and CD8+ T cells switch their metabolic programme towards glycolysis for faster ATP production, and they keep using the TCA cycle to produce intermediates for protein and lipid synthesis. In the first steps of the activation, TCR signalling activates PDHK1, which

inhibits the pyruvate dehydrogenase, and therefore, pyruvate is metabolized into lactate [26,64,65] instead of entering the TCA cycle [66]. However, by using a novel C^{13} tracing *in vivo* approach, Ma *et al.* [67] have shown that *in vivo* activated CD8$^+$ T cells use pyruvate also to fuel the TCA cycle. Similar to macrophages, different subpopulations of CD4$^+$ T cells have distinct metabolic programmes. Effector short-lived inflammatory populations, like Th1, Th2 and Th17 subsets, rely on glycolysis, while long-lived T cells, like Tregs and memory T cells, preferentially use OXPHOS as a source of ATP [68-70]. Thus, blocking glycolysis with 2-DG or galactose promotes the differentiation of activated CD4$^+$ T cells into regulatory and memory T cells [71-75]. Specifically, inhibiting glycolysis with 2-DG impairs Th1 and Th2 cells differentiation [76]. Surprisingly, limiting glycolysis does not block proliferation but cytokine production [72]. The metabolic switch of T cells is mainly controlled by the mTOR and c-Myc signalling pathways [77-80] that, upon activation, upregulate glucose transporters and glycolytic enzymes [26,77,81]. Inhibition of mTORC1 or mTORC2 prevents Th1 and Th2 differentiation, respectively, and simultaneous inhibition of both promotes Treg differentiation, demonstrating that mTOR is essential for effector differentiation of CD4$^+$ [79,82] and CD8$^+$ T cells [83]. T cells, like macrophages, activate HIF-1α to enable the glycolytic switch. Rapid ATP production and PPP occur when HIF-1α is activated, increasing the biosynthetic capacity of the cell [71]. HIF-1α is necessary for Th17 differentiation *in vitro* and, beyond promoting Th17 phenotype, inhibits Treg development [84,85]. Surprisingly, Th17 differentiation *in vivo* in murine colitis also requires OXPHOS [86]. Eliminating mTORC1 or HIF-1α in differentiating CD8$^+$ cells impairs their cytolytic function [26].

At the end of the inflammatory response, both macrophages and T cells need to acquire a pro-resolving phenotype in order to gradually shut down the inflammatory response. M2 macrophages, regulatory and memory T cells are key players in this phase. In contrast to M1 macrophages, M2 macrophages uptake fatty acids to fuel OXPHOS over glycolysis [87,88]. M2 formation is induced by IL-4 and IL-13 cytokines that activate Akt, preventing the stabilization of HIF-1α [89,90]. The phosphoglycerate dehydrogenase, the rate-limiting enzyme in *de novo* serine biosynthesis from glucose, is also required for M2 polarization [91]. In addition, M2 macrophages require glutamine to carry out their function since glutamine deprivation impairs M2 but not M1 polarization. In these cells, glutamine is required for the synthesis of UDP-GlcNAc, an essential molecule for M2 polarization

[92]. M2 macrophages still need glucose metabolism to fuel OXPHOS and FAO, since 2-DG impairs M2 differentiation [93,94].

In contrast to effector CD4$^+$ T cells, Tregs and memory T cells rely on OXPHOS and FAO [95]. Disruption of OXPHOS specifically in Tregs by deletion of mitochondrial complex III causes a premature autoimmune death [96,97]. In addition, boosting FAO enhances memory T-cell development after stimulation [98]. Moreover, IL-15, a cytokine that induces memory responses, promotes mitochondrial biogenesis and the expression of FAO enzymes [99], supporting that memory T cells use FAO as the principal source of ATP. This characteristic metabolism of Tregs and memory T cells biased towards OXPHOS and FAO is maintained by the AMPK and TRAF6 pathways [68,98,100]. Strikingly, it has been proposed that memory T cells do not uptake fatty acids but, instead, they generate them by *de novo* synthesis [101]. Although they depend on mitochondrial metabolism, memory T cells are capable of inducing the glycolytic switch much faster than naïve T cells [102,103].

As mentioned, during the glycolytic switch the hexosamine pathway is enhanced, and the end product of this pathway, the UDP-GlcNAc, serves for O-GlcNAcylation of several proteins. Strikingly, while the O-GlcNAcylation initiates an anti-inflammatory response in macrophages, it triggers pro-inflammatory effects in T cells through NF-κB and NFAT pathways [104,105]. Importantly, 2-DG disturbs glycosylation of proteins and activation of the endoplasmic reticulum (ER) stress response [76]. Since some of the experiments uncovering the metabolism of immune cells are based on the use of 2-DG, future experiments will be required to dissect the contribution of protein glycosylation or glycolysis inhibition of 2-DG.

Neuroinflammation and glycolysis

Neuroinflammation is a common feature to most neurodegenerative diseases such as multiple sclerosis (MS), and those clearly associated with ageing like Alzheimer's disease (AD) or Parkinson's disease (PD). Several types of cells participate in the neuroinflammatory process, highlighting microglia and astrocytes. Microglia are the innate immune cells of the brain, closely related to macrophages with whom they share inflammatory signalling pathways such as the inflammasome [106]. In the resting state, microglia scan their surroundings in search of any potentially damaging agent. Upon injury, they become activated and exert a number of responses dedicated to restore homoeostasis. As it occurs with other immune cells, resting

microglia mainly rely on OXPHOS and, upon activation, they switch on glycolysis and increase glucose uptake [107,108]. Recent studies have identified several mechanisms by which glycolysis influences pro-inflammatory gene transcription in microglia. One of these mechanisms involves the formation of advanced glycation end products (AGEs), which are modifications of proteins and lipids resulting from nonenzymatic reactions with sugars that typically accumulate during ageing [109]. Microglia express AGEs receptors which stimulate the expression of pro-inflammatory genes [110]. Importantly, chronic or aberrant activation of microglia can amplify the underlying neurodegenerative process and worsen the disease outcome [109,111]. A recent work by Baik *et al.* found that acute exposure to monomeric, oligomeric or fibrillary amyloid-β (Aβ) was able to shift OXPHOS to glycolysis through the mTOR/HIF-1α pathway in microglia. However, chronic exposure to Aβ had the opposite effect, turning down both glycolysis and OXPHOS, and impairing microglia responses. Interestingly, exposure to IFN-γ activated mTOR and glycolysis, improving microglia response to Aβ and ameliorating disease signs in an AD mouse model [112]. Similarly, microglia from AD-associated TREM-2 alleles and from TREM-2 knockout mice displayed decreased mTOR signalling, increased autophagy and impaired glycolysis [113]. These studies evidence the complexity of immune cell responses *in vivo* and how metabolic changes can induce different effects depending on the duration of the response. Importantly, glycolysis activation has also been recently found in activated microglia from MS patients [114] and actually targeting glycolysis and mitochondrial metabolism have been postulated as therapeutic options to treat MS [115]. Regarding this, neural stem cell (NSC) transplantation has been proved to show anti-inflammatory properties in experimental models of MS [116]. This beneficial effect would be mediated by the secretion of succinate, a TCA intermediate metabolite, by mononuclear phagocytes. Extracellular succinate binds its receptor, SUCNR1/GPR91, in NSCs, triggering the production and release of prostaglandin E2, a potent anti-inflammatory. Thereby, it impedes further entry of inflammatory monocytes into the central nervous system [117].

While microglia are the main executors of the inflammatory response in the brain, astrocytes also play a relevant role in this process. Astrocytes regulate the integrity and the permeability of the blood–brain barrier and also play a role in the control of immune cell trafficking and activation. In addition, astrocytes are immune-competent cells able to detect danger signals, release cytokines and chemokines and activate adaptive immune defence [118]. Cumulative studies have shown that glycolysis is an important pathway in reactive astrocytes [119]. An increase in their glycolytic rate and a subsequent increase in lactate production has been observed in AD [120] and during ischaemic insult [121]. Astrocyte-derived lactate is important for neuronal function and can actually boost memory formation [122]. Thus, activation of glycolysis by reactive astrocytes may be a mechanism to protect neurons from neuroinflammation. Recent evidences suggest that activated astrocytes also transit through different activation states. A1 astrocytes highly upregulate many classical complement cascade genes which are destructive to synapses, so they are postulated as harmful. In contrast, A2 astrocytes upregulate many neurotrophic factors, so it was hypothesized that they play a protective role during neurodegeneration [123]. A1 cells are abundant in several neurodegenerative diseases including AD, PD, Huntington's disease, amyotrophic lateral sclerosis or MS [124] as well as during normal ageing [125]. Since the discovery of A1/A2 astrocytes is very recent, whether changes in metabolic rates could have any impact on the acquisition of these phenotypes is still to be addressed.

How metabolism controls the inflammatory phenotype?

As a result of becoming glycolytic, cells accumulate certain metabolic intermediates typical of glycolysis and TCA cycle that define a specific metabolic profile. This metabolic state is characterized by an increased flux of glycolysis, high lactate production, augmented glutaminolysis, increased PPP and, in macrophages, broken TCA cycle [7]. In this scenario, the levels of several metabolites are unbalanced. In particular, phosphoenolpyruvate (PEP), succinate, citrate, itaconate, alpha-ketoglutarate (α-KG), lactate and 2-hydroxyglutarate (2-HG) have been demonstrated to impact the inflammatory state of cells. These metabolites modulate the inflammatory status by (a) activating signalling pathways, (b) promoting changes in the epigenetic landscape or (c) post-translational mechanisms. Metabolic enzymes also can (d) post-transcriptionally regulate the inflammatory outcome of cells (Fig. 2). Some of these metabolites, such as succinate, act through multiple of these mechanisms to modulate the inflammatory response (Fig. 3).

Signalling pathways regulated by metabolites

Phosphoenolpyruvate is generated during glycolysis by the enolase-1 and is accumulated in T cells upon
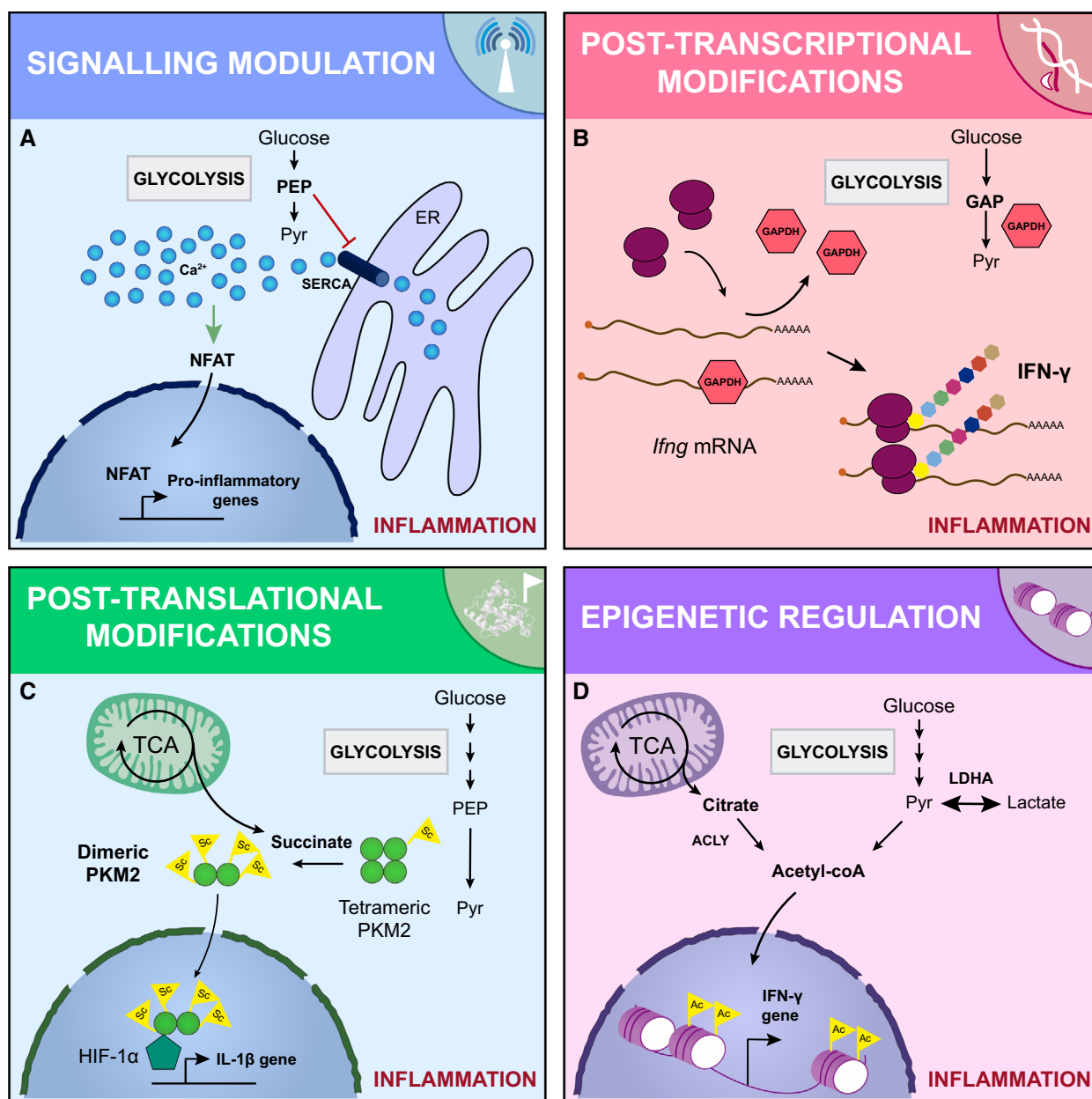
Fig. 2. Molecular mechanisms dictating the metabolic control of the inflammatory phenotype. The metabolic signature of inflammatory cells fuels their pro-inflammatory phenotype through different molecular mechanisms: (A) intracellular signalling events. For example, the PEP produced during glycolysis inhibits the ER calcium channel SERCA. The cytosolic calcium concentration is then increased, activating NFAT, which drives the expression of pro-inflammatory genes. (B) Sophisticated post-transcriptional modifications. Some glycolytic enzymes have a dual role and can act as RNA binding proteins. For example, GAPDH interacts with *Ifng* mRNA blocking its translation. When the glycolytic demand is high, GAPDH is required for glycolysis and the *Ifng* mRNA is released and translated, increasing the IFN-γ secretion. (C) Post-translational modifications. Certain metabolites can post-translationally modify proteins. For example, succinate is accumulated in the cytosol of activated cells, resulting in the addition of succinyl groups to the glycolytic enzyme PKM2, which loses its tetrameric conformation. The hypersuccinylated dimeric PKM2 enters the nucleus and complex with HIF-1α, enhancing the expression of the pro-inflammatory cytokine IL-1β. (D) Changes in the epigenome. The function of epigenetic remodelers is tightly modulated by the levels of certain metabolites. For example, upon activation, the intracellular levels of acetyl-CoA are increased. The acetyl-CoA is then used to acetylate histones enhancing the expression of pro-inflammatory cytokines. GAP, glyceraldehyde 3-phosphate; Pyr, pyruvate.
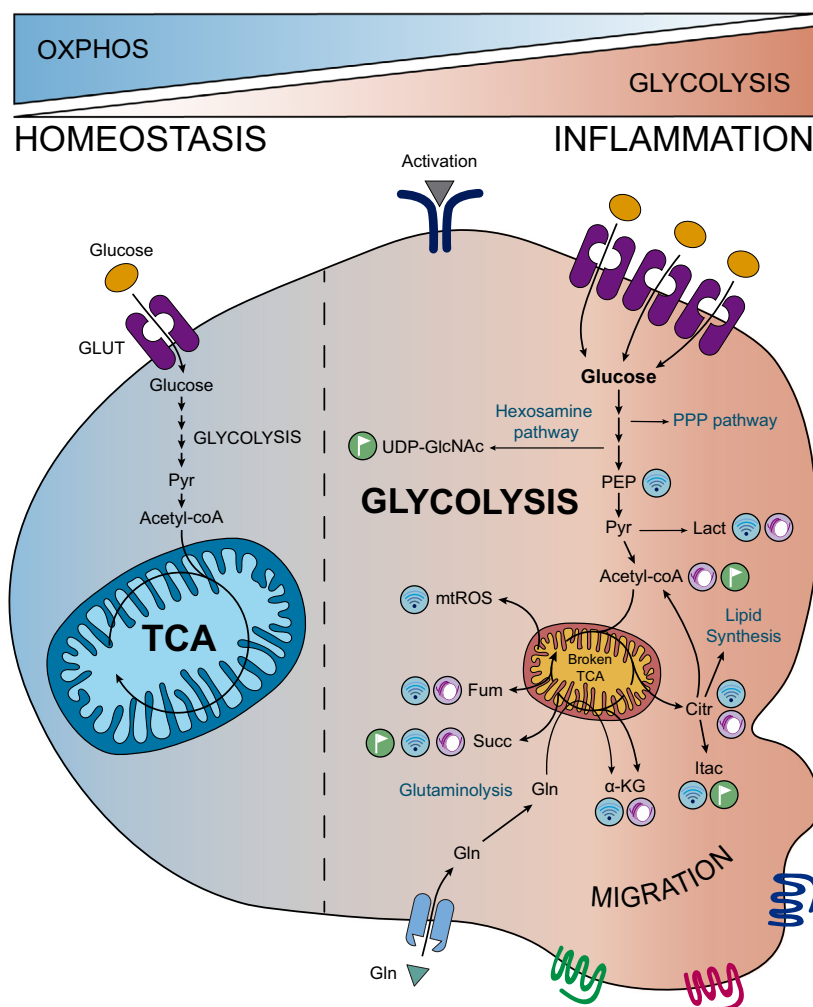
Fig. 3. Glycolysis orchestrates the behaviour of inflammatory cells. Upon inflammation, immune cells are reprogrammed towards glycolysis, enhancing this pathway at the expense of OXPHOS and, in macrophages, disbalancing the TCA cycle. As a consequence, glycolysis and broken TCA cycle-released metabolites such as lactate, citrate or succinate accumulate in immune cells promoting signalling, post-translational and epigenetic modifications which drive the inflammatory response. Moreover, glycolytic intermediates fuel other pathways such as the PPP, which provides biosynthetic molecules, and the hexosamine one, which generates UDP-GlcNAc for glycation reactions. Citr, citrate; Fum, fumarate; Gln, glutamine; Itac, itaconate; Lact, lactate; Pyr, pyruvate; Succ, succinate.

activation [126]. PEP contributes to inflammation by interfering with Ca^{2+} signalling. When T cells are stimulated through the TCR, Ca^{2+} enters from the extracellular space and triggers signalling pathways in the lymphocyte. PEP inhibits the ER calcium channel SERCA, avoiding Ca^{2+} flux to the ER. Consequently, Ca^{2+} levels increase in the cytoplasm, prolonging TCR signalling and activating the NFAT [127]. PEP accumulation has a similar pro-inflammatory action on macrophages, promoting M1 polarization and increasing pro-inflammatory cytokine expression [128]. The final product of glycolysis, lactate, is now known to display signalling properties during inflammation [129-131]. In this sense, lactate suppresses the immune response by impairing the metabolic reprogramming towards pro-inflammatory phenotypes and by blocking pro-inflammatory signalling pathways in monocytes, macrophages and DCs [132-135]. Lactic acid accumulation in DCs drives the switch to an anti-inflammatory phenotype by decreasing activation and increasing

IL-10 secretion [136]. Furthermore, it inhibits T-cell migration [137] as well as $CD8^{+}$ T-cell survival and cytotoxic activity by inhibiting rate-limiting glycolytic enzymes [137]. In addition, lactate also promotes the expansion of the Treg subpopulation [138]. However, it has been reported that lactate-enriched environments enhance Th17 responses [137] and NF-κB-dependent pro-inflammatory activities in macrophages [139]. In ECs, lactate is capable of entering the cells, stimulating the NF-κB/IL-8 pathway and inducing ROS production [140] (Table 1).

One of the metabolites accumulated as a consequence of the broken TCA cycle and high glutaminolysis is succinate. Succinate accumulates in macrophages both upon LPS exposure or in the synovium in rheumatoid arthritis [141]. Succinate accumulation leads to M1 polarization of macrophages by direct inhibition of the prolyl hydrolase, resulting in the stabilization of HIF-1α and IL-1β secretion [60,142]. Succinate also limits the production of the

anti-inflammatory cytokine IL-10 [143]. Some metabolites can act as extracellular signalling molecules. Inflammatory pathways can be modulated by the extracellular succinate levels through the binding to its receptor SUCNR1 [144]. Interestingly, both pro- and anti-inflammatory effects can be mediated by SUCNR1. As mentioned, extracellular succinate triggers the production of the anti-inflammatory prostaglandin E2 in NSCs [117]. In addition, SUCNR1 deletion in myeloid cells promoted a local pro-inflammatory phenotype in adipose tissue, demonstrating that succinate triggers an anti-inflammatory effect in adipose-tissue-resident macrophages [145]. In contrast, extracellular succinate causes a pro-inflammatory effect in different immune cells [146], increasing the migration and the secretion of the pro-inflammatory cytokines TNF-α and IL-1β in DCs and macrophages. In addition, upon DC presentation, succinate improves the production of IFN-γ and TNF-α in T cells without exerting a direct effect [147,148].

Another metabolite that plays a signalling role and is implicated in defining the inflammatory state of cells is citrate, which accumulates in LPS-stimulated macrophages [60,92]. Citrate is generated during the TCA cycle in the mitochondria and is exported by the citrate carrier (CIC) to the cytosol, where it is required for fatty acid synthesis. Once in the cytoplasm, citrate is metabolized by the ATP-citrate lyase (ACLY) to acetyl-CoA and oxaloacetate, precursors for lipid synthesis, ROS and NO. Reducing cytoplasmic citrate levels by deletion of CIC decreases the production of ROS, NO and prostaglandins, impairing the pro-inflammatory differentiation of cells and highlighting the importance that the different subcellular localization of certain metabolites has in the inflammatory response [149].

Itaconate is a metabolite derived from citrate that has been intensively studied due to its antibacterial properties [150]. Citrate can be transformed into cis-aconitate, which exits the mitochondria and is decarboxylated to itaconate. Itaconate is accumulated during the broken TCA cycle in M1 macrophages [150,151]. Interestingly, the absence of endogenous itaconate by genetic deletion of *Igr1* causes an increased production of pro-inflammatory cytokines [152]. In fact, despite itaconate is increased in macrophages upon LPS activation, it is considered as an anti-inflammatory metabolite [152,153]. Itaconate triggers two

Table 1. Metabolites accumulated in inflammatory cells impact the inflammatory pathways through different molecular mechanisms, including intracellular signalling events (indicated in blue), post-translational (in orange) or epigenetic (in purple) mechanisms.

Metabolite	Mechanism	Cell type	Reference
Phosphoenolpyruvate (PEP)	Increased cytosolic Ca^{2+}	T cells	[127]
	Pro-inflammatory cytokine expression (M1 polarization)	Macrophages	[128]
Lactate (Lact)	Multiple blockade of the immune response	Monocytes, macrophages, DCs, NK cells, T cells	[132-135]
	Th17 responses	T cells	[137]
	TLR4/NF-κB activation	Macrophages	[139]
	NF-κB/IL-8 activation	Endothelial cells	[140]
	Histone lactylation (M2 polarization)	Macrophages	[177]
Acetate/Acetyl-CoA	GAPDH acetylation	Memory CD8+ T cells	[162]
	Histone acetylation (Th1 responses)	Macrophages	[178-179,182]
Citrate (Citr)	ROS, NO, PGs	Macrophages	[149,181]
Itaconate (Itac)	SDH inhibition	Macrophages	[154]
	Keap1 alkylation (Nrf2 activation)	Macrophages	[156]
α-Ketoglutarate (α-KG)	mTORC1 activation (Th1 responses)	T cells	[158]
	Histone demethylation (M2 polarization)	Macrophages	[186]
	DNA methylation modification (Th1 responses)	T cells	[187]
2-Hydroxyglutarate (2-HG)	Histone and DNA methylation	T cells	[188,189]
Succinate (Succ)	HIF-1α stabilization (M1 polarization)	Macrophages	[60]
	Protein succinylation	Macrophages	[144,169]
	DNA methylase inhibition	T regulatory cells	[96]
Fumarate (Fum)	Keap1 modification (Nrf2 activation)	Microglia, DCs, T cells	[163-166]
	Demethylase inhibition	Monocytes	[184]
S-adenosylmethionine (SAM)	Histone methylation	Macrophages, T cells	[190,191]

different anti-inflammatory signalling effects in cells. Itaconate directly inhibits succinate dehydrogenase (SDH), stopping the pro-inflammatory signals from succinate dehydrogenation [154]. In addition, itaconate has been recently proved to activate the transcription factors Nrf2 and ATF3 in macrophages, repressing pro-inflammatory genes [155]. Knowledge about ita-conate needs to be carefully interpreted since some studies have used itaconate derivatives instead of ita-conate, such as dimethyl itaconate [153,155,156], which may trigger different effects in cells [157]. α-KG results from glutamine anaplerosis and is accumulated upon IL-2 signalling in Th1 CD4$^+$ and CD8$^+$ cells, increasing Th1 differentiation through mTORC1 activation. In addition, glutamine deprivation or α-KG limitation, have been shown to favour *in vitro* Treg differentiation, even under Th1 stimulating conditions [158].

Post-transcriptional regulation driven by glycolytic enzymes

In the last years, growing evidence supports that gly-colytic enzymes can act as post-transcriptional regula-tors of inflammatory genes. Pioneer work from the Pearce lab revealed that the classical glycolytic enzyme GAPDH is able to bind mRNA. In the naïve state of CD4$^+$ T cells, GAPDH binds IFN-γ and IL-2 mRNA, inhibiting their translation. Therefore, when glycolysis is incremented, GAPDH releases these mRNAs to catabolize substrates, allowing the translation of these inflammatory cytokines [72]. In monocytes, GAPDH carries out a similar regulatory function with TNF-α mRNA [159]. Accordingly, in a wide proteomic approach, glycolytic enzymes (such as GAPDH, pyru-vate kinase, lactate dehydrogenase, enolase and aldo-lase) and enzymes involved in the TCA cycle and lipid synthesis were identified as mRNA binding proteins [160].

The enolase-1 is codified by the gene *ENO1*, which encodes different transcripts besides the enolase-1 mRNA. One of them is a noncoding RNA that specifi-cally binds the promoter region of FoxP3, a master regulator of Tregs. This noncoding RNA impacts the splicing of FoxP3. Therefore, upon T-lymphocyte acti-vation, the expression of *ENO1* is enhanced to fulfil the glycolytic flux and the functional isoform of FoxP3 is repressed, thus inhibiting Treg differentiation [161].

Besides its metabolic function, PKM2 can enter into the nucleus controlling the transcription of certain pro-inflammatory genes. Upon LPS activation, PKM2 interacts with HIF-1α, and this complex can directly bind and activate the IL-1β promoter in bone marrow-derived macrophages (BMDMs) [58].

Post-translational modification of proteins

Some metabolites that accumulate in pro-inflammatory cells can dictate post-translational modifications of proteins such as acetylations, succinylations or lactyla-tions. These post-translational modifications can affect the epigenetic landscape but also other different pro-teins related to inflammation. The levels of acetyl-CoA determine the acetylation of several different proteins for instance histones, tubulin or p53, but also meta-bolic enzymes such as GAPDH. CD8$^+$ memory T cells uptake acetate *in vitro* and metabolize it into acetyl-CoA, which in turn is used to acetylate GAPDH. This acetylation increases GAPDH function, improving the glycolytic flux and increasing IFN-γ production [162]. Itaconate also alters protein function by inducing post-translational modifications. Itaconate directly alkylates cysteine residues of the KEAP1 protein. KEAP1 is usually associated with Nrf2 and promotes its degradation, but alkylation of KEAP1 permits Nrf2 function, enhancing the expression of antioxidant and anti-inflammatory genes [156]. Regarding Nrf2 function, a fumarate derivate, dimethyl fumarate, acti-vates Nrf2 by modifying KEAP1 in a similar way to itaconate. This modification reduces microglial activa-tion, DC maturation and T-cell activation [163-166]. The accumulation of succinate produces succinylation of proteins [167,168]. Succinyl groups can be added to lysines, neutralizing lysine positive charges and thereby likely resulting in conformational changes [167]. Suc-cinylation could be a mechanism for maintaining gly-colysis since several glycolytic enzymes have been found succinylated in macrophages activated with LPS [144]. In fact, succinylation of PKM2 increases IL-1β production in LPS-activated BMDMs [169].

Epigenetics

Epigenetics controls gene expression in several impor-tant biological processes, including inflammation [170,171]. The function of epigenetic remodelers is tightly modulated by the levels of metabolic intermedi-ates and cofactors [172,173]. Additionally, metabolic programmes can determine the epigenetic signature through the control of the NAD$^+$/NADH balance [174-176]. One quintessential example is the role of sir-tuins, histone deacetylases whose epigenetic function requires NAD$^+$. Therefore, metabolism can impact gene expression by regulating cellular metabolites required for epigenetic modifications or by affecting epigenetic remodelers.

Very recently, lactate has been shown to lactylate histones and modify gene expression. *Zhang et al.*

showed that lactate accumulation promotes the expression of M2-characteristic genes during M1 BMDM polarization. After the first hours of macrophages exposure to bacteria, lactate promoted the lactylation of histones, initiating the expression of homoeostatic genes that have been traditionally associated with M2-like macrophages [177]. Thus, lactate could lead a feedback mechanism to drive macrophages to an anti-inflammatory outcome for the resolution phase. In addition, deletion of lactate dehydrogenase A (LDHA), which catalyses the interconversion between pyruvate and lactate, causes a decrease of IFN-γ expression in T cells [178]. Surprisingly, LDHA controls the expression of IFN-γ by modulating the acetyl-CoA pool, which serves as a donor for histone acetylation [179,180]. In $CD4^+$ T cells, acetyl-CoA is used for histone acetylation in the IFN-γ promoter, promoting the differentiation towards Th1 subsets [178,179]. Both citrate and acetyl-CoA levels impact the inflammatory state of macrophages, T cells and microglia through epigenetic modifications. Reducing the acetyl-CoA pool by ACLY deletion decreases the expression of glycolytic enzymes such as hexokinase-2, PKF-1 and LDHA [181]. Increasing acetyl-CoA levels by acetate supplementation rescued this silencing [182]. In low-glucose conditions, acetate is taken up and converted into acetyl-CoA in $CD8^+$ T cells modifying the epigenetic landscape and increasing IFN-γ production [183]. Moreover, the levels of succinate influence the epigenetic state of cells through DNA modifications. Succinate inhibits the ten-eleven translocation (TET) family of DNA methylases in Tregs, blocking expression of suppressive genes but without altering cell proliferation [96].

Another metabolite implicated in epigenetic regulation is fumarate. In monocytes that have been stimulated with β-glucan, a second stimulation with LPS has been shown to trigger a more efficient response. This immunological training of monocytes is explained by an increase of fumarate levels upon β-glucan stimulation. Fumarate decreases the activity of KDM5 demethylases, enabling the expression of genes involved in the immune response and the migration of leucocytes [184]. Therefore, altering metabolism is a way to improve innate immune training that acts through epigenetic modifications.

Furthermore, α-KG is a cosubstrate for the histone demethylases family JmjC [185]. α-KG accumulation induces M2 polarization in BMDMs through histone demethylation of M2-specific genes, resulting in the expression of anti-inflammatory molecules [186]. However, α-KG seems to cause the opposite effect in T cells. This metabolite is accumulated after IL-2

signalling and favours Th1 differentiation, increasing the expression of pro-inflammatory and glycolytic genes by modifying DNA methylation [187]. 2-HG is a structural analogue of α-KG and acts as a competitive inhibitor of α-KG-dependent histone demethylases, promoting histone hypermethylation. 2-HG is accrued upon activation or hypoxia and promotes histone and DNA hypermethylation through the inhibition of Utx and Tet2, respectively. 2-HG increases the expression of CD62L and CD127, differentiating T cells to memory cells [188,189]. Upon activation, glucose-derived ribose and one-carbon metabolism converges in the generation and accumulation of *S*-adenosylmethionine (SAM) in macrophages and T cells. The production of SAM maintains a high SAM/S-adenosylhomocysteine ratio that allows the trimethylation of histones, increasing the expression of pro-inflammatory genes like IL-1β in LPS-stimulated macrophages. Consequently, impairment of SAM production promotes an anti-inflammatory phenotype in macrophages both *in vitro* and *in vivo* [190]. SAM also controls T-cell phenotype by modifying certain epigenetic marks. Decrease of SAM production by methionine restriction impairs T-cell proliferation and cytokine production in both *in vitro* and *in vivo* experiments. SAM restricted T cells showed reduced histone trimethylation and limited Th17 expansion in an EAE model [191].

Given that different metabolites can simultaneously influence cell epigenetics and that each metabolite can modulate the inflammatory status by different mechanisms, it must be considered that the ratio between metabolites will determine the final inflammatory outcome (Fig. 3).

Concluding remarks

A growing body of evidence has accumulated supporting the immunomodulatory properties of glycolysis and TCA cycle-released metabolites during inflammatory processes. Molecules such as succinate, citrate or lactate, which were at first thought to be mere by-products of cellular metabolism, are now regarded as paramount signalling, post-transcriptional, post-translational or epigenetic modulators that dictate the inflammatory response. In the light of the studies about immunometabolism, it is clear that immune cells adapt their metabolism to maintain or change their inflammatory phenotype. However, very recent findings point to a metabolic switch as a result and not as a cause of inflammation [192]. Inflammatory processes are a common feature to many age-associated diseases, such as cardiovascular and neurodegenerative disorders. The finding that immunometabolism could be a

master regulator of inflammation broadens the avenue for treating inflammation-related pathologies through the manipulation of the vascular and immune cell metabolism. Therefore, increased understanding of the metabolic pathways in inflammatory cells could be exploited therapeutically to dampen exacerbated inflammatory responses.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

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