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Review

Lysine Deacetylases and Regulated Glycolysis in Macrophages

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Regulated cellular metabolism has emerged as a fundamental process controlling macrophage functions, but there is still much to uncover about the precise signaling mechanisms involved. Lysine acetylation in macrophages regulates the activity, stability, and/or localization of metabolic enzymes, as well as inflammatory responses. Two protein families, the classical zinc-dependent histone deacetylases (HDACs) and the NAD-dependent HDACs (sirtuins, SIRTs), mediate lysine deacetylation. We describe here mechanisms by which classical HDACs and SIRTs directly regulate specific glycolytic enzymes, as well as evidence that links these protein deacetylases to the regulation of glycolysis-related genes. In these contexts, we discuss HDACs and SIRTs as key control points for regulating immunometabolism and inflammatory outputs from macrophages.

Highlights

Metabolic and immune pathways are intimately linked in that key metabolic enzymes and energy metabolites have a direct influence on pro- and anti-inflammatory responses of macrophages.

In response to danger signals, activated macrophages reprogram nutrient metabolic pathways, for example enhancing aerobic glycolysis, to meet heightened energy requirements and generate sufficient biomolecules to mount an effective innate immune response.

Lysine deacetylases are dual regulators of both metabolic pathways and inflammatory responses of macrophages.

The acetylation status of lysine residues on some key metabolic enzymes can control their enzymatic activity, protein stability, and/or subcellular localization.

Understanding the precise molecular basis of metabolic control of macrophage inflammatory processes could lead to novel approaches to target immunometabolism and inflammation.

Regulated Glycolysis in Myeloid Cell-Mediated Inflammation and Disease

Organismal survival requires the capacity to combat infection, metabolize nutrients, and store energy for times of need. It is therefore not surprising that metabolism and inflammation, two fundamental biological processes that are crucial for sustaining life, are intimately linked. Macrophages are key sentinel cells charged with the task of detecting and responding to homeostatic alterations such as pathogen invasion, metabolic stress, and/or tissue damage. Flexibility in metabolic pathways allows these cells to rapidly respond to changes in their environment. 'Naïve' or resting macrophages utilize glycolysis (Box 1) to generate pyruvate, which is oxidized into acetyl-CoA for use by the tricarboxylic acid (TCA) cycle, feeding electrons in the form of NADH and $FADH_2$ into mitochondrial oxidative phosphorylation (OXPHOS) to generate ATP. However, in response to proinflammatory stimuli, such as those that activate

Box 1. The Glycolysis Pathway

Glycolysis converts one molecule of glucose into two molecules of pyruvate, two molecules of ATP, and two molecules of NADH via a series of intermediate metabolites. Flux through this pathway is regulated in multiple ways: availability of substrate, amount of rate-limiting enzyme present, allosteric regulation by metabolites, and PTMs of the enzymes involved. Glucose uptake is mediated by cell-surface glucose transporters, and key control points in this pathway are the irreversible reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase. Hexokinase catalyzes the first step of glucose metabolism, the rate-limiting ATP-dependent phosphorylation of glucose into glucose-6-phosphate [33]. This phosphorylation event also effectively prevents glucose from exiting the cell and helps commit glucose-6-phosphate towards energy metabolism pathways such as glycolysis and the pentose phosphate pathway [33]. Phosphofructokinase catalyzes the third glycolytic reaction: transfer of a phosphoryl group from ATP to fructose-6-phosphate, yielding fructose-1, 6-bisphosphate (F-1, 6-BP). These two enzymatic reactions consume ATP; however, the second half of glycolysis generates four molecules of ATP, resulting in the net production of two ATP molecules through this metabolic pathway. Pyruvate kinase, which catalyzes the final step of glycolysis, converting phosphoenolpyruvate into pyruvate, is activated by the upstream metabolite F-1, 6-BP. In mammalian cells, pyruvate then has two fates: it is either reduced by lactate dehydrogenase to lactate or is converted by pyruvate dehydrogenase to acetyl-CoA for use in the TCA cycle.

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Toll-like receptors (TLRs), rapid energy and biochemical requirements are met by metabolic reprogramming that favors aerobic glycolysis over OXPHOS. This phenomenon, known as the Warburg effect [1], has been well studied in cancer cells and, more recently, in macrophages and other immune cells [2,3].

Specific alterations in levels of metabolites, as well as in the functions of metabolic enzymes, are associated with metabolic reprogramming in activated macrophages [4]. For example, TLR signaling instructs the cell to increase glucose uptake and convert it to lactate, whereas OXPHOS is suppressed by disruptions in the electron transport chain [5]. Carbon-tracing experiments [6] demonstrate fragmentation at specific points in the TCA cycle, resulting in a build-up of key metabolites such as citrate that can be used for fatty acid synthesis and/or converted to itaconate, which has both antimicrobial [7] and anti-inflammatory [8] properties. Succinate accumulates in activated macrophages, also as a consequence of TCA cycle disruption, and acts as a molecular switch for the initiation of inflammatory responses; this metabolite stabilizes the transcription factor HIF-1α, thus enabling inducible expression of proinflammatory and glycolytic genes [9]. Studies in proliferating cancer cells have been highly informative for understanding how metabolic enzymes control immunometabolism in immune cells. For example, in most cells, pyruvate kinase M (PKM) catalyzes the final step of glycolysis – the conversion of phosphoenolpyruvate to pyruvate. However, in rapidly dividing cells and activated immune cells, an alternatively spliced isoform of PKM, PKM2, acts in an alternative role as a transcriptional coactivator to drive expression of glycolytic and inflammatory genes. In this way, PKM2 plays a central role in driving the shift towards aerobic glycolysis. From a therapeutic standpoint, interfering with these metabolic adaptations, for example by inhibiting glycolytic flux using 2-deoxy-D-glucose [9,10] or by interfering with the transcriptional functions of PKM2 [11,12], reduces macrophage inflammatory outputs and inflammation-related pathology. Nonetheless, our understanding of the precise molecular mechanisms of metabolic control of innate immune processes is still rudimentary. Deciphering such mechanisms, together with identifying the key signaling metabolites and regulatory enzymes that control this metabolic switch, may unlock new opportunities to target immunometabolic circuits in immune cells to dampen inflammation-associated pathology. Lysine deacetylases are key suspects given their well-established roles as cancer- and inflammation-associated regulatory enzymes that can also be modulated pharmacologically. Intriguingly, the capacity of metabolic enzymes to be regulated by lysine acetylation seems to be a common mechanism of control (Figure 1, Key Figure), positing this post-translational modification as a possible molecular switch, and an important pharmacological target, for regulating cellular metabolism and inflammation.

Roles for Lysine Deacetylases in Immunometabolism in Macrophages

Reversible lysine acetylation is a common post-translational modification (PTM) that is now implicated in both immunological and metabolic pathways. This PTM is controlled by histone deacetylases (HDACs, that remove acetyl group from lysine residues) and histone acetyltransferases (HATs, that add acetyl groups to lysine residues). The HDAC family includes the classical zinc-dependent HDACs (classes I, II and IV) and the NAD^+-dependent class III HDACs, also known as silent mating-type information regulation 2 (sirtuins or SIRTs). Emerging evidence suggests that the classical HDACs and SIRTs modify the activity, stability, and localization of some metabolic enzymes to regulate immunometabolism. Furthermore, in carrying out their biochemical functions, HDACs also control the availability of certain intermediary metabolites. These can play important secondary signaling roles, directing the fate and function of a cell in response to external stressors and nutritional cues.

Like itaconate and succinate, acetyl-CoA is a metabolite used by cells to link nutrient availability and metabolic status with cellular signaling, chromatin structure, and gene expression.

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Key Figure

Examples of Metabolic Pathways Regulated by Deacetylation

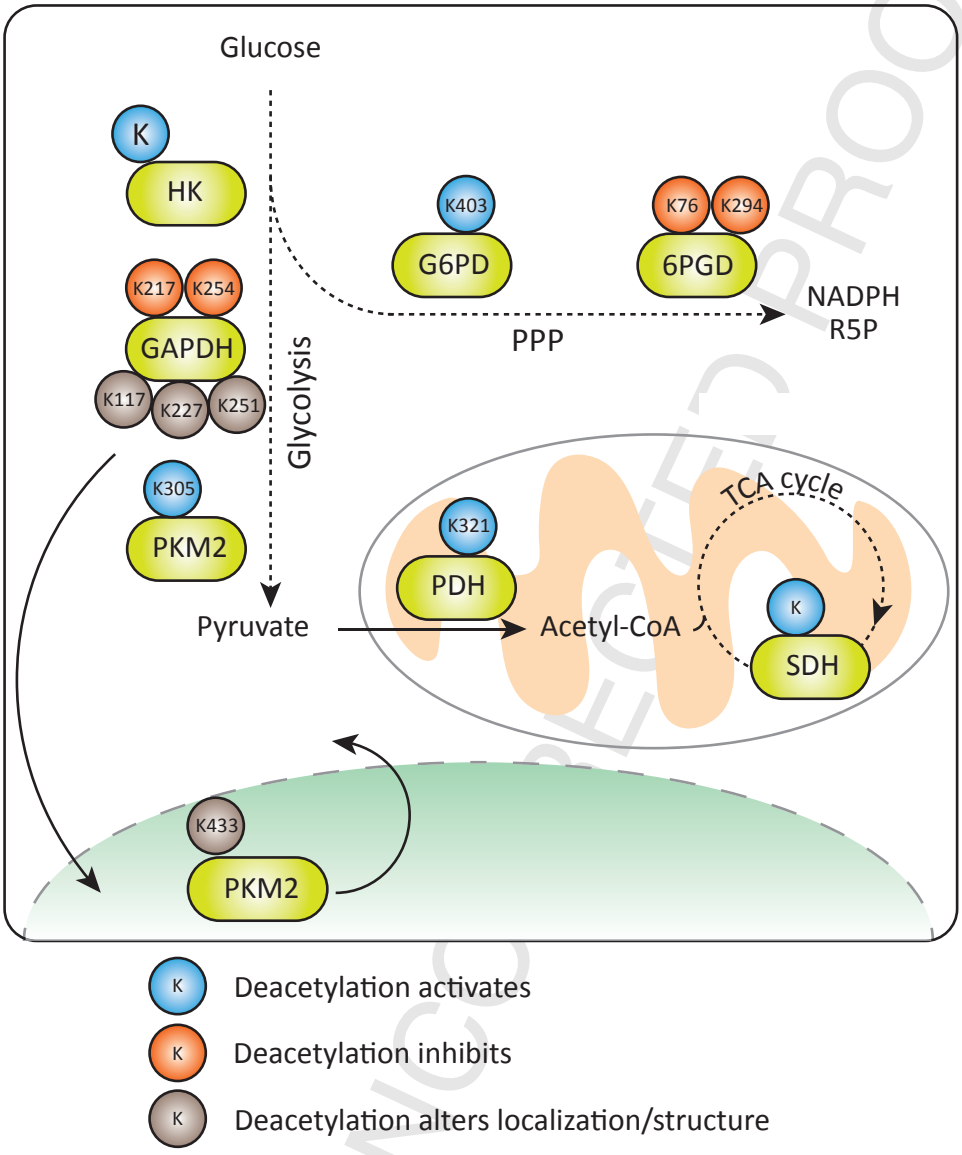


Figure 1. Examples are provided of how lysine deacetylation regulates metabolic enzymes that play key gatekeeping roles in metabolic pathways. Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; HK, hexokinase; PDH, pyruvate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; PKM2, pyruvate kinase M2; SDH, succinate dehydrogenase; TCA cycle, tricarboxylic acid cycle.

Numerous metabolic pathways, including glycolysis-derived pyruvate oxidation and β-oxidation of fatty acids, generate a mitochondrial pool of acetyl-CoA, which is metabolized via the TCA cycle to yield NADH. Distinct cytosolic and nuclear pools of acetyl-CoA can also be created from mitochondrial export or by other enzymatic reactions [13]. Acetyl-CoA in each compartment is used as the donor for HAT-mediated addition of acetyl groups to lysine residues of target proteins. Classical HDACs, which carry out the reverse reaction of lysine deacetylation, generate acetate that can then be converted by acetyl-CoA synthetases to acetyl-CoA [14,15], thus providing an additional means of generating this metabolite. As discussed in this review, deacetylation of lysine residues on rate-limiting metabolic enzymes could also either inhibit or increase enzyme activity to support nutrient flux through a particular pathway. Thus, by regulating both the cellular acetyl-CoA pool and the acetylation status of metabolic enzymes, HATs and HDACs can control cellular metabolism. In keeping with this concept, small-molecule inhibitors of classical HDACs alter cellular metabolism [16,17]. For example, HDAC inhibition in myeloma cells resulted in decreased glucose uptake over a 48 h period as a result of repression of glucose transporter expression and inhibition of the glycolytic enzyme hexokinase [16]. There is also some evidence to suggest that there is interplay between the metabolic shift and the proinflammatory functions of specific HDACs in activated macrophages [18].

NAD^+ is another metabolite used as a coenzyme in metabolic reactions, as well as a co-substrate for the SIRTs. During glycolysis, NAD^+ is reduced to NADH, and then oxidized back to NAD^+ during reduction of pyruvate to lactate or during mitochondrial OXPHOS. Cells therefore use the NAD^+/NADH ratio to monitor metabolic activity and redox state. SIRT-mediated deacetylation of target proteins is coupled to the cleavage of NAD^+ to nicotinamide (NAM), and the *O*-acetyl-ADP-ribose moiety of NAD^+ acts as the acceptor of the acetyl group. Dynamic changes occur in the NAD^+/NADH ratio during TLR activation of macrophages, and these are sensed by SIRTs to instruct the metabolic shift that supports early- and late-phase responses [19]. NAM acts as an endogenous feedback inhibitor of SIRT activity and has been shown in multiple systems to inhibit inflammatory pathways. For example, adequate NAD^+ was required for optimal lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF) production and NAM-inhibited SIRT activity to reduce, via translational control, the synthesis of this important inflammatory cytokine [20]. Thus, the acetylation status of metabolic enzymes, as well as levels of metabolic intermediates that are generated by the protein deacetylase activity of classical HDACs (acetyl-CoA) and SIRTs (NAM), control metabolic responses and inflammatory outputs from macrophages.

The Lysine Deacetylase Family

Classical HDACs

Members of the classical HDAC family (classes I, II and IV), encompassing HDAC1–11, utilize zinc as a cofactor in the catalytic site and are subdivided based on the homology of their deacetylase domains [21]. These enzymes developed before the evolution of histones, and it is now known that lysine deacetylation is a PTM that regulates many non-histone proteins. Class I HDACs (1, 2, 3, and 8) are mostly restricted to the nuclear compartment and are responsible for histone deacetylation and epigenetic control of gene expression. Class IIa HDACs (4, 5, 7, and 9) share high homology in their C-terminal deacetylase domains, and also contain an N-terminal protein interaction region and nuclear export and import sequences. These proteins shuttle between the nucleus and cytoplasm in response to a variety of cell signals such as growth factors. Some reports have also demonstrated that members of this subfamily can localize to other cellular compartments such as the mitochondria [22]. These properties suggest that class IIa HDACs likely exert at least some effects by acting on non-

histone substrates. HDAC6 and HDAC10, which comprise the class IIb subfamily, are cytoplasmic enzymes that are distinguished by their duplicated deacetylase domains. HDAC6 is known to deacetylate multiple cytosolic proteins such as tubulin, HSP90, and peroxiredoxins. The sole class IV member, HDAC11, shares sequence similarity with both class I and II HDACs. Although it has not been as extensively studied as the other classical HDACs, recent reports have demonstrated important functions for this enzyme in immune cells, for example in acting to constrain effector T cell function [23], to repress IL-10 in antigen presenting cells [24], and to regulate proinflammatory cytokines in neutrophils [25]. Small-molecule inhibitors of classical HDACs are approved clinically for oncological applications and have shown therapeutic promise in experimental models of inflammatory diseases [21]. The most widely studied of these are broad-spectrum inhibitors that target all or many of the classical HDACs by binding to zinc within the catalytic site. More recently, class or isoform-selective HDAC inhibitors have been developed by modification of the moiety that interacts with the rim region of the active site [26] or by taking advantage of residue differences in the active sites of class I and IIb versus IIa HDAC enzymes [27]. An in-depth understanding of how HDACs regulate metabolic pathways in immune cells may provide insights into the molecular basis for anti-inflammatory effects of broad-spectrum HDAC inhibitors, and should also help to guide the development of more specific inhibitors targeting individual HDACs for applications in inflammation-related diseases.

Sirtuins (SIRTs)

The sirtuins, or class III HDACs, are highly conserved from bacteria to humans [28]. They are NAD^+-dependent protein deacetylases and/or mono-ADP-ribosyltransferases. Mammals contain seven sirtuins (SIRT1–7) that have high sequence homology across their catalytic and NAD^+-binding domains [29]. However, each member differs in subcellular localization and catalytic activity (Table 1). SIRT1 and SIRT2 shuttle between the nucleus and cytoplasm in a cell cycle- and cell-type dependent manner. SIRT3–5 are mitochondrial sirtuins, while SIRT6 is found exclusively in the nucleus. SIRT7 is found in both the nucleus and cytoplasm. Substrates of nuclear sirtuins include histones and non-histone proteins such as nuclear transcription factors and cofactors, while cytoplasmic and mitochondrial sirtuins play important roles in deacetylating key enzymes involved in glycolysis, fatty acid oxidation (FAO), the TCA cycle, and other oxidative and metabolic pathways [29]. Because sirtuins are expressed throughout the body, they function as cellular energy sensors and modulate a wide range of physiological and metabolic processes. Dysregulation of their activity has been associated with various metabolic, infectious, and neurological diseases, while their activation reportedly has beneficial effects in some preclinical rodent models [30], making them promising targets for pharmacological intervention in human diseases.

HDACs as Signaling Hubs in Regulating Immunometabolism

Given that the functions of some key metabolic enzymes are regulated by lysine acetylation (below), and that metabolites generated through the actions of lysine deacetylases also control immune processes, these enzymes are likely to be important in controlling cellular metabolism and inflammatory responses. Numerous studies have linked specific classical HDACs [21] and SIRTs [31] to inflammatory responses, but whether these effects relate to effects on metabolic pathways has not yet been widely investigated. Below we describe studies across different biological systems that implicate regulated lysine deacetylation in the control of aerobic glycolysis and the pentose phosphate pathway, and describe how some of these modifications may be involved in regulating inflammatory outputs from innate immune cells such as macrophages.

Q11 Table 1. Human Sirtuins: Localization, Enzymatic Activity, and Roles in Glycolysis

Sirtuin	Location	Main enzymatic activities	Substrates involved in glycolysis and functions	Refs
SIRT1	Nucleus, cytoplasm	Deacetylation, ADP-ribosylation	Activates PGC-1α, which attenuates transcription of glycolytic genes. Regulates transcription of the *HIF1A* gene by deacetylating H3K14 and also deacetylates HIF-1α directly, leading to decreased glycolysis. Inhibits glycolysis by deacetylating the glycolytic enzyme PGAM-1	[65,71,94]
SIRT2	Nucleus, cytoplasm	Deacetylation	Deacetylates PEPCK that is required for maintaining glucose homeostasis. Deacetylates K305 of PKM2. Deacetylates HIF-1α	[54,66,95]
SIRT3	Nucleus, mitochondria	Deacetylation	Represses glycolysis by destabilizing HIF-1α. Overexpression of SIRT3 increases glycolysis and mitochondrial respiration. Deacetylates K321 of PDH, leading to activation of the PDC	[62,67,96]
SIRT4	Mitochondria	Deacetylation, ADP-ribosylation	ADP-ribosylates glutamate dehydrogenase (GDH), downregulating its activity and promoting glutamine/glutamate metabolism and insulin secretion. Overexpression of SIRT4 increases glycolysis and mitochondrial respiration	[96,97]
SIRT5	Mitochondria	Demalonylation, deacetylation	Protein demalonylation by SIRT5 maintains glycolysis, and malonylation of K184 of GAPDH results in reduced glycolysis. Overexpression of SIRT5 increases glycolysis and mitochondrial respiration	[96,98]
SIRT6	Nucleus	Deacetylation, ADP-ribosylation	Deacetylates H3K9 at the promoter of the *HIF1A* gene, thereby repressing *HIF1A* transcriptional activity leading to diminished glycolysis under homeostatic conditions. Deacetylates K433 of dimeric PKM2	[52,84]
SIRT7	Nucleus, cytoplasm	Deacetylation	No current evidence for regulation of glycolysis	

Lysine Deacetylases and the Control of Glycolysis

The Glycolysis Pathway

Although glycolysis is a relatively inefficient means of generating ATP, heightened flux through this pathway in macrophages is essential for the rapid generation of energy, biosynthetic intermediates, and cofactors for enzymes, all of which are required for inducible inflammatory responses. Lysine acetylation/deacetylation has a well-established role in controlling metabolic enzymes in the TCA cycle, particularly through the actions of sirtuins (Box 2). Although somewhat less studied, this PTM has also been implicated in regulating the stability and/or activity of some glycolytic enzymes (Figure 2). Below we describe known roles for specific glycolytic enzymes in the control of innate immune functions and evidence for regulated acetylation of these enzymes (Table 2 and Figure 3), as well as gene regulatory mechanisms by which classical HDACs and SIRTs influence glycolysis. Interestingly, the pentose phosphate pathway is coupled to glycolysis and inducible effector functions of macrophages, and enzymes in this pathway are also regulated by lysine deacetylases (Box 3 and Figure 2).

Box 2. Acetylation, the TCA Cycle, and Immunometabolism

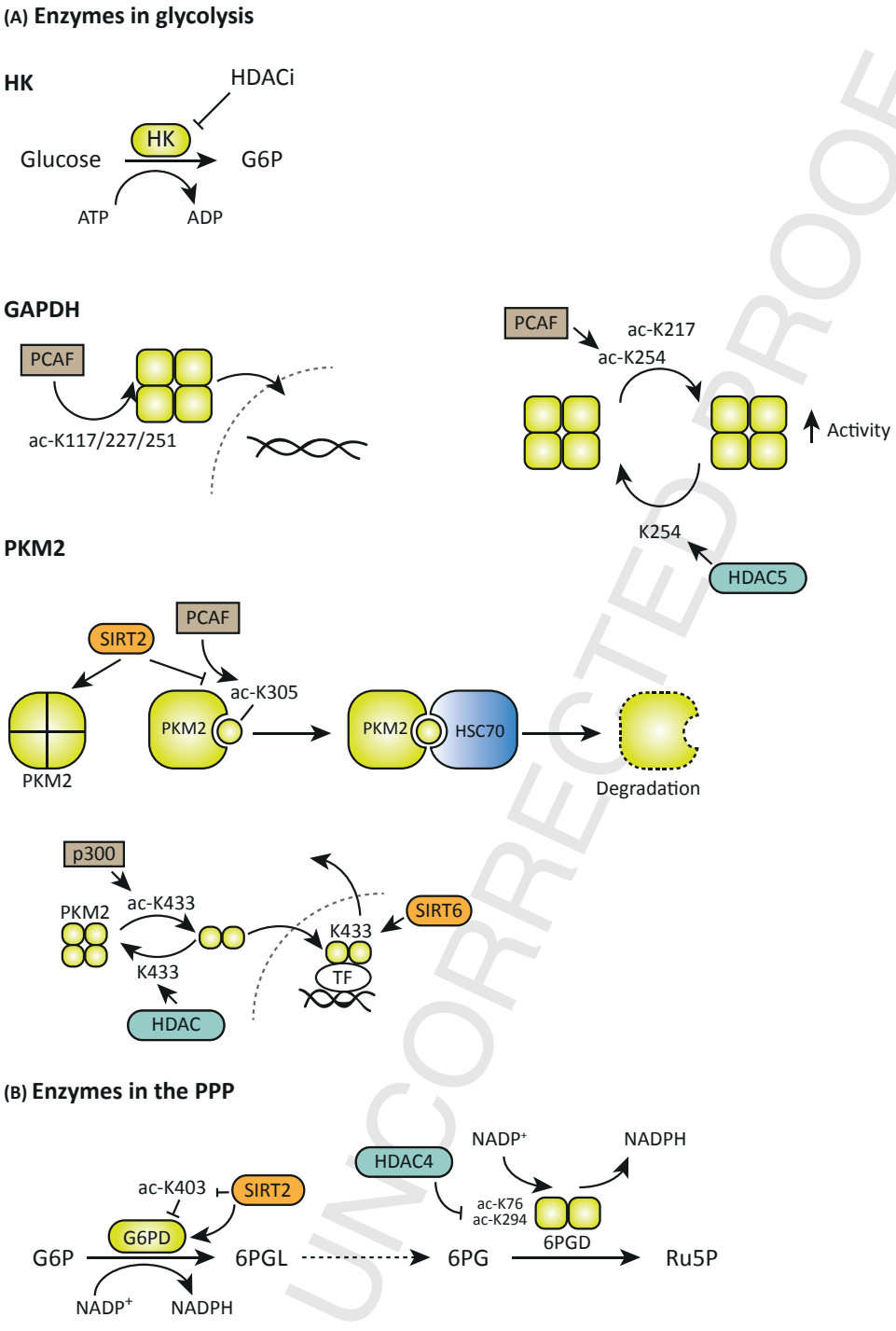
In the mitochondria, eight enzymatic reactions in the TCA cycle utilize pyruvate-derived acetyl-CoA to transfer electrons to NAD or FAD, generating two ATP, six NADH molecules, and two $FADH_2$ per glucose molecule for use by the ETC. Every TCA enzyme can be acetylated [85] and, similarly to glycolytic enzymes, distinct changes in TCA cycle enzyme expression and/or function are crucial for the accumulation of metabolic intermediates such as succinate, itaconate, malate, and fumarate that act as signaling molecules to regulate macrophage immunometabolism. For example, citrate exported from the mitochondria can be converted into cytosolic acetyl-CoA and used for histone acetylation and the expression of glycolytic genes [76]. Citrate can also be converted to itaconate, which inhibits succinate dehydrogenase (SDH) to promote accumulation of succinate [8]. This provides a mechanism to promote proinflammatory (e.g., IL-1β) and reduce anti-inflammatory (e.g., IL-10) gene expression [9,86], as well as to amplify ROS production [86]. SDH has dual functions: within the TCA cycle it oxidizes succinate to fumarate but also operates as complex II of the ETC. Acetylation of SDH at the active site interferes with substrate binding and decreases enzymatic activity [87], whereas deacetylation by mitochondrial SIRT3 increases SDH enzymatic activity [87,88]. Other mitochondrial enzymes that can be targeted by SIRT3 include acetyl-CoA synthetase 2, isocitrate dehydrogenase, and glutamate dehydrogenase. Thus, regulated acetylation provides a means of controlling the activity of SDH and other mitochondrial enzymes, and, in turn, immunometabolism.

Hexokinases (HKs)

The four isoforms of hexokinase (HK-I, HK-II, HK-III, and HK-IV, encoded by *HK1*, *HK2*, *HK3*, and *GCK*, respectively) vary in tissue expression and subcellular location, and also differ in their affinity towards glucose [32]. HK enzyme activity is inhibited by its enzymatic product, glucose-6-phosphate, enabling tight regulation through feedback inhibition [33]. Thus, dysregulated expression and/or activity of HKs is a major contributor to the increased glucose gorging and elevated glycolysis that facilitate cancer cell proliferation and metastasis [33,34]. HKs also have key functions in innate immunity. HK-I-dependent glycolysis was required for activation of the NLRP3 inflammasome in LPS-primed mouse macrophages [35]. By contrast, HK-II plays a dominant role in TLR-inducible glycolysis in mouse dendritic cells [36], implying that different HKs may control the glycolytic shift in different immune cell populations. This study also showed that localization of HK-II to mitochondria was important for the glycolytic shift in DC. Intriguingly, another study showed that peptidoglycan activated the NLRP3 inflammasome through *N*-acetylglucosamine-mediated inhibition of HK, resulting in its dissociation from mitochondrial outer membranes [37]. Thus, it would appear that the subcellular localization of HKs dictate their immunological functions. Whether such functions are controlled by lysine acetylation remains be determined. However, a global acetylomics screen identified HK-I and HK-II as acetylated proteins in human cell lines [38]. Furthermore, broad-spectrum classical HDAC inhibition in multiple myeloma cells decreased HK enzymatic activity [16], suggesting that one or more of the classical HDACs may contribute to deacetylation-mediated activation of this crucial step in the glycolytic pathway. These studies give impetus to future investigations on regulated deacetylation of HK during macrophage activation.

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)

GAPDH catalyzes the conversion of glyceraldehyde-3-phosphate (G3P) to 1,3-bisphospho-D-glycerate. This enzyme was originally thought to reside solely in the cytoplasm as a tetramer of four identical 37 kDa subunits that perform its well-characterized glycolytic function [39]. However, based on its oligomerization state, PTM, and subcellular localization, GAPDH can execute a multitude of other important cellular functions. Interestingly, in mammalian cancer cells GAPDH was recently predicted to be a key enzyme in regulating aerobic glycolysis [40]. That study proposed that the regulation of energy metabolism and cellular homeostasis by GAPDH is much more complex than was initially thought. Emerging evidence suggests that PTMs of GAPDH may control its subcellular localization and functions, acting as a molecular switch for both inducible glycolysis and proinflammatory mediator production.



Q9 Figure 2. Acetylation Regulates Glycolytic Enzyme Form and Function. (A) Examples of glycolytic enzymes regulated by acetylation, as well as the specific acetyltransferases/deacetylases implicated in these processes. Hexokinase (HK), a rate-limiting enzyme in the glycolysis pathway, phosphorylates glucose to generate glucose-6-phosphate (G6P). This enzyme is known to be lysine-acetylated [38], and histone deacetylase inhibition (HDACi) reduced the activity of this enzyme [16], implicating HK deacetylation in inducible glycolysis in activated macrophages. Multiple lysine residues of

(Figure legend continued on the bottom of the next page.)

In macrophages, binding of GAPDH to the 3′-untranslated region (UTR) of *TNF* mRNA couples expression of this key inflammatory cytokine with the cellular metabolic state. When glycolytic rate is low, binding of GAPDH represses *TNF* expression through post-transcriptional mechanisms. Enhanced glycolysis dissociates the complex, presumably by increasing the amount of GAPDH substrate (G3P) to compete at the enzymatic site, simultaneously enabling inducible TNF expression and regulated immunometabolism [41,42]. A similar mechanism occurs in T cells, where GAPDH directly binds to mRNA encoding IFN-γ and prevents its translation [43]. When T cells are activated, increased glucose influx promotes the disassociation of GAPDH from IFN-γ mRNA, allowing translation and secretion of this effector cytokine, as well as amplified aerobic glycolysis that is required for T cell proliferation [43]. Intriguingly, another study showed that GAPDH was recruited to the plasma membrane in activated macrophages. At the membrane it functioned as an immune receptor for plasminogen, controlling the recruitment of macrophages to sites of inflammation [44]. Collectively, these studies suggest that the cellular metabolic state and subcellular localization of GAPDH dictates its role in regulating immunometabolism.

Emerging evidence suggests that regulated lysine acetylation of GAPDH may be an important switch that controls its dual metabolic and immune regulatory functions. For example, in response to systemic bacterial infection, acetylation of GAPDH on lysine (K) 217 in mouse memory $CD8^+$ T cells supported GAPDH enzymatic activity. The enhanced glycolytic rate was required for optimal memory recall responses to *Listeria monocytogenes* [45]. In cancer cells, high glucose levels promoted the acetylation of GAPDH at K254, enhancing its enzymatic activity to support proliferation [46]. This inducible acetylation was mediated by the acetyltransferase PCAF, whereas glucose deprivation promoted an interaction with HDAC5, its deacetylation, and a reduction in GAPDH enzymatic activity [46]. Acetylation at other residues (K117, 227, and 251) was also required for GAPDH nuclear translocation under apoptotic stress in NIH3T3 cells [47]. Whether the regulation of GAPDH acetylation by glucose availability in macrophages alters its subcellular localization and/or interaction with cytokine mRNAs is unknown at this stage. Nonetheless, the effects of stimuli that control immunometabolism in macrophages, such as TLR ligands, on GAPDH acetylation/deacetylation warrant further investigation.

The Pyruvate Kinase M Isoform PKM2

The various non-glycolytic functions of PKM2 in inflammatory cells position it as a hallmark enzyme that bridges metabolism and immunity. In the glycolytic pathway, tetrameric PKM2 catalyzes the rate-limiting final step of glycolysis by transferring the phosphate group of phosphoenolpyruvate to ADP, producing pyruvate and ATP. This tetrameric form relies on the availability of the upstream intermediate fructose-1,6-bisphosphate (F-1,6-BP), and

GAPDH can be acetylated (ac). PCAF-mediated acetylation of GAPDH on K117/227/251 was required for nuclear translocation and subsequent moonlighting functions in gene regulation. Acetylation of lysine (K) 217 [45] and K254 [46] enhanced the glycolytic enzymatic activity of GAPDH, whereas deacetylation by HDAC5 reduced it [46]. Acetylation of lysine residues on PKM2 can regulate its functions in multiple ways. PCAF-mediated acetylation of K305 promotes its association with chaperone HSC70, targeting PKM2 for lysosomal degradation [53], and SIRT2 can deacetylate this residue to promote tetramer formation [54]. PCAF-mediated acetylation on K433 of the cytosolic, tetrameric form of PKM2 interferes with FBP binding and promotes PKM2 dimer formation [51]. Nuclear SIRT6 can deacetylate dimeric PKM2, enhancing its nuclear export [52]. (B) Key enzymes in the pentose phosphate pathway (PPP) are regulated by acetylation. Acetylation of K403 on G6PD inhibited enzymatic activity, whereas deacetylation by SIRT2 enhanced its activity [91]. 6PGD can be regulated by acetylation at two sites; K76 acetylation promotes $NADP^+$ binding and K294 acetylation promotes active dimer formation. HDAC4 can deacetylate both of these residues [93]. Abbreviations: G6P, glucose-6-phosphate; 6PGL, 6-phosphogluconolactone; 6PG, 6-phosphogluconate; Ru5P, ribulose-5-phosphate.

Table 2. Identification of Lysine Residues in Glycolytic Enzymes Regulated by Acetylation

Enzyme	Acetyl-lysine residue(s)	Functional consequence of lysine acetylation/ deacetylation	Refs
Hexokinase (HK)			
Hexokinase domain-containing 1	K173[a], K174[a]	Currently unknown, residues identified by global proteomic screening	[85]
HK-I	K453[a]	Currently unknown, residues identified by global proteomic screening	[38]
HK-II	K337[a], K346[a]		
Phosphofructokinase-1 (PFK1)			
6-Phosphofructokinase type C	K797[a]	Currently unknown, residues identified by global proteomic screening	[85]
Phosphofructokinase, muscle	K113[a]		
Phosphofructokinase, platelet	K776[a]		
6-Phosphofructokinase, liver type	K762[a]		
Aldolase	K12, K41, K146	Acetylation of K146 is predicted to decrease enzyme activity	[99]
Triosephosphate isomerase (TPI)	K222[a]	Currently unknown, residues identified by global proteomic screening	[99]
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	K117, K227, K251, K254	Acetylation of K117, K227, and K251 is required for nuclear translocation of GAPDH. HDAC5-mediated deacetylation of K254 decreases enzyme activity	[46,47]
Phosphoglycerate kinase-1 (PGK1)	K388	ARD1-mediated K388 acetylation of PGK1 increases kinase activity to phosphorylate Beclin1 S30 and promote autophagy initiation and tumorigenesis	[100]
Phosphoglycerate mutase (PGAM)			
PGAM1	K251, K253, K254	Acetylation enhances enzyme activity. SIRT1-mediated deacetylation decreases enzyme activity	[94]
PGAM2	K100	Acetylation decreases enzyme activity. SIRT2-mediated deacetylation increases enzyme activity	[101]
PGAM1/2	K100, K106, K113, K138	SIRT2-mediated deacetylation decreases enzyme activity	[102]
Enolase	K71[a]	Currently unknown, residues identified by global proteomic screening	[99]
Pyruvate kinase M2 (PKM2)	K305, K433	K305 acetylation decreases enzyme activity and increases lysosomal degradation. K433 acetylation prevents activation and promotes nuclear accumulation and kinase activity	[51,53]
Pyruvate dehydrogenase (PDHA1)	K321	SIRT3-mediated deacetylation increases PDHA1 enzyme activity and attenuates the Warburg effect	[62]
Lactate dehydrogenase A (LDHA)	K5	Acetylation decreases enzyme activity. SIRT2-mediated deacetylation increases enzyme activity. LDHA acetylation decreases cell proliferation and migration. SIRT3-mediated deacetylation increases enzyme activity	[103,104]

[a]Lysine residues identified using global proteomic screening in the indicated reference; peptide sequences were matched to reference sequences to identify the specific lysine residue(s) indicated here.

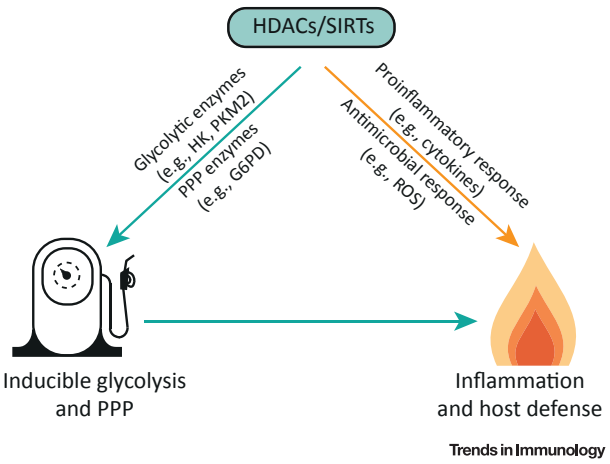


Figure 3. Deacetylases as Potential Links Between Regulated Metabolism and Inflammatory Outputs of Macrophages. Individual HDACs and SIRTs have both pro- and anti-inflammatory functions in macrophages. In the context of the former, these lysine deacetylases positively regulate the function or expression of key enzymes in the glycolysis pathway (e.g., HK, PKM2) and the PPP (e.g., G6PD), as well as specific functional responses of macrophages (e.g., proinflammatory cytokine production, antimicrobial responses). Thus, deacetylases are strong candidates as molecular links between regulated metabolism and biological responses of macrophages (teal arrows). In addition, HDACs and SIRTs are also likely to regulate some macrophage functions in host defense and inflammation independently of metabolic control (orange arrow). Abbreviations: HDAC, histone deacetylase; PPP, pentose phosphate pathway; ROS, reactive oxygen species; SIRT, sirtuin.

dissociation of the tetramer to a dimeric form occurs when concentrations of this intermediate are low [48] or when phosphorylation of Y105 disrupts binding of F-1,6-BP [48]. The reduced enzymatic activity of dimeric PKM2 allows build-up of key glycolytic intermediates to be used as precursors for protein, lipid, and nucleic acid synthesis. Extracellular signals such as growth factors and inflammatory stimuli can also induce the formation of dimeric PKM2, which reportedly has functions that are independent of its role as a glycolytic enzyme, including as a nuclear protein kinase and a coactivator of transcription factors [11]. For example, TLR4 signaling in macrophages induced association of PKM2 with HIF-1α in the nucleus to drive expression of glycolytic (e.g., *Ldha*) and inflammatory genes (e.g., *Il1b*) [11]. In addition, in macrophages from patients with coronary artery disease, nuclear translocation of dimeric

Box 3. Control of the Pentose Phosphate Pathway (PPP) by Lysine Acetylation

The PPP utilizes glycolysis-derived glucose-6-phosphate (G6P) to generate reducing equivalents in the form of NADPH and ribose-5-phosphate for nucleotide synthesis. In TLR-activated macrophages, flux through the PPP is enhanced to provide NADPH for the oxidative respiratory burst and to generate glutathione as a buffer for ROS. Glucose-6-phosphate dehydrogenase (G6PD), the first and rate-limiting enzyme in the PPP, has been extensively implicated in inflammatory responses and is regulated by acetylation. G6PD catalyzes the oxidation of G6P into 6-phosphoglucono-δ-lactone, with concomitant reduction of $NADP^+$ to NADPH. Active G6PD exists as a dimer of two identical monomers, with each monomer containing a G6P-binding site and a catalytic coenzyme site that binds $NADP^+$. LPS and free fatty acids stimulate G6PD expression in macrophages, and its expression is elevated in adipose tissue macrophages from obese patients. The elevated expression of this enzyme promoted oxidative stress and activated the p38 MAPK and NF-κB signaling pathways to drive expression of proinflammatory cytokines [89]. Conversely, deletion of G6PD attenuated macrophage inflammatory responses, improved insulin sensitivity, and reduced chronic tissue inflammation in a model of diet-induced obesity [90]. Targeting aberrant expression and/or activity of G6PD would thus be predicted to reduce inflammation-associated pathology. Proteomic acetylome profiling has identified seven lysine residues on G6PD that can be acetylated (K89, K171, K386, K403, K432, K497, and K514), and subsequent studies have identified a regulatory role for K403 acetylation. In HEK293 cells, active dimer formation was prevented by K403 acetylation, thereby reducing G6PD enzymatic activity [91]. Conversely, deacetylation of this residue by SIRT2 activated enzyme activity and enhanced NADPH production [91,92]. Given the importance of the respiratory burst for antimicrobial responses, a detailed understanding of how acetylation regulates PPP enzyme activity in macrophages may have implications for host-directed antimicrobial therapies.

PKM2 facilitated binding with STAT3 to promote the expression of IL-1β and IL-6 [12]. Despite such evidence (and that described below), the protein kinase activity of PKM2 remains somewhat controversial; for example, Hosios *et al*. [49] could find no evidence of direct transfer of phosphate from ATP to protein by PKM2. Interestingly, dimeric PKM2 was also detected in the circulation of cancer patients, and was shown to promote angiogenesis [50]. The contributions of extracellular PKM2 to inflammation are not well understood at this stage, and indeed there is still much to uncover about the specific molecular mechanisms by which intracellular PKM2 drives macrophage inflammatory responses.

Several lysine residues in PKM family members can be regulated by acetylation. K433 is located in PKM2-specific exon 10 and has been linked to the switch between its cytoplasmic metabolite kinase activity and nuclear protein kinase function. Acetylation of this residue by the HAT p300 interfered with the ability of F-1,6-BP to bind to PKM2, driving the switch to a dimer and promoting nuclear accumulation and its protein kinase activity [51]. In cancer cells, this promoted cell proliferation and tumorigenesis, and cotreatment with a pan inhibitor of classical HDACs (trichostatin A) and NAM enhanced this modification, implicating both HDACs and SIRTs in its regulation. Additional studies in cancer cells showed that nucleus-localized SIRT6 can deacetylate K433 of dimeric PKM2 to promote nuclear export and loss of its protein kinase activity [52]. Also in cancer cells, PCAF-mediated acetylation of K305 in response to high glucose decreased classical pyruvate kinase activity, enhanced the accumulation of glycolytic intermediates, and promoted autophagy-mediated degradation of PKM2 [53]. SIRT2 deacetylated this residue to promote tetrameric pyruvate kinase activity [54], and treatment with trichostatin A plus NAM decreased PKM2 protein expression [53], indicating that deacetylation of this residue is able to stabilize the protein. Finally, in breast cancer cells, another pan classical HDAC inhibitor, SAHA, induced acetylation of K305 [55]. No functional outcome of this was described, and a clear delineation of how classical HDACs and SIRTs regulate PKM2 acetylation in specific cell types is still required. Residues of PKM2 regulated by acetylation are yet to be identified in immune cells; however, a recent study in macrophages demonstrated that succinylation of K311 promoted formation of the nuclear dimer, enabling association with HIF-1α and inducible IL-1β expression [56]. SIRT5 was shown to be required for desuccinylation of this residue, inducing the tetrameric form and preventing inflammatory mediator production. In keeping with this regulatory role, *Sirt*$^{-/-}$ mice were highly susceptible to DSS-induced colitis [56]. Lysine succinylation frequently overlaps with acetylation [57], and this opens up the interesting possibility of crosstalk between different lysine modifications (e.g., acetylation, succinylation) on K311 or other lysine residues of PKM2 to control its stability and/or function. In this regard, HDACs/SIRTs may act as gatekeepers, deacetylating lysine residues to enable alternative PTMs.

Pyruvate Dehydrogenase (PDH)

The pyruvate dehydrogenase complex (PDC) is formed by the association of three catalytic enzymes; PDH (E1), dihydrolipoamide transacetylase (E2), and dihydrolipoamide dehydrogenase (E3), as well as the E3-binding protein that tethers them together [58]. When localized in the mitochondrial membrane, the PDC decarboxylates pyruvate into acetyl-CoA for use by the TCA cycle, linking glycolysis to the TCA cycle and determining whether pyruvate is used for OXPHOS or is reduced to lactate to sustain aerobic glycolysis. Intriguingly, despite its large size, intact and functional PDC can translocate across the mitochondrial membrane and shuttle to the nucleus, where it provides a localized nuclear pool of acetyl-CoA for histone acetylation and the control of gene expression [59]. Because PDH catalyzes the rate-limiting step of pyruvate decarboxylation, the activity of this subunit determines the rate of flux, which in turn replenishes the TCA cycle. Proinflammatory macrophages sustain pyruvate oxidation through PDH and use

it to generate citrate in the mitochondria, providing substrate for the production of itaconate and for lipogenesis [60]. The major inhibitory control point of PDC activity is phosphorylation on three serine residues of PDH by pyruvate dehydrogenase kinase (PDK) [61]. SIRT3-mediated deacetylation of PDH at K321 increased its enzymatic activity in cancer cells [62], and acetylation at this residue inhibited activity by recruiting PDK [63]. Thus, it would appear that deacetylation of PDH provides a means to activate the PDC. There is other evidence of indirect control of PDH activity via regulated lysine acetylation in cancer cells. Acetylation of K202 of pyruvate dehydrogenase phosphatase 1 (PDP1) inhibits its interaction with PDH, preventing dephosphorylation-mediated activation of PDH [63]. Thus, deacetylation of PDP1 would be predicted to favor PDH activation. ACAT1 and SIRT3 were identified as the responsible lysine acetylase and deacetylase, respectively, in this pathway.

HDAC/SIRT-Mediated Control of Glycolysis Through Gene Regulation

In addition to acetylation/deacetylation-mediated control of the activity of glycolytic enzymes, HDACs and SIRTs can also influence glycolysis by modulating the expression of glycolysis-associated genes, either by deacetylating transcription factors or histones (epigenetic mechanisms). For example, HIF-1α, a key transcription factor at the convergence of inflammatory and metabolic signals, regulates the expression of glycolytic genes such as glucose transporters (*Glut1*), *Hk2*, *Pkm*, and *Ldha*, thereby enhancing glycolytic flux [64]. HIF-1α is known to be regulated by acetylation; SIRT1 [65] and SIRT2 [66] can each directly bind to, deacetylate, and inactivate HIF-1α, while SIRT3-dependent reactive oxygen species (ROS) production stabilizes HIF-1α by targeting the prolyl hydroxylases that induce degradation [67]. Class I and II classical HDACs can also regulate HIF-1α localization, stability, and function to control glycolytic and inflammatory gene expression. Furthermore, the enzymatic activity of classical HDACs was required for HIF-1α-dependent transcriptional responses in TLR-activated macrophages [68]. The peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) and Forkhead box O (FoxO) transcription factors are other deacetylase-modulated targets that control glycolytic gene expression and regulate TLR4 signaling in macrophages [69,70]. In hepatocytes, PGC-1α is deacetylated by SIRT1 to modulate the balance of glycolytic and gluconeogenic genes [71]. Also in the liver, class IIa HDACs are necessary to recruit HDAC3 for deacetylation and the activation of FoxO transcription factors, thus enabling the expression of enzymes involved in gluconeogenesis [72]. In adipocytes, sirtuins can also directly deacetylate FoxO transcription factors to regulate cell metabolism; SIRT1-mediated deacetylation of FoxO1 resulted in increased adiponectin transcription [73], which in turn modulated glucose metabolism, FAO, and insulin levels. Whether these specific HDACs also deacetylate PGC-1α and/or FoxO to control regulated metabolism in macrophages remains an open question.

Numerous studies have demonstrated that metabolic input is integrated into changes at the level of chromatin structure. Lactate, the end-product of aerobic glycolysis, inhibits HDAC activity [74], thus providing a molecular mechanism by which the metabolic state of the cell can exert epigenetic control. In keeping with this, glycolytic metabolism promoted global histone acetylation and an open chromatin configuration in cancer cells [75], likely by providing additional acetyl-CoA for HATs [76,77] and by increased production of HDAC-inhibitory lactate [74]. Furthermore, extracellular signals that influence macrophage activation status can regulate gene expression through control of metabolites. For example, IL-4 signaling induced a nuclear pool of acetyl-CoA that was necessary for histone acetylation and the expression of genes associated with alternatively activated macrophages [78]. In addition, epigenetic reprogramming of monocytes in response to *Candida albicans* required high glucose consumption coupled with elevated lactate production and a high NAD^+/NADH ratio [79], with SIRT1 and 6 being implicated in this process [19,79]. With respect to specific epigenetic mechanisms,

SIRT1 directly regulates transcription via deacetylation of histones such as H1 [80], H3 [80,81], and H4 [80]. By deacetylating H3K14 at the promoter of the *HIF1A* gene, SIRT1 inhibited HIF-1α expression [82]. Similarly, in activated skeletal muscle stem cells, increased glycolysis was associated with reduced SIRT1-mediated deacetylation of H4K16 [83]. SIRT6 also regulates glucose homeostasis by deacetylation of H3K9 to inhibit the expression of various HIF-1α-dependent glycolytic genes [19,84]. Thus, multiple SIRT family members appear to be particularly important for the epigenetic control of glycolysis in several cellular systems.

Concluding Remarks

There is very strong evidence that specific HDAC/SIRT enzymes deacetylate metabolic enzymes and regulate metabolic pathways in cancer cells. While aerobic glycolysis is utilized by both cancer cells and activated macrophages, there are many questions that remain about how HDACs and SIRTs control macrophage immunometabolism (Outstanding Questions). For example, there are likely to be many differences in the expression and/or localization of individual lysine deacetylases between cancer cell lines and primary immune cells. Thus, detailed mechanistic studies will now be necessary to better understand the roles of individual HDAC/SIRT enzymes in regulating glycolysis in macrophages. Some of these enzymes have already been linked to the regulation of macrophage inflammatory responses, for example HDAC7 ~~that~~ promotes macrophage-mediated inflammatory responses [68] and SIRT5 ~~that~~ plays a regulatory role by constraining the inflammatory functions of PKM2 [56]. A more comprehensive molecular understanding of how specific lysine deacetylases control specific glycolytic enzymes in resting and activated macrophages is likely to deliver new opportunities for manipulating immunometabolism in these cells as an anti-inflammatory strategy for managing disease.

Outstanding Questions

Which HDACs and SIRTs control regulated glycolysis in macrophages, and via modifications on which metabolic enzymes? How does this differ from cancer cells?

In response to infectious or inflammatory stimuli, what are the molecular mechanisms that control lysine deacetylase enzyme activity in macrophages?

How does lysine acetylation regulate the subcellular localization of metabolic enzymes in macrophages and, conversely, do metabolites influence the subcellular localization of lysine deacetylases in macrophages?

Do HDACs, metabolic enzymes, and key transcription factors function together in protein complexes to regulate macrophage immunometabolism? Do deacetylases function as scaffolds or as enzymes in such complexes?

Does the ability of lysine deacetylases to influence other PTMs such as succinylation, malonylation, and SUMOylation regulate inducible glycolysis in macrophages?

Do pharmacological agents that target lysine deacetylases exert some of their anti-inflammatory effects via regulation of immunometabolism?

Acknowledgments

Q5 This work was supported by an ARC Discovery Project to M.J.S. (DP170102321), and a National Health and Medical Research Council of Australia (NHMRC) project grant to M.J.S. and D.P.F. (ID 1125316). M.J.S. is supported by an NHMRC Senior Research Fellowship (ID 1107914), D.P.F. is supported by an NHMRC Senior Principal Research Fellowship (ID 1117017), M.R.S. is supported by a University of Queensland Postdoctoral Research Fellowship. A.I. was supported by a University of Queensland Postdoctoral Research Fellowship, and D.P.F. and A.I. are supported by the ARC Centre of Excellence in Advanced Molecular Imaging (CE140100011). C.Y.C. is supported by Biomedical Research Council A*STAR Young Investigator Grant 1518251030, and A.S. is supported by SIgN A*STAR, A*STAR JCO-CDA 15302FG151, and Singapore-India joint grant 1518224018.

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