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Crosstalk between metabolism and epigenetics during macrophage polarization

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Abstract

Macrophage polarization is a dynamic process driven by a complex interplay of cytokine signaling, metabolism, and epigenetic modifications mediated by pathogens. Upon encountering specific environmental cues, monocytes differentiate into macrophages, adopting either a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype, depending on the cytokines present. M1 macrophages are induced by interferon-gamma (IFN-γ) and are characterized by their reliance on glycolysis and their role in host defense. In contrast, M2 macrophages, stimulated by interleukin-4 (IL-4) and interleukin-13 (IL-13), favor oxidative phosphorylation and participate in tissue repair and anti-inflammatory responses. Metabolism is tightly linked to epigenetic regulation, because key metabolic intermediates such as acetyl-coenzyme A (CoA), α-ketoglutarate (α-KG), S-adenosylmethionine (SAM), and nicotinamide adenine dinucleotide (NAD^+) serve as cofactors for chromatin-modifying enzymes, which in turn, directly influences histone acetylation, methylation, RNA/DNA methylation, and protein arginine methylation. These epigenetic modifications control gene expression by regulating chromatin accessibility, thereby modulating macrophage function and polarization. Histone acetylation generally promotes a more open chromatin structure conducive to gene activation, while histone methylation can either activate or repress gene expression depending on the specific residue and its methylation state. Crosstalk between histone modifications, such as acetylation and methylation, further fine-tunes macrophage phenotypes by regulating transcriptional networks in response to metabolic cues. While arginine methylation primarily functions in epigenetics by regulating gene expression through protein modifications, the degradation of methylated proteins releases arginine derivatives like asymmetric dimethylarginine (ADMA), which contribute directly to arginine metabolism—a key factor in macrophage polarization. This review explores the intricate relationships between metabolism and epigenetic regulation during macrophage polarization. A better understanding of this crosstalk will likely generate novel therapeutic insights for manipulating macrophage phenotypes during infections like tuberculosis and inflammatory diseases such as diabetes.

Keywords Macrophages, M1, M2, Epigenetics, Sirtuins, IFN-γ, IL-4, IL10, IL-13, Glucose metabolism, Metabolism, Macrophage polarization, Histones, Methylation, Acetylation

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Introduction

Macrophage differentiation, plasticity, and functional polarization

Typically, macrophages develop from monocytes which are produced in the bone marrow and circulate in the bloodstream before differentiating into macrophages when entering tissues to play critical roles in maintaining homeostasis and orchestrating the immune response [1]. An exception is that alveolar macrophages arise within the lungs. As key components of the innate immune system, macrophages are essential for pathogen recognition, phagocytosis, antigen presentation, and the regulation of inflammatory processes. Their ability to adapt to diverse environmental stimuli enables them to fulfill distinct functional roles, ranging from pro-inflammatory activities to anti-inflammatory and tissue-repair functions [2]. A defining feature of macrophages is their plasticity, which allows them to polarize into different phenotypes based on external cues. The two most widely studied phenotypes are classically activated (M1) macrophages and alternatively activated (M2) macrophages [3, 4]. These differentiation processes can be driven by growth factors and cytokines [5]. For instance, growth factors like macrophage colony-stimulating factor (M-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) promote the differentiation of monocytes into macrophages. Further, interferon-gamma (IFN-γ) and lipopolysaccharide (LPS), alone or together, promotes the formation of M1 macrophages, which are associated with pro-inflammatory cytokines including IL-1β, IL-6 and tumor necrosis factor α (TNFα), for pathogen elimination [6–10]. Conversely, cytokines like IL-4 and IL-13 induce M2a macrophages, which are involved in tissue repair, fibrosis, and inflammation resolution. Other M2 subtypes include M2b, which arises in response to immune complexes and IL-1R signaling and plays a regulatory role in immune responses and adaptive immunity, and M2c, which is driven by IL-10, TGF-β, and glucocorticoids, contributing to immune suppression and inflammation resolution [11]. It is noted that the M1/M2 macrophage polarization concept has been widely adopted in immunology as a broad model to understand macrophage responses to different stimuli. Therefore, throughout this review, we use the M1/M2 framework to describe macrophage functional states while acknowledging the complexity and plasticity of macrophage polarization.

The role of macrophages in tumors, metabolism, and epigenetic regulation

Besides their roles in infection and inflammation, macrophages are pivotal players in the tumor microenvironment (TME) [12]. Tumor-associated macrophages (TAMs), which often exhibit M2-like characteristics, promote tumor progression through several mechanisms. These include immune suppression, enhancement of angiogenesis, remodeling of the extracellular matrix, and facilitation of metastatic dissemination. TAMs can inhibit anti-tumor immune responses by producing immunosuppressive cytokines and modulating T-cell function, thereby contributing to immune evasion. Conversely, macrophages can exhibit anti-tumor properties under certain conditions, particularly when polarized to an M1-like phenotype [13]. This dual role underscores the complexity of macrophage function within the TME and highlights their potential as therapeutic targets [14].

Cytokines drive macrophage differentiation by binding to specific cell surface receptors, which activate intracellular signaling pathways such as JAK-STAT, NF-κB, and MAPK [15]. These pathways change gene expression and activate transcription factors (TFs) that dictate macrophage polarization. For example, IFN-γ activates TFs like IRF5 to drive M1 macrophages [16], whereas IL-4 and IL-13 activate STAT6 to promote M2 macrophages [17]. Interestingly, recent research has uncovered that macrophage function and polarization are tightly regulated by metabolic and epigenetic mechanisms [18, 19]. Metabolic pathways, such as glycolysis, the tricarboxylic acid (TCA) cycle, fatty acid oxidation, and the pentose phosphate pathway (PPP), dictate the energy requirements and biosynthetic precursors necessary for macrophage activation. For instance, M1 macrophages rely heavily on glycolysis to fuel their pro-inflammatory responses and reactive oxygen species (ROS) production, while exhibiting a disrupted TCA cycle with increased succinate accumulation. Succinate stabilizes hypoxia-inducible factor-1α (HIF-1α), promoting the production of inflammatory cytokines like interleukin-1β (IL-1β) [20]. In contrast, M2 macrophages depend on oxidative metabolism and fatty acid oxidation to sustain their anti-inflammatory functions and maintain mitochondrial integrity. The PPP supports NADPH production in both phenotypes but is differentially regulated to meet their specific metabolic demands.

Concurrently, epigenetic modifications, including histone acetylation, methylation, and succinylation, and DNA methylation, modulate gene expression programs that guide macrophage differentiation and stabilize macrophage phenotypes [21, 22]. For example, histone acetylation at promoter regions of inflammatory genes enhances their transcription in M1 macrophages, while M2 macrophages exhibit histone H3K27 demethylation that promotes anti-inflammatory gene expression [23]. DNA methylation and hydroxymethylation, regulated by factors such as ten-eleven translocation (TET) methylcytosine dioxygenases and methyltransferases, further influence macrophage polarization and function. Additionally, metabolites like α-ketoglutarate (α-KG), succinate, acetyl coenzyme A (acetyl-CoA),

S-adenosylmethionine (SAM), and nicotinamide adenine dinucleotide (NAD^+) serve as cofactors for these epigenetic regulators, linking metabolism directly to epigenetic control.

Understanding the metabolic-epigenetic axis in macrophages has significant implications for human health and disease. In the context of cancer, metabolic reprogramming and epigenetic alterations in TAMs can shift their phenotype toward tumor-promoting states, contributing to immune evasion and resistance to therapy [24]. Similarly, in infectious diseases such as tuberculosis, metabolic and epigenetic changes induced by pathogens can impair macrophage function, leading to immune dysregulation and chronic infections [25]. Emerging evidence also implicates macrophages in metabolic disorders, such as obesity and diabetes, where their polarization states influence systemic inflammation and insulin resistance [26]. Thus, targeting the metabolic and epigenetic networks that govern macrophage polarization offers promising therapeutic avenues for modulating immune responses in diverse pathological conditions.

Metabolic regulation of histone acetylation and methylation in gene expression and macrophage function

Histone methylation is a key epigenetic modification involving the addition of methyl groups to specific amino acid residues, predominantly lysine and arginine, on histone proteins. The patterns of histone methylation are intricate and highly specific, influencing gene expression by either activating or repressing transcription depending on the modified residue and the degree of methylation [27, 28]. For instance, tri-methylation of lysine 4 on histone H3 (H3K4me3) is commonly associated with active gene transcription, transcriptional pause-release, and elongation [29–31], whereas tri-methylation of lysine 9 (H3K9me3) and lysine 27 (H3K27me3) on histone H3 are typically linked to gene repression [32, 33]. Histone H4 K20 mono-methylation facilitates chromatin openness, particularly during mitosis [34–36], while di- and tri-methylation (H4K20me2 and H4K20me3), interacting with H3K9me3, increase quiescence and promote chromatin compaction [37, 38]. Additionally, methylation of histone H3 lysine 36 (H3K36me) plays a critical role in the regulation of alternative splicing of RNA and RNA elongation [39, 40], and methylation of histone H3 lysine 79 (H3K79me) is involved in the DNA damage response [41].

Histone acetylation also regulates gene expression and chromatin dynamics [42]. The acetylation process involves the addition of acetyl groups to lysine residues on histone proteins, which neutralizes their positive charge and reduces their affinity for the negatively charged DNA [43]. As a result, the chromatin structure becomes more relaxed and open, allowing greater 'accessibility' for the transcriptional machinery. This openness facilitates the binding of TFs and RNA polymerase to specific gene promoters, thereby enhancing gene activation and expression [44]. Moreover, acetylation of histones is instrumental in the recruitment of transcriptional coactivators and other regulatory proteins, which are essential for the initiation and maintenance of gene transcription [45]. This process is crucial for various cellular functions, including cell differentiation and development. In particular, histone acetylation influences the differentiation of macrophages in different functional states from homeostasis to host defense [21]. In addition, histone acetylation impacts DNA repair and replication [46]. The relaxed chromatin structure resulting from acetylation facilitates the accessibility of damaged DNA to repair enzymes, thereby supporting efficient DNA repair mechanisms. In addition, acetylation plays a role in DNA replication by regulating chromatin structure during the cell cycle, ensuring proper genome duplication [42]. It is noted here that histone acetylation and methylation are not isolated processes; rather, they often interact through complex crosstalk that influences chromatin structure and gene expression. These two types of histone modifications can affect each other's actions and work in tandem to regulate cellular processes [47]. For instance, the presence of acetyl groups on histones can influence the binding of methyltransferases or demethylases, and conversely, specific methylation marks can affect the efficiency of acetylation processes [48]. Moreover, certain histone marks can create a "histone code" that guides the recruitment of other regulatory proteins, thereby integrating signals from both acetylation and methylation to fine tune gene expression and chromatin dynamics [49]. This interplay is crucial for maintaining the balance between gene activation and repression, influencing processes such as gene silencing, transcriptional activation, and cellular differentiation. Overall, the crosstalk between histone acetylation and methylation underscores the complexity of epigenetic regulation and its impact on cellular functions [47], including macrophage polarization [50].

Interestingly, histone acetylation and methylation are deeply intertwined with cellular metabolism, creating a dynamic relationship [51, 52]. Intriguingly, histone acetylation depends on acetyl-CoA, a metabolite derived from glycolysis, fatty acid oxidation, deacetylation of acetylated molecules, and ATP citrate lyase (ACLY)-mediated citrate cleavage. As metabolic activity changes, such as during increased glycolysis or fatty acid β-oxidation in stem cells or developing cells, the availability of acetyl-CoA can lead to altered levels of histone acetylation [53–57]. This modification relaxes chromatin structure, facilitating gene activation. Histone acetylation can be removed by histone deacetylases (HDACs). Specifically, sirtuins, a family of NAD^+ HDACs (Type III HDACs),

play a crucial role in metabolism [58, 59]. In addition to NAD^+, sirtuins bind to zinc and, therefore, sirtuin signaling is zinc dependent and requires an adequate level of zinc for proper enzymatic activity [60, 61]. Sirtuins adjust histone acetylation in response to the cellular energy status and nutrient availability, linking nutrient intake and metabolic states to transcriptional regulation [59]. By deacetylation of histones and other proteins, sirtuin consume NAD^+ by breaking down the NAD^+ molecule into nicotinamide and ADP-ribose.

On the other hand, histone methylation is regulated by SAM, which serves as the primary methyl donor for histone methyltransferases. The availability of SAM, which is influenced by one-carbon metabolism and dietary nutrients like folate and vitamin B_{12}, directly impacts histone methylation patterns [62]. SAM levels, especially the SAM/SAH (S-adenosylhomocysteine) ratio, controls histone methylation, influencing gene expression and cellular functions [63, 64]. Moreover, cellular metabolic states such as hypoxia can affect histone methylation by altering the expression or activation of histone methyltransferases and demethylases involved during methylation processes [65]. Under hypoxic conditions, changes in metabolic pathways, including glycolysis and one-carbon metabolism, can lead to a shift in histone methylation pattern, linking environmental stress to epigenetic modifications [66].

Histone demethylation is also intricately connected to cellular metabolism, as metabolic states directly influence the activity of histone demethylases [67]. Key demethylases, such as those in the Jumonji C (JmjC) domain family, rely on alpha-ketoglutarate (α-KG) as a cofactor, a metabolite from the TCA cycle. Changes in α-KG levels can impact the efficiency of these demethylases, thereby altering histone methylation patterns and gene expression [68]. Additionally, the availability of other metabolites involved in one-carbon metabolism, such as folate, also affects demethylase activity [69]. Nutrient availability can therefore modulate these metabolites which in turn, influence histone demethylation. Conditions such as hypoxia, which alter metabolic pathways, further affect the activity of histone demethylases and the associated epigenetic modifications [65, 70, 71]. The interplay between metabolism and histone demethylation allows cells to adjust their epigenetic landscape in response to varying metabolic conditions, thereby integrating metabolic signals into gene regulation and cellular adaptation.

The metabolic and epigenetic landscapes in M1 and M2 macrophages

The metabolic and epigenetic landscapes of M1 and M2 macrophages are intricately linked to their contrasting functional roles within the immune system. M1 macrophages are equipped for aggressive immune responses and inflammation, characterized by glycolytic metabolism, a metabolic pathway that produces energy (ATP) rapidly and generates ROS. ROS drive inflammatory signaling by acting as redox-sensitive modulators of key cellular pathways: They activate TFs like NF-κB by oxidizing cysteine residues in regulatory proteins, leading to the expression of pro-inflammatory cytokines [72]. ROS also activate the NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3) inflammasome by destabilizing mitochondrial membranes and releasing activators such as thioredoxin-interacting protein (TXNIP), enhancing the maturation of IL-1β and IL-18 [73]. Additionally, ROS generate reactive aldehydes (e.g., 4-hydroxynonenal (4-HNE)) from membrane lipids, which function as signaling molecules to further promote inflammation [74]. They also stimulate MAPK pathways [75], driving inflammatory gene transcription and modulating epigenetic processes such as histone modifications and DNA methylation [76, 77]. Furthermore, ROS amplify their production through positive feedback loops involving nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and mitochondrial dysfunction, intensifying cellular stress and immune cell recruitment [78, 79]. The shift toward glycolysis in M1 macrophages, even in the presence of oxygen, supports their role in pro-inflammatory responses and pathogen elimination [19]. Moreover, arginine is metabolized into citrulline releasing nitric oxide (NO) to form reactive nitrogen species (RNS), by activated iNOS enzyme; RNS are major antimicrobial agents. M1 macrophages also produce metabolites like succinate, which stabilizes hypoxia-inducible factor 1-alpha (HIF-1α), further enhancing their inflammatory responses [80]. The epigenetic landscape of M1 macrophages is characterized by specific histone modifications that facilitate the expression of pro-inflammatory genes. For instance, increased histone acetylation at the promoters of IL-6 and TNF-α enhances their accessibility of TFs and expression [81, 82]. Other key TFs, such as NF-κB/RelA and STAT1, are also activated in M1 macrophages to drive the expression of cytokines and other factors involved in inflammation and immune activation [83, 84]. In addition, DNA methylation patterns in M1 macrophages can also influence the accessibility and expression of inflammatory genes, contributing to their functional state [85, 86]. In contrast, M2 macrophages, also known as alternatively activated macrophages, rely predominantly on oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) to meet their energy demands and support their anti-inflammatory functions, such as tissue repair and wound healing [19]. Unlike the glycolytic metabolism of pro-inflammatory M1 macrophages, M2 macrophages utilize FAO to fuel the TCA cycle, providing substrates for OXPHOS [87]. This metabolic reprogramming is driven by cytokines like IL-4 and IL-13,

which activate the STAT6 and PPARγ pathways, upregulating key enzymes involved in FAO [87, 88]. Mitochondrial biogenesis, regulated by PPAR Gamma Coactivator 1β (PGC-1β), is essential for maintaining OXPHOS, and mitochondrial integrity is critical for M2 polarization, as dysfunctional mitochondria impair their function [89]. Additionally, the metabolic shift towards FAO and OXPHOS is coupled with epigenetic modifications, such as histone acetylation, which promote the transcription of M2-specific genes [90]. The epigenetic landscape of M2 macrophages is distinct, with histone modifications that promote the expression of anti-inflammatory genes and support tissue repair [91]. TFs, such as STAT6 and PPAR-γ, play crucial roles in regulating genes, including Arg1, Dectin-1, Mrc1, Fizz1, and Ym1 [4]. The expression of these genes or proteins is increased by IL-4 stimulation but reduced by treatment with either LPS or IFN-γ [92]. Among these regulatory genes, Mrc1 (Mediator of Replication Checkpoint 1) is a replisome-associated histone chaperone essential for the symmetrical transfer of parental histones, particularly H3-H4 tetramers, to newly replicated DNA strands. Through distinct effects on the histone-binding domain (HBD) and other structural domains, Mrc1 coordinates with replisome components (FPC, Cdc45, MCM2, and POLE) to ensure balanced histone recycling and epigenetic inheritance during DNA replication [93]. Higher expression of Mrc1 is potentially correlated with increased expression of histone proteins and the MCM (minichromosome maintenance) complex, which together support elevated DNA replication activity. In M2 macrophages, arginine is metabolized by ARG1 into ornithine, which is used to synthesize polyamine including spermine, spermidine, and putrescine. Polyamine differentially regulates histone methylation: putrescine increases H3K9me3 and decreases H3K9ac, favoring M2 macrophages [94]. Spermine also favors M2 macrophages, while spermidine favors M1 macrophages [94]. In addition to histone modifications, DNA methylation patterns in M2 macrophages also help maintain the expression of genes that support tissue repair and the resolution of inflammation, reflecting their role during immune regulation and tissue homeostasis [50, 95]. Finally, M2 macrophages produce lower levels of ROS and RNS, aligning with their function in promoting healing rather than inflammation [96]. Conversely, this weakens their antimicrobial functions. Other metabolic pathways, including PPP, glutamine and arginine metabolism, tryptophan metabolism, and one-carbon metabolism, are also distinctly regulated between M1 and M2 macrophages. These pathways support the metabolic demands and functional specialization of macrophages during polarization. We have extensively studied and reviewed these metabolic alterations in prior publications, providing detailed insights into their mechanisms of macrophage function and polarization [97–99].

Acetyl-CoA dependent histone acetylation during macrophage polarization

Acetyl-CoA, a critical metabolic intermediate, is generated through several key metabolic pathways. It can be produced through the oxidative decarboxylation of pyruvate derived from glycolysis, the oxidation of long-chain fatty acids in the mitochondria, the oxidative degradation of certain amino acids, and the deacetylation of acetylated biomolecules [97]. In addition, acetyl-CoA can be synthesized by ACLY, which converts citrate into acetyl-CoA and oxaloacetate, both of which play key roles in cellular metabolism and energy production. Furthermore, two acetyl-CoA synthetase enzymes, acetyl-CoA synthetase 1 and 2 (ACSS1 and ACSS2), also contribute to acetyl-CoA production by catalyzing the condensation of acetate with thiol groups, a reaction that occurs in both the cytoplasm and the mitochondria [99].

As the macrophage's energy demands change, particularly during polarization, metabolic intermediates like acetyl-CoA serve as both a fuel source and a signaling molecule. This dual role of acetyl-CoA is essential for maintaining macrophage activation and ensuring proper immune responses. In the early phase of stimulation of macrophages (0.5–2 h post-stimulation with LPS), glycolysis is increased, driving pyruvate oxidation by the pyruvate dehydrogenase complex (PDC) to produce acetyl-CoA which either enters TCA or is used for fatty acid synthesis and protein acetylation. This also leads to enhanced citrate production and its cleavage by activated ACLY, resulting in elevated acetyl-CoA levels and increased global histone acetylation [100, 101].

In macrophages, the generation of acetyl-CoA and its subsequent use in histone acetylation is tightly regulated during polarization. During the early phase of macrophage stimulation (0.5–2 h post-stimulation with LPS), macrophages experience an upregulation of glycolysis, which is a hallmark of M1 polarization [19]. Increased glycolytic flux leads to an accumulation of pyruvate, which is shuttled into the mitochondria where it is oxidized by PDC to produce acetyl-CoA. This acetyl-CoA can either enter the TCA cycle, fueling oxidative phosphorylation for energy production, or it can be used for other cellular processes such as fatty acid synthesis or protein acetylation [98]. The increased acetyl-CoA levels, driven by both glycolysis and citrate cleavage, enable the acetylation of histones and non-histone proteins, which supports the transcription of inflammatory cytokines and other immune effector molecules that promote the pro-inflammatory M1 state [99]. Thus, the balance of acetyl-CoA production via glycolysis and mitochondrial activity directly influences the macrophage's ability

to respond to external stimuli and polarize toward M1 or M2 phenotypes. However, in the late phase (48–72 h post-stimulation with LPS and IFN-γ), NO inhibits PDC and the oxoglutarate/α-KG complex (OGDC), impairing glycolysis and the TCA cycle [102–104]. This reduces acetyl-CoA production, along with histone deacetylation, leading to a decrease in global histone acetylation. Therefore, global histone acetylation in M1 macrophages follows a biphasic pattern upon stimulation.

Lactate-mediated regulation of macrophage polarization: a negative feedback mechanism

As glycolysis is upregulated in M1 macrophages, lactate production also increases, leading to enhanced histone lactylation [105]. Increased lactate production from pyruvate generates NAD^+ to maintain NAD^+ homeostasis, compensating for the consumption of NAD^+ during glycolysis and the decreased *de novo* biosynthesis from tryptophan metabolism [98, 99]. However, accumulated lactate, in turn, has negative feedback effects on macrophage polarization. Lactate accumulation can induce the expression of immune suppressive markers and inhibit further M1 polarization by activating the lactate receptor, GPR81, which has been shown to inhibit NF-κB signaling and inflammatory cytokine production [106]. Additionally, histone lactylation activates genes linked to anti-inflammatory functions favoring an M2-like phenotype in tumor and chronic inflammatory environments [105]. Interestingly, lactate can enhance histone acetylation by inhibiting HDACs due to its structural similarity to pyruvate, resulting in a combined effect on lactylation and acetylation that stabilizes the M2 phenotype and suppresses pro-inflammatory pathways [107–109]. In the tumor microenvironment, lactate drives M2 macrophage polarization via a TCA cycle-dependent mechanism that requires mitochondrial pyruvate uptake and ACLY activity for histone acetylation and immune-suppressive gene expression [110]. Disruption of this lactate-driven pathway impairs M2 polarization and tumor progression, highlighting a critical metabolic-epigenetic link during immune evasion [110, 111].

NAD^+-dependent histone deacetylation by sirtuin proteins during macrophage polarization

Sirtuin proteins play a critical role in macrophage polarization, particularly in diseases like tuberculosis, as reviewed by us previously [99]. Among the seven members of the sirtuin protein family, SIRT2 is prominently expressed in M2 macrophages, supporting an anti-inflammatory phenotype, whereas SIRT5 is more prevalent in M1 macrophages associated with pro-inflammatory responses [8]. Moreover, SIRT2 deletion promotes inflammatory responses with enhanced expression of pro-inflammatory cytokines related to M1 macrophages by increasing NF-κB acetylation and reducing the M2-associated anti-inflammatory pathways [112]. This review will therefore focus on these two proteins, SIRT2 and SIRT5, and their distinct roles in macrophage polarization from a broad perspective.

SIRT2 in macrophage polarization: epigenetic, metabolic, and therapeutic implications

SIRT2 is the most expressed sirtuin in myeloid cells and macrophages. It plays a significant role in macrophage development and functional polarization [97, 113]. During macrophage development, SIRT2 governs key signal transduction pathways and transcriptional programs that determine macrophage polarization [112]. For instance, SIRT2 is highly expressed in M2 macrophages, where it promotes anti-inflammatory and tissue-reparative functions. Mechanistically, RelA/p65 forms a complex with SIRT2 and is deacetylated by SIRT2 at lysine 310 (K310) in the cytoplasm [112, 114]. Upon stimulation by TNFα, RelA translocates to the nucleus to function as a transcriptional activator. Inhibition of this deacetylation at K310 leads to increased expression of a subset of NF-κB-dependent genes [114]. These NF-κB-regulated genes include IL-1β, IL-6, IL-12, IL-23, TNFα, and iNOS, which are key markers of pro-inflammatory M1 macrophages [112, 115–118]. SIRT2 depletion seems to have the same effect as its inhibition, which results in hyperacetylation of RelA at Lys310 in bone marrow-derived macrophages (BMDMs), promoting inflammatory responses characteristic of M1 macrophages while reducing the expression of anti-inflammatory genes associated with M2 macrophages [112]. However, one contradictory finding has also been reported: decrease of SIRT2 expression in BMDMs reduced LPS-induced pro-inflammatory or M1-related cytokine expression, iNOS/NO production, through inhibition of RelA activation due to SIRT2-induced reduction of RelA phosphorylation and translocation [119].

SIRT2 also intersects with metabolic pathways pivotal for macrophage function. By modulating glycolysis, mitochondrial respiration, and autophagy, SIRT2 ensures the metabolic flexibility of macrophages, enabling them to adapt to various stress conditions. Predominantly localized in the cytoplasm, SIRT2 deacetylates key enzymes in glycolysis and other metabolic pathways, thereby regulating their activity and influencing macrophage polarization and function. The role of SIRT2 (and other sirtuin proteins)-dependent metabolism, particularly in macrophages during mycobacterium tuberculosis (*Mtb*) infection, has been comprehensively reviewed by us elsewhere [99].

In addition to its metabolic functions, SIRT2 can be transported from the cytosol to the nucleus by importin-7 in response to bacterial infection [120]. In the

nucleus, SIRT2 exerts epigenetic regulation by deacetylating histones. It modulates chromatin accessibility and transcriptional programs by targeting specific histone residues, such as H3K18ac and H4K16ac [120–125]. These modifications are essential for defining the transcriptional landscapes that underpin M1 or M2 phenotypes. Therefore, SIRT2 interplays among epigenetics, metabolism, and the signaling pathways underscoring its multifaceted role in macrophage polarization.

Targeting SIRT2 offers significant therapeutic potential in modulating macrophage polarization for various diseases. Enhancing SIRT2 activity may promote M2 polarization, aiding in tissue repair and resolution of chronic inflammation. On the other hand, inhibiting SIRT2 could strengthen M1-mediated immune responses, providing benefits in combating infections and tumors. For example, inhibition of SIRT2 has been shown to significantly suppress the growth of *Mtb* [126–129]. Individuals with diabetes are more susceptible to bacterial infections such as *Mtb* and often experience worse infection outcomes compared to non-diabetic individuals [130–132]. This heightened susceptibility is linked to factors such as the availability of glucose as a carbon source for pathogen growth, increased gluconeogenesis and impaired glucose utilization, decreased ketoacidosis, and weakened GLUT1-facilitated phagocytosis [133], which reduces the production of ROS and RNS [134]. Diabetes is also associated with alterations in pro-inflammatory cytokine profiles during infection, though the literature presents conflicting findings. Some studies report elevated pro-inflammatory cytokine expression, while others show the opposite [135–138]. This suggests that cytokine production in diabetic individuals may vary depending on the severity of the disease or blood glucose management. Inhibition of SIRT2 has the potential to enhance pro-inflammatory cytokine production and promote M1 macrophage polarization, potentially improving pathogenic bacterial clearance. However, this inhibition also carries the risk of exacerbating insulin resistance due to heightened pro-inflammatory cytokine production, despite the ability of SIRT2 to negatively regulate insulin resistance [139, 140]. Resolving this dilemma—enhancing pathogen killing while preventing insulin resistance—poses a critical research challenge.

SIRT5 in mitochondrial metabolism and macrophage polarization

SIRT5, a non-nuclear sirtuin protein localized exclusively in mitochondria, plays a crucial role as a desuccinylase, regulating key enzymes involved in mitochondrial metabolism. The substrates of SIRT5 have been extensively identified through affinity mass spectrometry in mouse liver tissue [141–143]. SIRT5's enzymatic activities include the deacetylation and desuccinylation of key metabolic enzymes, such as pyruvate kinase 1 and 2 (PKM1/2) in glycolysis [144], the pyruvate dehydrogenase complex (PDC), and succinate dehydrogenase (SDH), along with other dehydrogenases involved in the TCA cycle [141, 142]. Additionally, SIRT5 targets hydroxyacyl-CoA dehydrogenase (HADH) in fatty acid beta-oxidation, 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) in ketogenesis, and complexes I, II, and IV of the electron transport chain [141, 143]. SIRT5 also regulates crucial components of the urea cycle and extended arginine metabolism, including carbamoylphosphate synthase 1 (CPS1), argininosuccinate synthase 1 (ASS1), ornithine aminotransferase (OAT), glutamate-oxaloacetic transaminase (GOT1/2), and glutaminase (GLS) [98, 143, 145–147].

While most of SIRT5's substrates and desuccinylation sites have been identified in mouse liver tissue, as well as in yeast, *Escherichia coli* and various cell lines [141–143, 148, 149], it remains unclear whether these sites overlap with those in human immune cells. It is particularly unknown if distinct modification sites exist in immune cells, especially during macrophage polarization. However, SIRT5 has been observed to desuccinylate and activate PKM2, which in turn blocks IL-1β production in macrophages [144]. In contrast, SIRT5 has been found to promote M2 macrophage polarization, contributing to mucosal edema and exacerbated Th2-type inflammation in chronic rhinosinusitis with nasal polyps (CRSwNP) [150]. Interestingly, while SIRT5 deficiency does not appear to compromise innate immune responses to bacterial infections such as endotoxemia, *E.coli* peritonitis, *Streptococcus pneumoniae* pneumonia, *Klebsiella pneumoniae* infection, or listeriosis (its impact on tuberculosis remains unknown) [151], inhibition of SIRT5 with the desuccinylation inhibitor MC3482 has been shown to decrease inflammation. This occurs by increasing M2 macrophage levels and reducing M1 macrophage levels in complete Freund's adjuvant (CFA)-induced adjuvant arthritis models, via the SIRT5-GLUD1-α-KG axis [152]. Further research is necessary to fully understand SIRT5's role in macrophage polarization and its responses to various microenvironments. For example, an area of research would be whether treatment with SIRT5 inhibitors or activators promotes M1 or M2 macrophage polarization.

Though SIRT5 is primarily recognized for its role in mitochondrial metabolism rather than direct involvement in epigenetic regulation, it competes with other sirtuin proteins for acetylation sites and NAD^+, which is required for its enzymatic activity. This competition affects metabolic pathways, influencing the production of acetyl-CoA, methyl donors, and the consumption and regeneration of NAD^+. Consequently, while SIRT5 does not directly impact epigenetic modifications, its influence on these metabolic processes may indirectly affect

the epigenetic landscape. Lysine succinylation often overlaps with acetylation, frequently appearing at the same modification sites in mitochondrial proteins [148]. Given that SIRT2 is primarily a cytosolic protein and SIRT5 is specific to mitochondria, the distribution of lysine acetylation and succinylation varies across different cell compartments [148]. Acetyl-CoA is predominantly produced through glycolysis, while succinyl-CoA originates from the TCA cycle [148, 153]. Glycolysis is upregulated in M1 macrophages, whereas the TCA cycle is upregulated in M2 macrophages [19, 97]. Our observations indicate that SIRT5 is upregulated in M1 macrophages, while SIRT2 is upregulated in M2 macrophages [8]. Considering the differential distribution of enzymes and cofactors in M1 and M2 macrophages—specifically, high SIRT5 and low SIRT2 with high acetyl-CoA in M1 macrophages, versus low SIRT5 and high SIRT2 with high succinyl-CoA in M2 macrophages—we predict that lysine acetylation would be high and succinylation low in M1 macrophages, and the reverse would be true in M2 macrophages (Fig. 1). However, the stoichiometry of lysine acetylation and succinylation during macrophage polarization still requires experimental validation through mass spectrometry and immunological methods. Notably, SIRT5 and SIRT2 may compete to regulate the acetylation state of NF-κB p65/RelA, thereby influencing the activation of the NF-κB pathway and its downstream cytokines [154, 155].

Regulation of Sirtuin activity by NAD$^+$ and its impact on macrophage polarization

NAD$^+$ is a vital coenzyme involved in redox reactions and serves as a substrate for various enzymes, including sirtuins and poly(ADP-ribose) polymerases (PARPs). Its subcellular distribution spans the cytoplasm, mitochondria, and nucleus, each maintaining distinct NAD$^+$ pools that are interconnected and buffered and tightly regulated [156]. This compartmentalization is crucial for modulating metabolic pathways, DNA repair, chromatin remodeling, and other cellular processes. Recent research has illuminated the significant role of NAD$^+$ metabolism in influencing macrophage function and polarization [157].

One pathway of interest is the *de novo* NAD$^+$ synthesis via the kynurenine pathway (KP) in macrophages. Activation of the KP leads to the production of quinolinic acid (QA), which is subsequently converted to NAD$^+$ by the enzyme quinolinate phosphoribosyltransferase (QPRT). Studies have shown that during aging, there is a suppression of QPRT expression, resulting in decreased

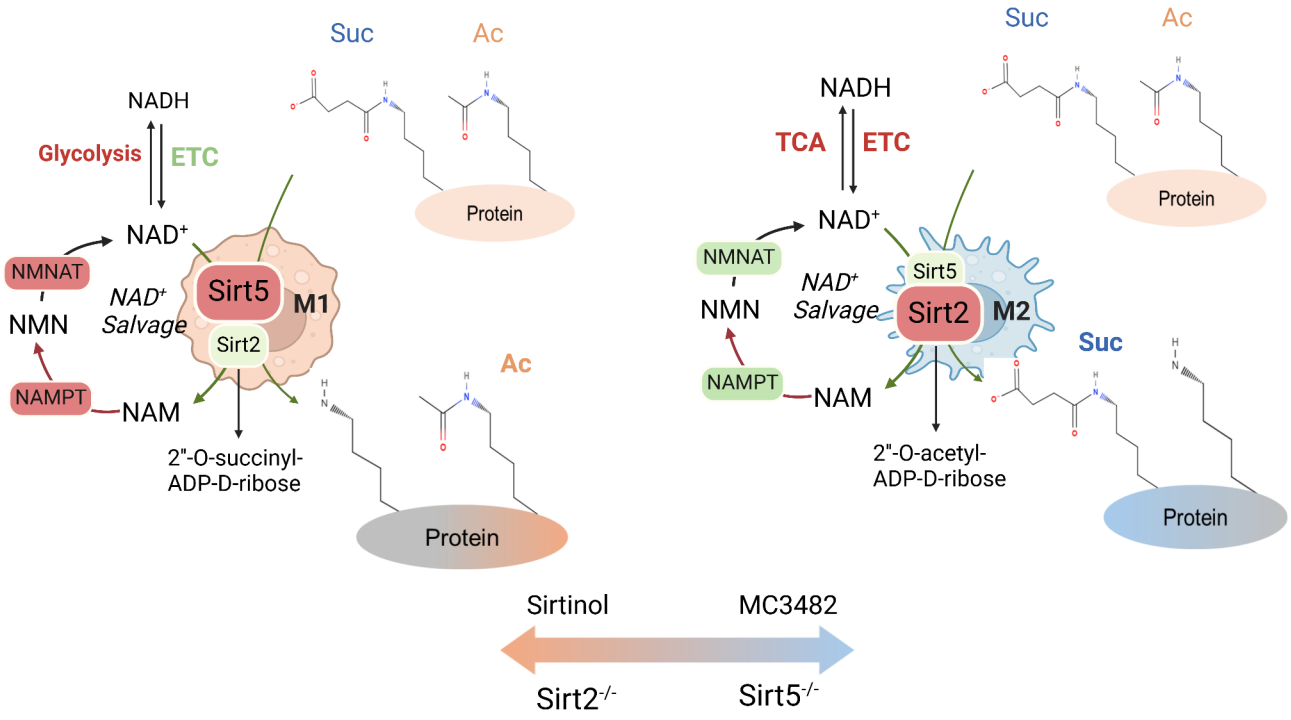

Fig. 1 The stoichiometry of lysine acetylation and succinylation regulated by SIRT2 and SIRT5 determines the polarization states of macrophages. SIRT5 is highly expressed in M1 macrophages, it desuccinylates protein succinylation, resulting in hyperacetylation and hyposuccinylation; SIRT2 is highly expressed in M2 macrophages, it deacetylates protein acetylation resulting in hypersuccinylation and hypoacetylation. Modulation of the expression or activity of SIRT2 and SIRT5 drives macrophage polarization towards either M1 or M2. Activation of SIRT2 and SIRT5 is dependent of homeostatic level of NAD$^+$, which is regulated by the NAD+de novo synthesis from tryptophan catabolism and the salvage pathway [98]. NAD$^+$ is consumed by glycolysis and TCA, but can be regenerated through the Electron-Transporting-Complexes (ETC). Sirtinol: SIRT2 inhibitor; MC3482: SIRT5 inhibitor. Suc: succinylation; Ac: acetylation. Red color: up-regulated proteins or pathways; green color: down-regulated proteins or pathways

NAD^+ levels. This decline impairs mitochondrial function and skews macrophages toward a pro-inflammatory state or the M1-like state but with a declined phagocytosis activity [158]. Restoring QPRT expression in aged macrophages has been demonstrated to replenish NAD^+ levels, enhance mitochondrial respiration, and promote a more balanced immune response [158]. Contradictory to the non-stimulated macrophages [97], stimulated macrophages with LPS increases the expression of IDO1 while reducing the expression of QPRT, resulting in an impeded conversion of QA to NAD^+ and the accumulation of QA [158]. In addition to the *de novo* NAD^+ synthesis, salvage pathways are crucial for macrophage activation as well. A decrease in *de novo* NAD^+ synthesis coupled with an increase in the salvage pathway is normally associated with M1 macrophage polarization [98]. For instance, inhibiting IDO1 or QPRT, which are involved in tryptophan metabolism for NAD^+ production, leads to increased production of pro-inflammatory cytokines, including TNF-α, IL-1β, LPS, and IFN-γ [99, 159, 160]. These cytokines activate nicotinamide phosphoribosyltransferase (NAMPT) expression, essential for NAD^+ production and required for CD38 (a NAD^+ hydrolase) consumption and ROS-induced DNA damage [161, 162]. CD38 is upregulated in both mouse and human M1 macrophages [163–165]. CD38 activity contributes to increased autophagy, which mediates early control of *Mtb* proliferation in lungs [166]. *Mtb* infection depletes NAD^+, which is critical for glycolysis and immune cell protection. Supplementation with NAM can limit *Mtb* proliferation by restoring NAD^+ levels [167, 168]. However, there are contradictory findings regarding NAD^+ supplementation. Increasing NAD^+ production through niacin (NA) or its metabolite NAM via NAMPT can shift macrophage polarization towards the M2 phenotype, resulting in reduced iNOS and NO production [169–171]. This discrepancy suggests that the host's NAD^+ homeostasis critically influences the balance between M1 and M2 macrophages that impacts the outcome of bacterial infection [98, 99]. One possible explanation is that during the early phase of *Mtb* infection, upregulated glycolysis, and reduced *de novo* NAD^+ biosynthesis leads to NAD^+ depletion, reduced sirtuin activity, and increased histone acetylation. Over time, NAD^+ levels are rebalanced through the salvage pathway, with upregulated NAMPT in M1-macrophages restoring sirtuin activity [172], which in turn reduces global histone acetylation and acetylation of CPS1 [97–99, 146]. Therefore, we propose that the balance between NAD^+ biosynthesis and salvage pathways and other metabolic pathways that consume or produce NAD^+ from NADH, together with the activation of SIRT2 and SIRT5, plays a crucial role macrophage polarization (Fig. 2).

In summary, sirtuin proteins, particularly SIRT2 and SIRT5, together with NAD^+ metabolism, play critical roles in regulating macrophage polarization through metabolic, epigenetic, and immune pathways. SIRT2, highly expressed in M2 macrophages, promotes anti-inflammatory responses by deacetylating NF-κB/RelA and histones, enhancing tissue repair but suppressing pro-inflammatory cytokines. In contrast, SIRT5, predominantly found in M1 macrophages, regulates mitochondrial metabolism via desuccinylation of key metabolic enzymes, influencing inflammatory responses and macrophage function. SIRT2 inhibition fosters M1 polarization, enhancing glycolysis, histone acetylation, and immune responses against infections, such as *Mtb*, though it may exacerbate insulin resistance for diabetes patients who are more vulnerable to *Mtb* infection. SIRT5 modulates lysine acetylation and succinylation stoichiometry, indirectly shaping macrophage metabolism and polarization states (Fig. 1). Both sirtuins compete for NAD^+ and acetylation sites, highlighting their interplay during macrophage polarization. However, the distribution of NAD^+ and sirtuins (beyond SIRT2 and SIRT5) across different cellular compartments—such as the cytosol, mitochondria, and nucleus—and their roles in regulating metabolism, energy production, redox reactions, DNA repair, and epigenetics during macrophage development and polarization remain poorly understood. This compartmentalization appears crucial for the immune response to foreign invaders but poses significant challenges for investigation as techniques to measure sirtuin targets and compartment-specific NAD^+ levels are limited and not yet widely accessible [156, 173].

SAM-dependent methylation during macrophage polarization

SAM serves as a critical methyl donor in cellular methylation reactions and consists of two primary components: an adenosyl group and methionine, the latter can donate a methyl group. The adenosyl group contains adenosine composed of a ribose sugar and a purine base. Ribose, an essential sugar component of adenosine, is derived from the PPP pathway and the degradation of nucleosides and nucleotides. Purine and methionine, the two essential precursors of SAM, can either be obtained directly from nutrients or synthesized internally. Purine synthesis involves a complex pathway relying on intermediates from the PPP and folate metabolism, while methionine synthesis depends on the one-carbon metabolism pathway, which provides the methyl groups necessary for methionine's conversion to SAM.

SAM serves as a cofactor for numerous methyltransferases, including histone lysine methyltransferases (HMTs), protein arginine methyltransferases (PRMTs), DNA methyltransferases (DNMTs), RNA methyltransferases,

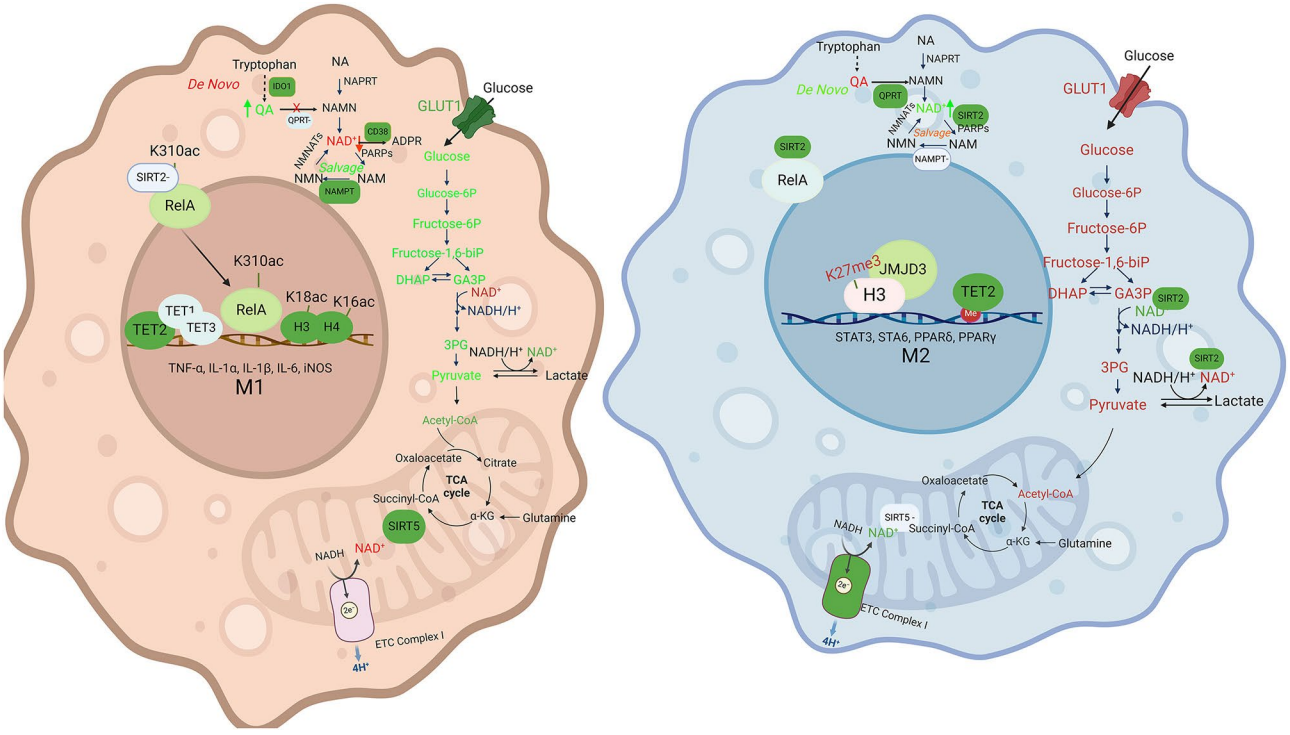

Fig. 2 Regulation of NAD^+level and SIRT2/5 expression in M1 and M2 Macrophages. M1 Macrophages (Left): The pro-inflammatory M1 phenotype exhibits enhanced glycolysis, as indicated by the upregulation of GLUT1 and key glycolytic intermediates (NAD^+ consumption), leading to lactate production (NAD^+ production). NAD^+ biosynthesis is downregulated via the de novo (tryptophan-derived quinolinic acid pathway) due to reduced expression of QPRT, though it may be compensated by salvage pathways (mediated by increased NAMPT). SIRT5 supports ETC function and TCA cycle flux by modulating NAD^+ levels. Epigenetically, TET2 (the most abundant in macrophages compared to TET1 and TET3) enhances transcription of TNF-α, IL-1β, and other pro-inflammatory cytokines through DNA demethylation, while the enhanced acetylation of histones and NF-κB/RelA (K18ac, K310ac) due to abolished NAD^+-dependent deacetylase, SIRT2, promotes cytokine gene expression. M2 Macrophages (Right): The anti-inflammatory M2 phenotype relies on oxidative metabolism and fatty acid oxidation, driven by TCA cycle flux. Glycolysis proceeds minimally, with limited lactate production. NAD^+ levels are increased by unregulated *de novo* synthesis and ETC complex I, and decreased consumption by glycolysis. Together with high expression of SIRT2, acetylation of histones and RelA is decreased resulting in suppression of pro-inflammatory cytokine expression. In contrast, histone H3K27me3 marks are removed by JMJD3, enabling transcription of STAT3, STAT6, PPARδ, and PPARγ, which is also facilitated by TET2 demethylation, contributing to the expression of M2-specific genes. Green color: Up-regulated; Red color: Down-regulated

and various metabolic methyltransferases. The synthesis of SAM through pathways such as the PPP, SSP, and one-carbon metabolism is synergistically regulated during LPS-induced inflammation and is essential for activating the pro-inflammatory gene IL-1β via H3K36 tri-methylation [174]. Interestingly, extracellular treatment with a high concentration of SAM (500 μM) has been shown to repress the expression of LPS-induced pro-inflammatory genes, such as TNF-α, while increasing the expression of the anti-inflammatory gene IL-10 and enhancing global DNA methylation [175]. While more studies on histone, DNA, and RNA methylation during macrophage polarization can be found elsewhere [22, 176], this review will focus specifically on protein arginine methylation, which not only depends on SAM as a methyl donor but also generates degradation products—monomethylarginine (MMA), symmetrical dimethylarginine (SDMA), and asymmetrical dimethylarginine (ADMA)—that are involved in arginine metabolism and NO production [98, 177].

Protein arginine methylation: a link between epigenetics, arginine metabolism, and host-pathogen interactions

Protein arginine methylation, regulated by a family of 11 identified protein arginine methyltransferases (PRMTs), plays a crucial role in both immune responses and host-pathogen interactions. Among the enzymes in the PRMT family, PRMT1, PRMT5, CARM1/PRMT4 were the predominant proteins identified in proteomic analyses of human monocyte-derived macrophages (Data are available via ProteomeXchange with identifier PXD058308, and our recently unpublished data) [178]. Extensive studies have highlighted their critical roles in macrophages, particularly in modulating immune responses to pathogens and in cancer invasion [179, 180]. Therefore, in this review, we will center our discussion exclusively on these three PRMTs. PRMT1, an asymmetrical dimethyltransferase, catalyzes the formation of ADMA during protein degradation. ADMA serves as an endogenous inhibitor

of nitric oxide synthase (NOS) in both circulating blood and macrophages, thereby reducing NO production [181]. PRMT1 is closely linked to the regulation of inflammation and M2 macrophage polarization (Fig. 3A). For instance, PRMT1 knockout has been shown to increase the production of pro-inflammatory cytokines, a hallmark of M1 macrophages [182]. Mechanistically, PRMT1 regulates PPARγ gene expression by methylating histone H4 at arginine 3 (H4R3me2a) on the PPARγ promoter, thereby promoting M2 macrophage polarization

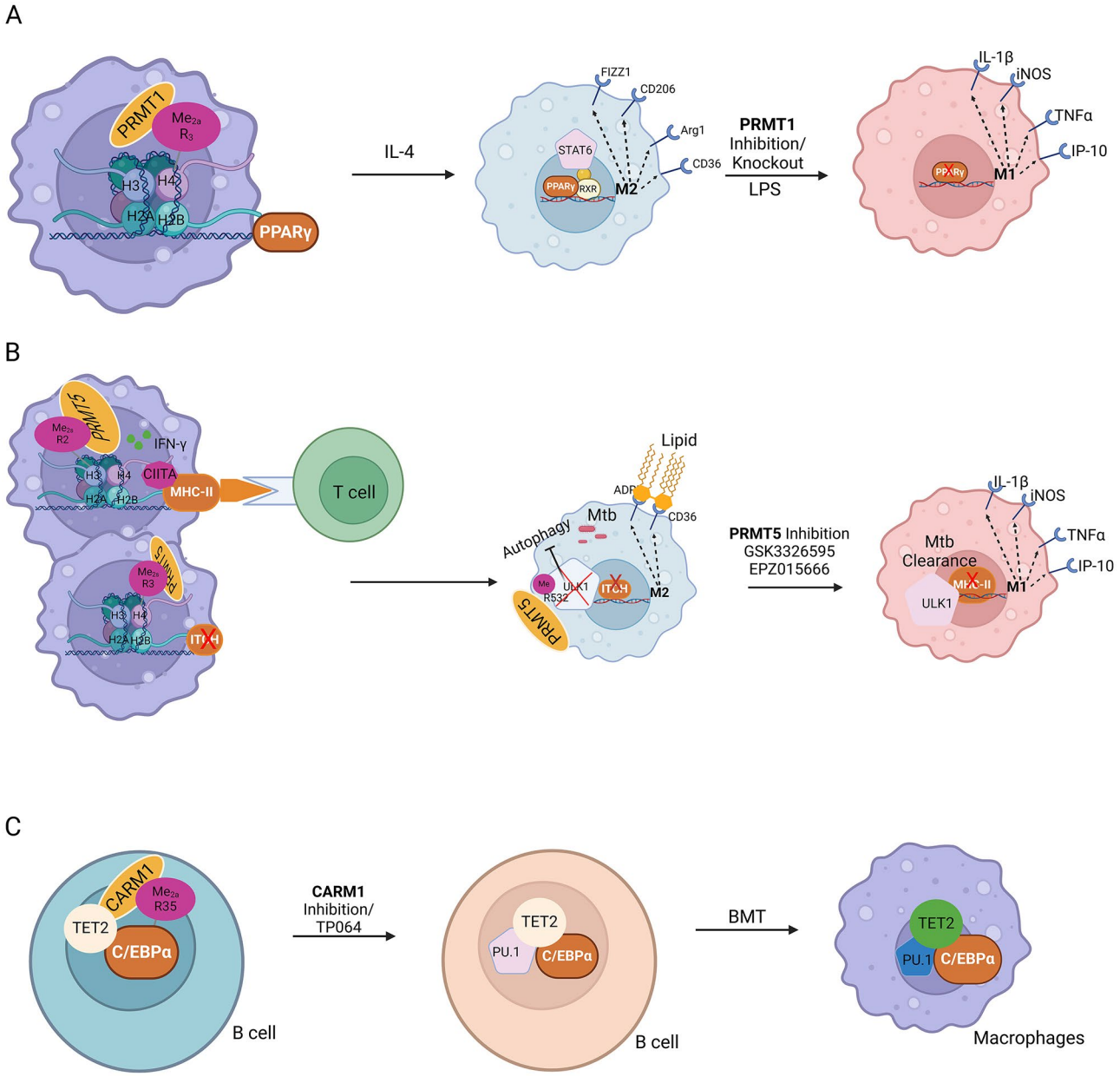

Fig. 3 Regulation of Macrophage Polarization and Function by Protein/Histone Arginine Methylation. (**A**) PRMT1-mediated arginine methylation of histones and non-histone proteins promotes IL-4-induced M2 macrophage polarization via STAT6 and PPARγ activation. Inhibition or knockout of PRMT1 shifts macrophages from the anti-inflammatory M2 phenotype towards the pro-inflammatory M1 phenotype, characterized by increased TNF-α secretion and increased expression of IL-1β, iNOS, and IP-10. (**B**) PRMT5 plays a dual role in macrophage responses during *Mtb* infection. It enhances IFN-γ-induced MHC II transcription by symmetrically dimethylating histone H3R2, promoting antigen presentation to T cells. Simultaneously, PRMT5 represses ITCH expression via H4R3me2s methylation, stabilizing lipid accumulation proteins (ADRP, CD36) to support foam cell formation and *Mtb* survival. PRMT5 also suppresses autophagy by monomethylating ULK1 at R532. Inhibition of PRMT5 with EPZ015666 reduces lipid accumulation, restores autophagy, and shifts macrophages toward the pro-inflammatory M1 phenotype, enhancing *Mtb* clearance. (**C**) CARM1 methylates R35 of the transcription factor C/EBPα in B cells. Inhibition of CARM1, via pharmacological agents such as TP064, promotes the differentiation of B cells into macrophage-like cells through bone marrow transplantation (BMT), involving PU.1 and C/EBPα demethylation by TET2

[182]. Furthermore, PRMT1 acts as a negative regulator of anti-tumor immunity by suppressing MHC-I expression, a crucial molecule for presenting tumor antigens to immune cells [183]. Inhibition or knockout of PRMT1 enhances MHC-I expression and boosts the efficacy of anti-PD-1 immunotherapy, suggesting that targeting PRMT1 could improve the outcome of cancer immunotherapy [183].

PRMT5, another key enzyme, plays a significant role in immune regulation by symmetrically dimethylating histone H3 at arginine 2 (H3R2me2s) following IFN-γ stimulation, which in turn regulates CIITA-mediated MHC II transcription. This modification accumulates on the MHC II promoter, marking it as active for transcription, and facilitates the recruitment of CIITA, a critical transcriptional coactivator for MHC II gene expression. This synergy enhances MHC II transcription, which is vital for macrophage-mediated presentation of antigenic peptides to helper T cells and initiating an effective adaptive immune response [184]. Inhibition of PRMT5 with GSK3326595 has been shown to reduce MHC II expression in IFN-γ-stimulated peritoneal macrophages. However, PRMT5 inhibition also primes macrophages for IFN-γ-induced M1 polarization, leading to elevated mRNA expression levels of M1 markers such as TNF-α, IL-1β, iNOS, and IP-10 (Fig. 3B) [185].

Arginine methylation of histones has also emerged as a crucial determinant of *Mtb* survival within host cells. A significant example of this is the epigenetic regulation of the E3 ubiquitin ligase ITCH through arginine methylation of histone H4 by PRMT5, which profoundly impacts lipid accumulation during mycobacterial infection [186]. Lipid accumulation within the host is a pro-mycobacterial process that facilitates pathogen persistence and dormancy. *Mtb* infection represses the expression of ITCH via PRMT5, which induces repressive H4R3me2s methylation on the ITCH promoter, thereby inhibiting ITCH expression. This inhibition prevents the proteasomal degradation of key lipid accumulation molecules, such as Adipose Differentiation-Related Protein (ADRP) and CD36. Knockdown of PRMT5 via siRNA rescues ITCH expression, leading to reduced levels of *Mtb*-induced ADRP and CD36, thus limiting foam cell (FM) formation during *Mtb* infection. Furthermore, inhibition of PRMT5 enzyme activity with the PRMT5 inhibitor EPZ015666 results in reduced lipid levels and decreased mycobacterial survival in mouse peritoneal macrophages (ex vivo) and in a therapeutic mouse model of *Mtb* infection (in vivo) [186]. Additionally, this process may involve autophagy; genetic ablation or pharmacological inhibition of PRMT5 has been shown to trigger cytoprotective autophagy. Indeed, in breast cancer cells, PRMT5 catalyzes the monomethylation of ULK1 at R532, thereby suppressing ULK1 activation and attenuating autophagy (Fig. 3B) [187]. This underscores the complex role of PRMT5 in modulating autophagy and immune responses, with implications for various diseases [185].

CARM1, also known as PRMT4, is unique among PRMTs as it is the only one that methylates arginine residues in proteins with a nearby proline-rich sequence. Although its substrates may not be fully identified, CARM1 is known to target proteins involved in gene expression, chromatin organization and remodeling, mRNA metabolism, lipid metabolism via PPARα, and white adipocyte differentiation [188]. In addition to acting as a transcriptional coactivator, CARM1 plays a role in cell development and differentiation, is implicated in cancer progression and overexpressed in type 2 diabetes [189, 190]. Furthermore, CARM1 acts as a nutritional sensor by increasing in response to glucose and amino acid starvation. This increase is driven by the repression of its degradation via the SKP2-containing SCF E3 ubiquitin ligase complex in the nucleus. As a result, CARM1 binding to TFEB on autophagy gene promoters increases, where it catalyzes the asymmetric dimethylation of histone H3 at arginine 17 (H3R17me2a); this modification enhances transcriptional activation of autophagy genes [191]. Furthermore, CARM1 methylates the cofactor Pontin, which enhances Pontin's interaction with FOXO3a and promotes the recruitment of the histone acetyltransferase Tip60 to the enhancer regions of autophagy genes [192]. This recruitment boosts histone H4 acetylation, creating a dual activation mechanism: CARM1 activates autophagy genes both at the promoter (through TFEB binding and H3R17me2a) and the enhancer (through FOXO3a-Pontin-Tip60 interaction). Together, these actions significantly upregulate autophagy under nutrient-deprived conditions, providing a comprehensive adaptive response to starvation [191, 192].

In summary, both PRMT1 and PRMT5 are seemingly associated with M2 macrophage polarization and host immune responses. Circulating monomethyl arginine (MMA) and ADMA inhibit NOS, reducing NO production [181, 193]. Inhibiting PRMT1 or PRMT5 alleviates the suppression of iNOS, elevating NO levels. This increase in NO production, combined with the upregulation of pro-inflammatory cytokines in M1 macrophages, enhances the immune response, aiding in the defense against infections and the destruction of cancer cells. Moreover, targeting PRMT5 may serve as a potential strategy for controlling mycobacterial infections by reducing lipid accumulation in macrophages and enhancing their autophagic activity [186].

Although CARM1 increase autophagy of cancer cells under nutrition stresses [191, 192], the inhibition of CARM1 enhances the efficacy of immunotherapy against resistant tumors by improving T-cell function, preserving

memory T-cell populations, and amplifying tumor cell-intrinsic type 1 interferon responses [194]. Additionally, CARM1 methylates the transcription factor C/EBPα, which regulates B-cell trans-differentiation into macrophages (Fig. 3C) [195]. However, its role in macrophage polarization remains unknown, and further studies are needed to clarify the role of CARM1 in immune regulation, particularly during macrophage activation and polarization.

Linking α-Ketoglutarate (α-KG) to histone and DNA demethylation during macrophage polarization

α-KG, a rate-determining intermediate in the TCA cycle and a key metabolite produced through glutaminolysis, plays a crucial role in linking metabolism to epigenetic regulation, particularly in macrophage function. This anti-inflammatory metabolite not only enhances M2 macrophage activation but also regulates their metabolic reprogramming via mechanisms dependent on the demethylase Jumonji domain-containing 3 (JMJD3) [196]. The balance between α-KG and succinate levels is pivotal: a high α-KG/succinate ratio favors M2 macrophage polarization, while a low ratio promotes the pro-inflammatory M1 macrophage phenotype [196].

Role of α-KG in histone demethylation

α-KG serves as a cofactor for several histone demethylases, including JMJD3, which specifically demethylates trimethylated lysine 27 on histone H3 (H3K27me3), a repressive mark associated with gene silencing. IL-4 induces up-regulation of JMJD3 and JMJD3-mediated demethylation at the IRF4 locus activates this gene, promoting M2 macrophage polarization in response to stimuli such as helminth infection and chitin exposure [4, 23]. The JMJD3-IRF4 axis also plays a role in modulating type I interferon signaling in M1 macrophages, and this modulation influenced by the α-KG/succinate ratio, which is in turn regulated by IFN-β signaling [197]. Additionally, during *Mtb* infection, the RNA-binding protein MUSASHI (MSI) targets JMJD3 expression, facilitating the generation of lipid-laden foam macrophages and promoting M2 polarization through a TLR2-dependent mechanism [198].

Role of α-KG in DNA demethylation

In DNA, α-KG acts as a cofactor for TET enzymes including TET, TET2 and TET3, which are responsible for converting 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), a key step in DNA demethylation. This process is essential for regulating gene expression in macrophages. For example, TET1 has been shown to increase 5-hmC levels globally and at the TNF-α promoter during the differentiation of monocytes into macrophages, with further increases upon LPS stimulation. Knockout studies of TET1 reveal its role in promoting pro-inflammatory gene expression by reducing TNF-α levels [199]. In contrast, TET2 has been found to restrain pro-inflammatory gene expression, modulating the inflammatory response without significantly affecting IL-4-induced M2 macrophage polarization, and loss of TET2 leads to increased expression of inflammatory genes [200]. Therefore, TET1 promotes inflammatory gene expression and TET2 restrain it. The discrepancy between TET1 and TET2 may be due to their distinct biological roles, despite both being involved in DNA oxidation/demethylation. TET1 is primarily responsible for maintaining demethylation at promoters and CpG islands [201], helping to regulate gene expression, particularly in stem cell pluripotency [202] and in enhancing pro-inflammatory gene expression in macrophages [199]. On the other hand, TET2 has a broader scope, being active at enhancers and involved in both DNA and RNA methylation. TET2 regulates chromatin state and gene activation by mediating RNA m^5C methylation, particularly in repetitive elements like LTR repeat RNAs, as its loss leads to global DNA hypomethylation resulting in an open chromatin state and transcription by antagonizing the m^5C-MBD6-BAP1 gene activation pathway [203, 204]. TET3, like TET1, promotes the expression of pro-inflammatory genes in macrophages, playing a key role in regulating immune responses [205]. TET2 is significantly more expressed compared to TET1 and TET3 in mouse [200] and possibly human macrophages as suggested by our unpublished proteomics data, and its mRNA expression is notably induced during *Mtb* infection [206]. TET2 is essential for hematopoietic cell maturation, and its loss or mutation is associated with leukemogenesis [207]. It regulates immune tolerance in chronically activated mast cells [208]. It is important for immune cell differentiation via suppressing the production of MIF (macrophage migration inhibitory factor) through interaction with histone deacetylation and transcription factor EGR1 (early growth response 1) [209]. In addition, TET2 plays an important role in B cell development, T cell development and differentiation [210]. Interestingly, TET2 facilitates the de-repression of myeloid target genes during C/EBPα-induced transdifferentiation of pre-B cells [211]. This mechanism suggests a potential collaboration of TET2 with CARM1, as previous discussions have highlighted a similar role for CARM1 in the transdifferentiation of B cells to macrophages (Fig. 3C) [195]. This possibility is further supported by the fact that TET2 is one of the methylation substrates of CARM1 [188]. In macrophages, TET2 expression is induced by LPS but not IL-4 [200], and it interacts with RelA to activate TNF-α, a key component of M1 polarization [206]. Conversely, TET2 can modulate M2 polarization by recruiting HDAC2 to suppress IL-6 [200, 212]

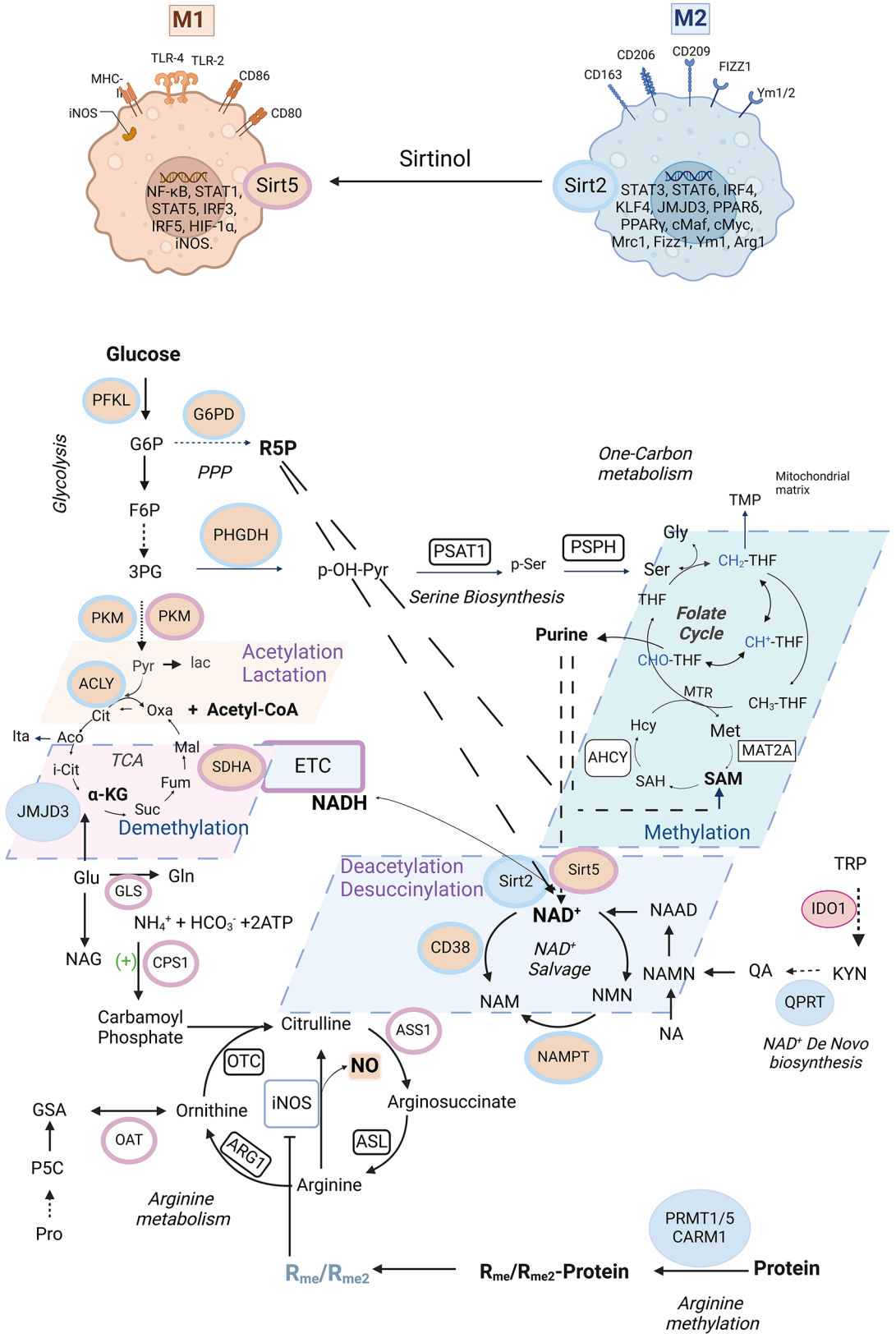

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Fig. 4 Metabolic pathways that links epigenetics in macrophages. In M1 macrophages, SIRT5 is highly expressed and targets key enzymes involved in glycolysis, ETC, and glutamine/arginine metabolism. These targets include PKM in glycolysis, SDHA (succinate dehydrogenase) in the ETC, and GLS (glutaminase), CPS1 (carbamoyl-phosphate synthase 1), ASS1 (argininosuccinate synthase), and OAT (ornithine aminotransferase) in amino acid metabolism. In contrast, SIRT2 is highly expressed in M2 macrophages, where it regulates glycolytic enzymes such as PFKL (phosphofructokinase) and PKM, for acetyl-CoA synthesis, G6PD (glucose-6-phosphate dehydrogenase) in the pentose phosphate pathway, PHGDH (phosphoglycerate dehydrogenase) in serine biosynthesis, and CD38 and NAMPT in the NAD^+ salvage pathway. SIRT2 activity is linked to epigenetic regulation, including the upregulation of JMJD3, a histone H3K27 demethylase that requires α-KG from the TCA cycle, IDO1 (indoleamine 2,3-dioxygenase) involved in tryptophan metabolism and NAD^+ biosynthesis, and PRMT1 and PRMT5, which mediate arginine methylation. These metabolic pathways are closely tied to histone acetylation, succinylation, deacetylation, arginine methylation, RNA/DNA/histone methylation and demethylation, linking metabolic states with epigenetic modifications in macrophages. Enzymes targeted by SIRT5 are marked with a purple border, those targeted by SIRT2 with a blue border, while upregulated enzymes are filled in orange for M1 macrophages and blue for M2 macrophages, highlighting the metabolic-epigenetic interplay in macrophage polarization

or regulating mRNA 5-methylcytosine methylation [213], in order to resolve inflammation [212].

Concluding remarks

Macrophage polarization is intricately regulated by both metabolism and epigenetic modifications. Pro-inflammatory M1 macrophages rely on glycolysis, while anti-inflammatory M2 macrophages favor oxidative phosphorylation and fatty acid oxidation, highlighting the metabolic shift essential for their functions [19]. Key metabolic pathways, such as glycolysis, TCA cycle, the PPP pathway, arginine metabolism, serine biosynthesis, and one-carbon metabolism for purine and methionine synthesis, are differentially activated in M1 and M2 macrophages, influencing their polarization states (Fig. 4). Epigenetic mechanisms, including histone acetylation, deacetylation, methylation, and demethylation, as well as DNA and RNA methylation, further shape macrophage function. Increased acetyl-CoA and histone acetylation enhances pro-inflammatory gene expression in M1 macrophages, while histone deacetylation represses inflammatory genes in M2 macrophages [98, 112]. Methylation patterns, such as DNA hypermethylation in the IL-12/IFN-γ signaling pathway, suppress pro-inflammatory responses [214], whereas demethylation by TET1 supports M1 polarization and demthylation by TET2 favors M2 macrophages [199]. RNA methylation, particularly N^6-methyladenosine (m^6A), also regulates inflammatory mRNA stability and translation [215]. Sirtuin proteins, like SIRT2 and SIRT5, integrate metabolic and epigenetic control in macrophages, with SIRT2 promoting M2 polarization and SIRT5 influencing arginine and glutamine metabolism with PRMT-catalyzed protein arginine methylation. Metabolites such as acetyl-CoA, SAM, α-KG, and NAD^+ link cellular metabolism to epigenetic regulation, as they act as substrates or cofactors for enzymes driving acetylation, methylation, deacetylation/desuccinylation, and demethylation (Fig. 4). During early infection of pathogens or simulation with LPS, the balance between these epigenetic modifications defines the polarization state of macrophages, with M1 macrophages exhibiting active histone acetylation and reduced histone and DNA methylation while M2 macrophages showing increased histone deacetylation and histone/DNA methylation. The epigenetic landscape is dynamic, influenced by metabolites produced following stimulation, such as lactate and TCA cycle intermediates. Targeting these metabolic and epigenetic pathways offers therapeutic potential to modulate macrophage function in infectious and inflammatory diseases, such as tuberculosis and diabetes.

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Author contributions
KZ: Wrote. CJ: Edited.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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