Sirtuins are crucial regulators of T cell metabolism and functions

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INTRODUCTION

Overview of the T cell-mediated adaptive immune response

The adaptive immune response elicited by T cells is crucial in mounting specific immune responses against foreign pathogens. T cells develop in the thymus and, upon maturation, are classified by their expression of either CD4 or CD8 receptors. CD4+ and CD8+ T cells exist in several subsets that perform unique functions during an immune response and are characterized by specific surface receptors, cytokine secretion, and expression of lineage-defining transcription factors (Fig. 1)1,2. Depending on the pathogen type and the cytokines generated by antigen-presenting cells (APCs), CD4+ T cells can become activated and differentiate into functionally distinct T helper (Th) 1, Th2, Th17, Th9, or T regulatory (Treg) cells3, while CD8+ T cells differentiate into cytotoxic T lymphocytes (CTLs)4. Th1 cells, which produce interferon-gamma (IFN-γ), interleukin (IL)-2, and tumor necrosis factor-beta (TNF-β), evoke cell-mediated immunity and phagocyte-dependent inflammation and are involved in the elimination of intracellular pathogens5. Th2 cells, which produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, are known to provide immunity against extracellular parasites, including helminths, and play major roles in the induction and persistence of asthma as well as other allergic diseases6. Th9 cells are known to primarily produce IL-9 and facilitate the immune response against helminth parasites7. Th17 cells are characterized by the production of IL-17 and are involved in host protection against microbial infections that are not resolved by Th1 or Th2 immunity, including infections with a subset of extracellular bacteria and some fungi8. Retinoic acid receptor-related orphan nuclear receptor gamma (RORYt) is considered to be one of the master regulators of the development of Th17 cells9. In contrast to the proinflammatory phenotype of Th cells, Treg cells participate in the prevention of uncontrollable inflammatory responses, autoimmune diseases and allergies by suppressing T effector (TEFF) cells and other immune cells10. Treg cells are defined by the expression of the forkhead box P3 (Foxp3) transcription factor10. This cell subset secretes key anti-inflammatory cytokines, including IL-10, tumor growth factor-β (TGF-β), and IL-35. CTLs are CD8+ T cells that participate in cellular immunity, and their role is to directly kill infected or malignant cells through the release of cytotoxic cytokines, including TNF-α and IFN-γ, as well as granules including perforin and granzymes11.

Metabolic shifts in T cells

Naive T (T N) cells are quiescent and require energy only for survival and circulation. These cells rely predominantly on mitochondrial oxidative phosphorylation (OxPhos) metabolism to generate ATP from glucose or fatty acids (FAs)11. Activation of T cells occurs after stimulation of the T cell antigen receptor (TCR) by a ligand in association with costimulatory signals, which initiates several signaling pathways that promote cell differentiation and growth. This activation is associated with a transition from relatively quiescent oxidative metabolism to intense glycolytic metabolism to support energetic needs for proliferation and cytokine production11–13. In addition to increased glucose catabolism in activated T cells, glutamine uptake and glutaminolysis are also upregulated to supply the tricarboxylic acid (TCA) cycle for ATP generation14,15. The metabolic profile of each specialized T cell subset is optimized to support their unique functions. TEFF cells, including Th1, Th2, and Th17 cells and CTLs, principally rely on aerobic glycolysis and glutaminolysis to promote their rapid growth, proliferation, and effector functions16,17. In contrast, Treg cells rely primarily on FA oxidation (FAO) and glutaminolysis to support their suppressive activity18,19.
rapidly respond to the same antigen. These cells are long-lived, metabolism (TME)22. To partially rescue their effector functions, TILs have been shown to increase FAO metabolism as a way to utilize alternative fuel sources23.

Due to competition for glucose and glutamine with cancer cells, alternative fuel sources23.

Increased evidence indicates that immune cell identity, functions, and metabolism are mediated by overlapping signaling pathways that are controlled, at least in part, via epigenetic and posttranslational modification (PTM) mechanisms. Sirtuins are key epigenetic and PTM regulators in T cells. In this review, we will provide relevant insights into the current understanding of T cell-specific immune response regulation by sirtuins and the therapeutic potential of sirtuin modulators in immune-related diseases.

**EPIGENETIC MECHANISMS**

In multicellular organisms, all cells contain the same genome; however, gene expression profiles vary from cell to cell, which contributes to differentiation into various cell types within the same organism24. This cell type differentiation is mostly regulated by epigenetic mechanisms, which result in dynamic changes in gene expression patterns without changing the DNA sequence25. Epigenetic regulation of gene expression is known to occur at the DNA, histone, and RNA levels. In this context, DNA methylation, histone methylation, acetylation, ubiquitination, phosphorylation, and microRNA-dependent gene silencing have been well characterized26. Methylation of DNA is mediated by DNA methyltransferases and consists of the addition of a methyl group to cysteine residues within DNA regions that are rich in cysteine-guanine dinucleotides. DNA methylation can act either to inhibit gene transcription if methylation occurs within a promoter region or to promote gene transcription if methylation occurs within the gene body27. Noncoding microRNAs are single-stranded RNA fragments that interact with target messenger (m)RNAs via a perfectly complementary base sequence, resulting in mRNA cleavage and translational repression28. Another important and common epigenetic mechanism that contributes to gene expression regulation is the modification of histones. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are the two classes of enzymes involved in the dynamic regulation of histone acetylation29. Acetylated histones maintain an open and fluid chromatin structure, called euchromatin, promoting gene transcription, while deacetylated histones often form a tightly packed chromatin structure that prevents gene transcription, called heterochromatin30.

**POSTTRANSLATIONAL MODIFICATION**

PTM is a biochemical mechanism in which amino acid residues from a protein are covalently modified after translation to regulate protein folding, degradation, signaling, localization, stability, enzymatic activity or protein–protein interactions31. There are over 400 known PTM types, including phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation, and proteolysis, which influence almost all aspects of physiological and pathological cell processes32. In contrast to epigenetic mechanisms, PTM allows rapid changes in protein properties in response to cellular needs during acute stress phases. In addition, multiosite PTM leads to a vast variety of potential molecular states33. Phosphorylation is the most widely studied PTM and can regulate signaling pathways, the cell cycle, metabolism, the immune response, and cellular growth and differentiation34. Ubiquitin (Ub) is a small and highly conserved protein made of 76 amino acids that can be attached to a lysine substrate through a complex conjugation process requiring a Ub-activating enzyme (E1), Ub-conjugating enzyme (E2) and Ub ligase (E3). Ubiquitination plays important regulatory roles in the life cycle of proteins by leading to the degradation of target substrates35. This process can be reversed by deubiquitinating enzymes (DUBs), which remove conjugated Ub molecules from target substrates36. Protein acetylation has emerged as a key PTM participating in cellular regulation, particularly through the modification of histones, nuclear transcription regulators and metabolic enzymes37. Lysine acetylation is the prevalent modification of metabolic enzymes, and virtually every enzyme in glycolysis, gluconeogenesis, the TCA cycle, the urea cycle, fatty acid metabolism, and glycogen metabolism has been found to be acetylated in human liver tissue38. Protein acetylation status is regulated by a highly dynamic equilibrium between HATs and HDACs39.

**OVERVIEW ON SIRTUINS**

The sirtuin proteins are classified within class III HDACs, which require nicotinamide adenine dinucleotide (NAD\(^+\)) as a cofactor for their deacetylase activity. Although initially identified as HDACs, later studies revealed that sirtuins can deacetylate a variety of nonhistone proteins40. Moreover, sirtuins show additional enzymatic functions other than deacetylation.

In mammals, seven sirtuins (Sirt1–7) are ubiquitously expressed with distinct subcellular localization and functions (Fig. 2)41–43. Sirt1 is the most studied mammalian sirtuin and is primarily localized in the nucleus. However, under specific conditions, Sirt1 can be transported to the cytoplasm44. Sirt2 is a predominantly cytosolic sirtuin that can migrate to the nucleus during mitosis45. Sirt6 is exclusively localized in the nucleus, whereas Sirt7 is localized in the nucleolus46. All the remaining sirtuins (3–5) are localized in mitochondria47. All sirtuins except Sirt5 have mono-ADP-ribosyl transferase activity, whereas Sirt5 catalyzes the
removal of succinyl, malonyl, and glutaryl groups from protein targets. The first sirtuin protein was discovered in yeast as silent information regulator 2 (Sir2), whose homolog in mammals is known as Sirt1. Since it was discovered that Sir2 extends yeast lifespan in response to calorie restriction, sirtuins have received considerable attention as anti-aging regulators. Furthermore, sirtuins have been described to regulate a variety of cell processes, including genome stability and cell metabolism, and are currently gaining extensive interest for their role in mediating several diseases associated with inflammation, metabolic disorders, and cancers. As sirtuins depend on NAD⁺ (a critical cofactor of metabolism) for their enzymatic activity, any fluctuations in NAD⁺ levels related to nutrient availability affect their functions. Hence, sirtuins serve as energy sensors that directly link environmental signals to cellular metabolic homeostasis. Given the link between metabolic reprogramming, nutrient availability and T cell effector function, sirtuins are speculated to be potential regulators of the immune response, impacting the ability of the immune system to combat foreign pathogens or malignant cells. While each of the sirtuins has been broadly studied and demonstrated to be involved in a number of diseases associated with inflammation, investigations of the roles of sirtuins in adaptive immunity have been limited to certain aspects of the immune response, and the most intense attention has been given to Sirt1 and Sirt3. This review will discuss the latest advances in the roles of the seven mammalian sirtuins in the T cell adaptive response and include our considerations for targeting sirtuins to manipulate the immune response.

SIRT1

Most studies on Sirt1 have indicated that decreased Sirt1 expression or activity contributes to the enhancement of T cell activation, thereby leading to the occurrence of autoimmune diseases. The immunoregulatory function of Sirt1 is dependent on the cell types and specific substrates that are targeted within the immune response. In addition, Sirt1-mediated regulation of metabolic processes is critical for optimal immune cell function. c-Jun is upregulated after T cell activation to induce IL-2 production, cell proliferation and differentiation. An early study indicated that Sirt1 inhibits the immune response by blocking c-Jun transcription factor activity, which supports IL-2 production and thereby decreases Th1 cell activation. Indeed, enhanced expression and activity of Sirt1 induced by resveratrol treatment impeded CD4⁺ T cell activation and IFN-γ production, further confirming that Sirt1 negatively impacts Th1 differentiation and IFN-γ secretion. More recently, in a mouse model of ovarian cancer, Th1 cell differentiation of CD4⁺ T cells was found to be increased after treatment with artesunate, a promoter of microRNA-142 expression that downregulates Sirt1 expression, again confirming the suppressive role of Sirt1 in Th1 cell differentiation.

Studies in Sirt1 knockout (Sirt1−/−) mice showed that Sirt1−/− T cells display increased proliferation and IL-2 production, and Sirt1−/− mice are more susceptible to developing autoimmune diseases. Further studies indicated that IL-2 can reverse T cell anergy by suppressing Sirt1 transcription via cytosolic sequestration of its upstream transcription factor, FoxO3a. The expression of a constitutively active form of FoxO3a blocks IL-2-mediated reversal of T cell tolerance by retaining Sirt1 expression.

B-cell lymphoma 2-associated factor 1 (Bclaf1), primarily considered a promoter of cellular apoptosis, has been found to be critical for T cell activation. Kong et al. reported that Sirt1 suppresses Bclaf1 activity by deacetylating histone lysine residues at the Bclaf1 promoter region, resulting in decreased IL-2 gene transcription. Accordingly, Sirt1−/− T cells displayed higher expression of the Bclaf1 gene and IL-2 production, and specific knockdown of Bclaf1 reversed the increase in IL-2 production and proliferation observed in Sirt1−/− T cells.

Early studies using ovalbumin-induced asthma models in mice demonstrated that pharmacologic inhibition of Sirt1 reduced allergic reactions compared with mock treatment. Furthermore, Sirt1 inhibition was found to suppress the differentiation of Th2 cells through the B cell lymphoma/leukemia 11B (Bcl11b) transcriptional activator. Bcl11b is essential for Th2 differentiation, and mice lacking Bcl11b in mature T cells have a diminished capacity to mount Th2 responses during helminth infection and allergic asthma. Sirt1 interacts directly with Bcl11b and is recruited to the promoter template in a Bcl11b-dependent manner to deacetylate histones, leading to transcriptional repression.

Hypoxia-inducible factor 1-alpha (HIF-1α) activity has consistently been associated with the generation of proinflammatory cytokines and restriction of anti-inflammatory cytokines. Sirt1 inhibition was found to promote Th9 cell differentiation and IL-9 production via the Sirt1-mTOR-HIF-1α axis. In the same study, Wang et al. indicated that Sirt1-dependent regulation of glycolysis was critical for directing the differentiation of Th9 cells, highlighting the importance of metabolic reprogramming in controlling T cell fate.

The effects of Sirt1 on Th17 cells are controversial, and more studies are needed to dissect its precise role. A few studies have indicated that the differentiation of Th17 cells is affected by the degree of STAT3 deacetylation. Sirt1 activators such as metformin have been shown to impede Th17 cell differentiation and reduce IL-17A and RORγt expression via deacetylation of the STAT3 transcription factor. STAT3 deacetylation restricts its ability to translocate into the nucleus to induce RORγt transcription. Another study showed that in vivo activation of Sirt1 using NAD⁺ supplementation delayed the onset of experimental autoimmune encephalomyelitis (EAE). This protection was hypothesized to be the result of the decrease in Th17-mediated inflammatory responses induced by enhanced Sirt1 expression. Moreover, treatment with methylene blue, another Sirt1 activator, alleviated Th17 responses and significantly reduced the clinical scores of EAE in mice. In contrast, other researchers have shown that Sirt1 activation promotes the Th17 phenotype via RORγt deacetylation. Deacetylated RORγt appears to have stronger transcriptional...
activity than Foxp3, thus strengthening the Th17 proinflammatory phenotype and suggesting that Sirt1 inhibitors may protect against autoimmune diseases.77

Foxp3 is a master regulator of Treg cell development and function and has three lysine acetylation sites (K31, K262, and K267) targeted by Sirt1.78 Hyperacetylation of Foxp3 prevents its polyubiquitination and proteasomal degradation. Accordingly, Sirt1 deacetylase activity has been found to reduce Foxp3 protein levels, and treatment with Sirt1 inhibitors results in increased functional Treg cells.79 In addition to the posttranslational control of Foxp3 by Sirt1, some studies reported that genetic deletion or pharmacologic inhibition of Sirt1 increased the number and suppressive activity of Foxp3 Treg cells by increasing Foxp3 mRNA levels.80,81 In contrast, Sirt1 was reported to promote Treg cell survival by stabilizing the Notch1 intracellular domain proximal to the membrane, given that the Notch receptor is essential for Treg cell survival within caloric-restricted conditions.82

Basic leucine-zipper ATF-like transcription factor (BATF) regulates multiple aspects of the T cell immune response.83 Sirt1 was reported to impact CD8+ T cell differentiation and effector functions under the influence of BATF. Indeed, BATF has been shown to transcriptionally inhibit Sirt1 expression, resulting in increased histone acetylation at the T-bet locus. In turn, high levels of T-bet expression promote CD8+ T cell differentiation, and loss of BATF consequently inhibits CD8+ T cell differentiation.84

The metabolic switch toward FAO is a characteristic of Th1 cell differentiation, and this switch is known to be supported by the transcriptional coactivator PGC-1, which is involved in mitochondrial biogenesis and OxPhos metabolism.85 Sirt1-mediated deacetylation has been shown to increase the transcriptional activity of the PGC-1α and PGC-1β cofactors,86,87 suggesting a potential role for Sirt1 in promoting Th1 cell formation. A previous report indicated a decrease in Sirt1 expression levels in terminally differentiated CD8+CD28− T cells, which concomitantly displayed enhanced glycolytic and cytotoxic capabilities,88 suggesting that Sirt1 may restrain glycolytic metabolism in T cells (Fig. 3).

SIRT2

Until recently, our knowledge on the role of Sirt2 within the immune system was limited to its anti-inflammatory function via negative regulation of the NF-κB p65 subunit.89 Accordingly, Sirt2-deficient mice were found to develop severe forms of dextran sodium sulfate (DSS)-induced colitis via polarization of bone marrow-derived macrophages.90 Furthermore, that study observed a greater proportion of activated CD4+CD69+ T cells in the mesenteric lymph nodes of Sirt2−/− mice in response to DSS-induced colitis, suggesting a role for Sirt2 in limiting CD4+ T cell functions.91

A recent publication uncovered a new function of Sirt2 as a master regulator of T cell metabolism and functions. Hamadi et al. found increased proliferation of CD4+ and CD8+ Sirt2−/− T cells upon TCR activation and activated CD8+ T cells displayed increased IFN-γ production and granzyme B expression, leading to superior cytotoxic activity. This hyperreactive phenotype of Sirt2−/− T cells endowed mice with superior antitumor immunity upon tumor challenge in vivo.92

Mechanistically, this hyperactivation and enhanced function of Sirt2−/− T cells is linked to Sirt2 regulation of multiple metabolic pathways that play crucial roles in T cell effector functions. Sirt2 deacetylates and negatively impacts the activity of key metabolic enzymes of the glycolysis, TCA cycle, FAO and glutaminolysis pathways. Accordingly, Sirt2 deletion during T cell activation was followed by hyperacetylation of multiple metabolic enzymes and amplification of their activities, leading to increased aerobic glycolysis, OxPhos, FAO, and glutaminolysis (Fig. 3)93.

**Fig. 3 Mammalian sirtuins and metabolic reprogramming.** The entry of glucose into the cell is mediated by the glucose transporter Glut1. Glucose is then metabolized to pyruvate, which enters the mitochondrial tricarboxylic acid (TCA) cycle and generates reducing equivalents for ATP production via oxidative phosphorylation (OxPhos). During aerobic glycolysis, pyruvate is fermented into lactate in the cytoplasm despite the availability of oxygen for complete glucose oxidation; this process is called the Warburg effect. Glutamine enters cells using alanine, serine, cysteine-prefering transporter 2 (ASCT2), and it is converted into glutamate by glutaminase (GLS) and into α-ketoglutarate (α-KG) by glutamate dehydrogenase (GDH), which enters the TCA cycle for ATP production via OxPhos. Fatty acid molecules are catabolized into acetyl-CoA via fatty acid oxidation (FAO), and acetyl-CoA enters the TCA cycle for ATP production. Sirtuins are metabolic sensors that modulate a variety of metabolic pathways. Sirt1, Sirt3, and Sirt6 restrain the glycolytic pathway through hypoxia-inducible factor 1-alpha (HIF-1α) inhibition or direct effects. Sirt3 upregulates the OxPhos pathway by enhancing the activity of mitochondrial complexes I, II, and III and dampening reactive oxygen species (ROS) production. Sirt1 is also able to increase FAO by activating PPAR-α and PGC1, while Sirt3 upregulates FAO under caloric restriction conditions. Sirt2 deacetylates and inhibits the activity of many metabolic enzymes involved in glycolysis, glutaminolyis, the TCA cycle and FAO. Sirt3 and Sirt4 activate and inhibit glutaminolyis, respectively, by regulating GDH activity. Sirt6 impacts the glutaminolyis pathway by regulating c-Myc transcriptional activity. Sirt5 increases glycolysis by increasing the activity of the glycolytic enzyme GAPDH. Sirt7 can also repress HIF-1α and, therefore, may inhibit the transcription of glycolytic genes.

Sirt2 was found upregulated in Tm cell stages. Tm cells rely on FAO metabolism, and Sirt2 negatively impacts FA catabolism; thus, Sirt2 deletion promoted CD4+ and CD8+ Tm cell formation ex vivo, which was associated with superior cell survival and decreased apoptosis. Consistently, increased accumulation of Tm cells within the TME and the secondary lymphoid organs of Sirt2−/− mice was observed upon tumor challenge, whereas no qualitative or quantitative phenotypic differences were observed between unchallenged WT and Sirt2−/− control mice.94

That study investigated the role of Sirt2 during T cell activation and maturation and within the antitumor immune response.95 However, the role of Sirt2 in different CD4+ T cell subsets with distinct metabolic programs, including Treg, Th1, Th2, Th9 and Th17 cell subsets, requires further investigation.

**SIRT3**

Sirt3 localizes mainly to the mitochondrial matrix and plays an important role in regulating elements of mitochondrial metabolism, including the TCA cycle, urea cycle, FAO, and reactive oxygen species (ROS) detoxification (Fig. 3).96 Sirt3 promotes energy
Sirt4

Sirt4 is a mitochondrial sirtuin with ADP-ribosylation activity. No deacetylase activity of Sirt4 has yet been identified. By ADP-ribosylating glutamate dehydrogenase (GDH), Sirt4 represses its enzymatic activity, which limits the metabolism of glutamine into glutamate to generate ATP (Fig. 3)\(^2\). It is thus conceivable that T cell metabolic activity and effector functions can be enhanced by Sirt4 inhibition via increasing glutaminolysis, another ATP-generating pathway used by activated T cells. Furthermore, given the role of glutaminolysis in Th17 cell differentiation\(^9\) and Treg cell development\(^8,19\), we would also expect a proinflammatory activity of in vitro suppression assay and an in vivo allograft model, and HDAC9 deletion was found to increase Treg suppressive activity by increasing Sirt3 expression\(^93\).

Sirt3 is also involved in CD8\(^+\) T cell function. Sirt3\(^−/−\) CD8\(^+\) T cells exhibited reduced ROS production and CXCR3 expression, with no significant impact on cytokine secretion or cytotoxic functions\(^91\).

Sirt5

Sirt5 is a chromatin-associated sirtuin implicated in numerous biological functions, including transcriptional repression, glucose homeostasis, DNA repair, telomeric function, cellular differentiation, mitosis, and meiosis\(^104\). Although Sirt6 has mono-ADP-ribosylase activity, the most robust activity of Sirt6 is histone deacetylation. Sirt6 deacetylases histone H3 lysine 9 (H3K9), H3K18, and H3K56 to induce transcriptional repression. Sirt6 can also remove the long-chain fatty acyl group from lysine\(^105\). Recent studies have revealed that Sirt6 possesses anti-inflammatory properties. Sirt6 represses proinflammatory gene transcription by affecting chromatin structure rather than by directly deacetylating NF-κB, while Sirt1 and Sirt2 directly deacetylate p65 and inhibit its transcriptional activity\(^97,106\). Although Sirt6 physically associates with the p65 subunit, it modulates its transcriptional activity by deacetylating H3K9 on the promoter of selected NF-κB target genes\(^107\). Sirt6 was also reported to interact with JUN and deacetylate H3K9 at the promoter of proinflammatory genes whose expression involves JUN. The same study also indicated that Sirt6-null mice develop chronic liver inflammation attributable to Sirt6 deficiency in immune cells and macrophages, and these cells express increased levels of MCP1, IL-6, and TNF\(^108\). While Sirt6 appears to exert anti-inflammatory action when acting at the transcriptional level, an anti-inflammatory protective role of Sirt5 was reported in a DSS-induced colitis mouse model. Sirt5 was found to suppress IL-1β production and proinflammatory responses in macrophages by regulating pyruvate kinase M2 (PKM2) succinylation. Sirt5-dependent succinylation promotes PKM2 entry into the nucleus, where the PKM2-HIF1α complex is formed at the promoter of the IL-1β gene to stimulate its transcription\(^100\). Paradoxically, proinflammatory activity of Sirt5 was described during the acute and immunosuppressive phases of sepsis. The induction and persistence of a hypoinflammatory and immunosuppressive state in severe sepsis are commonly associated with increased risks of secondary infections and mortality. Sirt5 was found to rescue the innate inflammatory response of endotoxin-tolerant macrophages by promoting acetylation of NF-κB p65. Mechanistically, Sirt5 competes with Sirt2 to interact with NF-κB p65 and block its deacetylation by Sirt2, which consequently leads to increased acetylation of p65 and activation of the NF-κB pathway with its downstream cytokines\(^101\). Although little work has been done to study Sirt5 in the adaptive immune response, a recent study has demonstrated a pivotal role of Sirt5 in regulating T cell activation and differentiation\(^102\). Indeed, Sirt5 deficiency was found to promote mouse naive T cell activation and increased IFN-γ production upon TCR ligation and to further influence T cell differentiation. Sirt5 deletion enhanced Th1 cell and CTL differentiation and decreased CD4 Treg cell differentiation, whereas no qualitative or quantitative phenotypic differences were observed in the peripheral lymphoid organs of WT and Sirt5\(^−/−\) mice under steady state conditions\(^102\). More importantly, even though Sirt5\(^−/−\) mice were found to be highly susceptible to DSS-induced colitis\(^100\), Sirt5\(^−/−\) mice were resistant to colorectal tumorigenesis following experimentally induced colitis, and this resistance was related to increased IFN-γ production in the colon tissue by immune cells\(^102\). While these data indicate the importance of Sirt5 in T cell activation and antitumor functions, the molecular mechanisms of Sirt5 activity need to be further elucidated.

Remarkably, studies on a large panel of preclinical mouse models of sepsis showed that Sirt5 deficiency has no impact on antimicrobial host immune defenses\(^103\), suggesting that Sirt5 plays a limited role in Treg cell functions. On the other hand, these observations support the assumption that therapies directed against Sirt5 do not impair antibacterial host defenses.
scattered evidence seems to suggest that Sirt6 induces proinflammatory effects by modulating distinct intracellular signaling factors, such as Ca\textsuperscript{2+} homeostasis\cite{109} and mRNA translation\cite{110}.

Indeed, Sirt6 has been shown to promote the expression of cytokines and chemokines, including IL-6, TNF, CXCL2, and IFN-γ, and to positively regulate TNF secretion by increasing mRNA translational efficiency in several immune cells\cite{110,112}.

Little is known about the role of Sirt6 in the adaptive immune response. HIF-1α is a transcriptional regulator of glycolysis and is known to regulate the production of several cytokines\cite{115} and to enhance Th17 cell differentiation while concomitantly inhibiting Treg cell function\cite{116,117}. Furthermore, Myc is a global regulator of the immune response and plays a crucial role in glutamine metabolism\cite{118}. Glycolysis and glutaminolysis are critical metabolic pathways on which T\textsubscript{FF} cells depend to meet their energy needs. Dynamic switching between these metabolic pathways needs to be tightly regulated to achieve optimal function within immune cells.

Sirt6 appears to strongly oppose glycolysis by inhibiting the transcription of many glycolytic genes\cite{119}. Sirt6 has been shown to physically interact with HIF-1α and to coexpress its transcriptional activity by deacetylating histone H3K9 at the promoters of several glycolytic genes\cite{120}. Furthermore, Sirt6 also regulates glutaminase expression, thus modulating the glutaminolysis pathway. Sirt6 interacts with Myc and reduces its transcriptional activity by deacetylating histones at the promoter region of Myc target genes\cite{121}. Collectively, these observations suggest that Sirt6 potentially has an immunomodulatory effect on T cell metabolism and functions by regulating the HIF-1α and Myc pathways (Fig. 3).

**SIRT7**

Sirt7 is the last and least studied member of sirtuins. Sirt7 is a vital regulator of ribosome biogenesis, and it is enriched in nucleoli, where it facilitates RNA polymerase I-dependent transcription of ribosomal (r)RNA genes\cite{122}. Sirt7 expression is linked to cell proliferation and oncogenic activity, connecting Sirt7-dependent regulation of ribosome biogenesis with cell cycle progression, metabolic homeostasis, stress resistance, aging and tumorigenesis\cite{120-122}.

Recent studies suggest that Sirt7 is also involved in inflammation. Vakhrusheva et al. showed that Sirt7 deletion predisposes mice to heart hypertrophy and cardiac inflammation. These authors observed an increased infiltration of immune cells, with higher levels of proinflammatory cytokine production\cite{122}. Information regarding the possible involvement of Sirt7 in the adaptive immune response is currently very limited.

**CONCLUDING REMARKS AND FUTURE PERSPECTIVES**

The well-documented capacity of sirtuins to promote the resolution of the inflammatory response has led many research groups to focus on developing therapeutic strategies to target their enzymatic activity in vivo. However, to achieve the desired clinical outcomes, it is paramount to decide whether to stimulate or block sirtuin activity and how to modulate a specific sirtuin within a specific T cell subset. All these challenges are worthy of further study due to the ubiquity of sirtuins. In addition, these proteins have intricate and partially unknown functions within an organism, reflecting the immense challenge of targeting any of them.

For example, enhancing Treg cell suppressive activity with Sirt3 agonists to attenuate inflammation represents a potential strategy to treat autoimmunity. Similarly, the use of Sirt1 activators to suppress inflammation could help treat autoimmune diseases. Metformin, classically prescribed for the management of type 2 diabetes, appears to have a stimulatory effect on sirtuins. Sirt1 activation by metformin has been shown to decrease inflammation by decreasing Th17 cell differentiation\cite{74}. Resveratrol, a compound with sirtuin-activating effects, is currently of intense interest within both scientific and lay communities for its anti-inflammatory properties\cite{124}. Resveratrol stimulates Sirt1 activity, which decreases c-Jun acetylation, consequently restraining T cell activation\cite{62}. Resveratrol has been shown to improve outcomes in several experimental autoimmunity models\cite{125}. For instance, administration of resveratrol protected mice against experimental rheumatoid arthritis by inhibiting Th17 cell differentiation\cite{126}. Furthermore, resveratrol was proven to have both preventative and therapeutic effects by reverting the advanced stages of insulitis in a nonobese diabetic (NOD) mouse model due to reduced Th17 cells\cite{127}. Moreover, resveratrol induced protective effects against high-fat diet-induced obesity in mice in part by increasing total Treg cells\cite{128}.

Despite the obvious progress in the organ transplantation field, the commonly required immunosuppressive therapies are known to ultimately induce cancer and quite often infections. Presently, sirtuins are being thoroughly studied for their role in promoting organ transplant tolerance. Treg and Th17 cells drive transplant tolerance, and in relation to these T cell subsets, Sirt1 has emerged as an eligible target for clinical interventions in transplantation. Indeed, Sirt1 deletion improved Treg cell suppressive activity in vitro and in vivo, and mice with specific Sirt1 deletion in Foxp3+

Treg cells exhibited prolonged survival after cardiac allografts concomitant with increased Treg cell infiltration. In the same study, similar results were obtained when using Sirt1 inhibitors in vivo\cite{129}. Later, comparable findings were reported after kidney transplantation from BALB/c mice into C57BL/6 recipient mice treated with EX-527, a Sirt1 inhibitor\cite{130}.

Recently, the effect of sirtinol, a Sirt1 inhibitor, on heart allograft survival was evaluated, and it was found that sirtinol-treated recipients exhibited increased numbers of Foxp3 Treg cells and reduced numbers of Th17 cells in addition to decreased expression of the IL-17A and RORγt transcription factor\cite{131}. Therefore, Sirt1 inhibition might affect the Th17/Treg cell ratio in favor of Treg cells. In contrast, Sirt1 activators such as metformin were found to decrease IL-17A and RORγt expression in mouse models as described earlier\cite{74}. Certainly, more studies are required to define the appropriate action of Sirt1 targeting for allograft retention.

To date, the research community has focused on promoting the anti-inflammatory activity of sirtuins to treat a multitude of autoimmune diseases and other chronic diseases associated with inflammation. However, we must extend our interest toward blocking the activity of sirtuins to promote the effector activity of T cells in the context of antitumor immunity. Indeed, enhancing tumor rejection by boosting tumor-reactive T cell activity would be tremendously effective in fighting cancer. Recent reports demonstrating the immunotherapeutic potential of Sirt2 and Sirt5 blockade in T cells to reject tumor challenge\cite{89} and to protect against colorectal tumorigenesis in murine models, highlight the expected effectiveness of such strategy\cite{102}.

In addition, sirtuins have been shown to play an oncogenic role in many tumor types\cite{132}, and sirtuin inhibitor drugs have been shown to have tumor-suppressive activity on cancer cells, suggesting an intriguing possibility that in certain cancer patients, targeting sirtuins may suppress tumor proliferation while simultaneously boosting antitumor immunity. Alternatively, manipulation of sirtuins could be achieved by gene editing during TIL expansion or chimeric antigen receptor (CAR) T cell generation ex vivo, thus selectively targeting T cells in the context of immune cell therapy\cite{133,134}.

Many reports indicate that exhausted tumor-infiltrating lymphocytes display dysregulated metabolism within the metabolically restricted tumor microenvironment\cite{122,22,135,136}. Modulation of sirtuins to enhance the metabolic activity of T cells could provide a viable strategy to restore exhausted tumor-reactive T cells.
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COMPETING INTERESTS
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ADDITIONAL INFORMATION
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