

Sirtuin deacylases: a molecular link between metabolism and immunity

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RECEIVED NOVEMBER 4, 2012; REVISED DECEMBER 27, 2012; ACCEPTED DECEMBER 30, 2012. DOI: 10.1189/jlb.1112557

ABSTRACT

Lysine deacetylation by the NAD⁺-dependent family of sirtuins has been recognized as an important post-translational modification regulating a wide range of cellular processes. These lysine deacetylases have attracted much interest based on their ability to promote survival in response to stress. Sirtuins require NAD⁺ for their enzymatic activity, suggesting that these enzymes may represent molecular links between cell metabolism and several human disorders, including diabetes and cancer. Inflammation represents a pathological situation with clear connections to metabolism and aging in humans, raising the possibility that sirtuins may also play an important role during a normal and/or a pathological immune response. A growing body of data has confirmed the immunomodulatory properties of sirtuins, although often with contrasting and opposing conclusions. These observations will be summarized herein and the possible strategies that may lead to the development of novel therapeutic approaches to treat inflammation briefly discussed. *J. Leukoc. Biol.* **93**: 669–680; 2013.

Introduction

Since its initial characterization in the yeast *Saccharomyces cerevisiae*, the *silent information regulator 2* (*Sir2*) protein has attracted increasing interest in the scientific community, mostly based on its putative ability to extend lifespan in several model organisms [1]. Despite some controversy concerning their role in organisms longevity [2], it is generally assumed that members of this family of enzymes (called sirtuins) act as stress-response and survival genes, exerting beneficial effects against age-related diseases. In mammals, in silico phylogenetic analy-

sis has led to the identification of seven members [3] with distinct subcellular localization, including the nucleus (SIRT1, -6, and -7), mitochondria (SIRT3, -4, and -5), and cytoplasm (SIRT1 and -2). Although sirtuins were identified originally as histone lysine deacetylases, regulating gene expression through chromatin modification, numerous nonhistone substrates have been identified in the last decade in most cellular compartments, expanding the potential role of this class of enzymes to virtually all cellular processes known to date. Recent studies have also revealed important differences in the enzymatic activity of sirtuins, suggesting, in particular, that SIRT4, and possibly SIRT6, may represent ADP-ribosyl transferases, whereas SIRT5 may preferably act as a lysine demalonylase and desuccinylase [4, 5]. One of the key features of this class of enzymes is their tight and specific requirement for the cofactor NAD⁺, conferring to sirtuins the ability to act as molecular links between cellular metabolism and the regulation of numerous cellular functions. In particular, sirtuins play an important role in maintaining cellular homeostasis by regulating energy status and stress resistance [6]. Accordingly, their enzymatic activity is modulated in response to various stress conditions, such as hypoxia [7–9], changes in nutrient availability [10], and oxidative insults [11]. Infection and its consequences, such as cell death, inflammation, and the initiation of an immune response, can also be considered as important cell stressors, causing brisk changes in cellular physiology and/or environment, thus suggesting a possible role for sirtuins during an immune and/or inflammatory response. Recent experimental evidence, confirming a role for several members of the sirtuin family in the regulation of multiple aspects of the immune and inflammatory responses, will be summarized briefly and discussed in this review.

NAD⁺ METABOLISM AND THE CONTROL OF INFLAMMATORY RESPONSES

As for most enzymes, the expression and activity of sirtuins are controlled tightly through general regulatory mechanisms (in-

Abbreviations: FOXO3/P3=forkhead box O3/P3, H3K9/56=histone 3 lysine 9/56, HFD=high-fat diet, HIF-1 α =hypoxia-inducible transcription factor 1 α , Nam=nicotinamide, NAMPT=nicotinamide phosphoribosyltransferase, NMN=nicotinamide mononucleotide, NMNAT=nicotinamide mononucleotide adenylyltransferase, NR=nicotinamide riboside, PARP=poly ADP-ribose polymerase, PBEF=pre-B cell enhancing factor, PGC-1=peroxisome proliferator-activated receptor- γ coactivator 1, PPAR- γ =peroxisome proliferator-activated receptor- γ , siRNA=small interfering RNA, SIRT=silent mating type information regulation 2 homolog (sirtuin), Treg=regulatory T cell

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cluding transcriptional, microRNA-dependent, post-transcriptional, and post-translational regulation), affecting their subcellular localization, tissue distribution, and enzymatic activity. NAD^+ dependency represents a unique feature of the sirtuin family [12]. In most tissues, NAD^+ is produced mainly by the salvage pathway counterbalancing the high NAD^+ turnover caused by a wide range of enzymes using NAD^+ as a substrate (Fig. 1). Nam, resulting from the consumption of NAD^+ , is transformed into NMN by the NAMPT and then converted back into NAD^+ by NMNAT1–3 [16]. This salvage pathway has been strongly associated with intracellular sirtuin activity in yeast [17] and mammalian cells [18]. Nam, the end-product of all enzymatic reactions catalyzed by NAD^+ -consuming enzymes, including sirtuins, represents a NAD^+ precursor and an endogenous, noncompetitive sirtuin inhibitor [19].

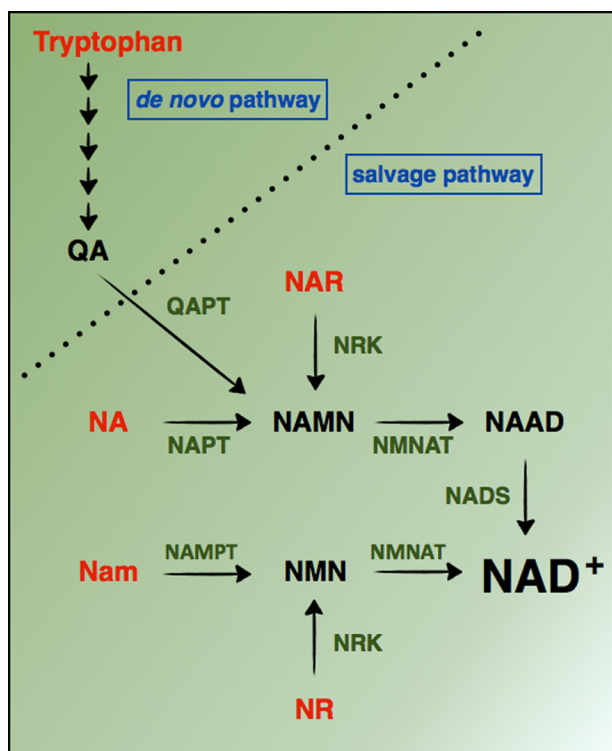


Figure 1. NAD^+ biosynthesis in mammals. Five precursors (in red) can initiate NAD^+ biosynthesis in mammals: tryptophan, Nam, nicotinic acid (NA), NR, and nicotinic acid riboside (NAR) [13]. Tryptophan can be degraded into quinolinic acid (QA) via the de novo NAD^+ biosynthesis pathway, also known as the kynurenine pathway, but tryptophan alone is not sufficient to support physiological NAD^+ levels in mammals [14, 15], indicating the important role of the salvage pathway in insuring NAD^+ biosynthesis under physiological settings. The quinolinic acid phosphoribosyltransferase (QAPT) links the de novo pathway to the salvage pathway. The pyridine bases Nam and nicotinic acid (NA) are converted to the corresponding mononucleotides (NMN and NAMN, respectively) by NAMPT and nicotinic acid phosphoribosyltransferase (NAPT), respectively. NMN and NAMN are then converted to the corresponding dinucleotide (NAAD or NAD^+) by NMNAT. NAAD is finally amidated to NAD^+ by NAD synthetase (NADS). NMN and NAMN are also generated through phosphorylation of, respectively, NR and NAR by NR kinase (NRK).

In mammals, therefore, the NAMPT-catalyzed reaction contributes to the recycling of Nam into NAD^+ , potentially creating a favorable biochemical environment (high NAD^+ : Nam ratio) for sirtuin activity. Moreover, the sensitivity of sirtuins to NAD^+ concentration is highlighted by their high Michaelis-Menten constant for NAD^+ compared with other NAD^+ -consuming enzymes [20, 21]. As a consequence, small variations of intracellular NAD^+ levels are thought to affect sirtuin activity, thus supporting their role as important metabolic cellular sensors.

Although expressed ubiquitously and constitutively, NAMPT has been found overexpressed by immune cells during inflammatory response, suggesting, even though indirectly at first, a possible role for sirtuins during an immune response. Noteworthy, NAMPT has been associated with immune functions since its original discovery as a putative cytokine involved in pre-B cell differentiation (hence, termed PBEF) [22]. Since then, elevated NAMPT expression has been found in activated T cells, neutrophils, and monocytes [16, 23, 24]. Although extracellular forms of this protein (called eNAMPT) have been described [25], its major function appears to be the recycling of Nam into the NAD^+ biosynthetic pathway. Whether this protein also possesses cytokine-like properties, i.e., binding and signaling through an extracellular receptor, is still a matter of debate. The very same protein was also rediscovered as a potential secreted adipokine and named visfatin from its elevated expression in visceral fat [26]. Despite some controversy surrounding the original publication (later retracted in ref. [27]), high levels of circulating PBEF/NAMPT/visfatin have been found in patients suffering from acute lung injury [28], rheumatoid arthritis [29], psoriasis [30], osteoarthritis [31], systemic lupus erythematosus [32], sepsis [33], and inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis [34]. Although the physiological relevance of these findings remains to be established, as the increase in circulating NAMPT may simply result from enhanced cell lysis, they further illustrate the existing link between NAD^+ metabolism and inflammation. A more direct immunoregulatory role for NAD^+ has been uncovered by a series of studies demonstrating the therapeutic efficacy of a pharmacological NAMPT inhibitor in several inflammatory models. For instance, the administration of the potent and specific NAMPT inhibitor FK866 to mice with collagen-induced rheumatoid arthritis reduced the disease severity with comparable effect to TNF inhibitors [35]. The anti-inflammatory properties of FK866 have been associated to its ability to inhibit several proinflammatory cytokines, including IL-6, TNF, and IFN- γ [35–37]. Similarly, the administration of FK866 after the induction of spinal cord injury inhibited proinflammatory cytokine secretions, such as TNF and IL-1 β , and partly reduced permanent damage [38]. Moreover, knockdown of NAMPT attenuates inflammatory profiles of lung epithelium infected by the pandemic virus H1N1 2009, as estimated by the release of IL-6, IL-8, TNF, and CXCL10 [39]. Mechanistically, reduced NAD^+ levels have been shown to affect proinflammatory gene expression at the transcriptional and post-transcriptional levels, although the fine details of these regulatory pathways remain to be firmly established. In some instances, the beneficial effect of NAD^+

lowering agents could be reproduced using sirtuin inhibitors, in keeping with a possible functional link among NAMPT expression, sirtuin activity, and control of inflammatory response [36, 37, 40].

In contrast to these observations pointing to a possible pro-inflammatory role for the NAMPT-sirtuin axis, studies performed in mice exposed to altered diet regimens appear to rather support a protective role for NAMPT and by consequence, sirtuins against inflammation. High caloric diets, a regimen favorable to the development of a proinflammatory profile, generally lead to a reduced expression of NAMPT, as determined in adipose tissues [41, 42], liver [42], and skeletal muscles [42], whereas fasting and glucose restriction cause increased NAMPT expression in selected tissues, such as liver [43, 44] and muscle cells [45]. Interestingly, mice subjected to calorie restriction are more resistant to LPS-induced hepatitis, correlated to reduced levels of serum IL-1 β , TNF, and IL-6 [46], further strengthening a possible positive association between high NAMPT levels and reduced inflammatory conditions.

Thus, and although a comprehensive analysis of the intricate interplay among NAMPT, sirtuins, and metabolism is beyond the scope of the present review, experiments performed with NAD⁺-lowering pharmacological agents suggest a proinflammatory role for NAMPT, whereas diet-induced modulation of the NAD⁺ metabolism rather suggests a favorable, anti-inflammatory effect of the NAMPT-sirtuin axis. The reason for this apparent contradiction is presently not clear but may be related to the recently reported contrasting effects of selected members of the sirtuin family on immune responses, as detailed in the following paragraphs.

THE CONTRASTING ROLE OF SIRTUINS IN INFLAMMATION

As discussed previously, the functional link between NAD⁺ and inflammation is best explained by assuming that NAD⁺-dependent enzymes, in particular, sirtuins, play a role in the control of an immune response. Surprisingly however, sirtuins appear to exert contrasting effects on the immune system, including pro- and anti-inflammatory functions, as summarized in the following section.

SIRT1

Signal transduction of many proinflammatory stimuli, including cytokines and PAMPs, converges on the transcription factor NF- κ B, considered therefore as a master regulator of inflammation. The biological activity of this transcription factor is regulated by post-translational modifications, including acetylation-deacetylation cycles [47]. RELA lysine 310 acetylation enhances the transactivational activity of NF- κ B, and SIRT1 is able to antagonize this modification [48], acting therefore as a negative regulator of this important proinflammatory factor. Since this initial observation, the biological relevance of this mechanism has been confirmed extensively. HIV transcriptional activator Tat is a telling example, showing how the virus targets the SIRT1-NF- κ B interplay to

create a permissive environment for HIV1 propagation [49]. The HIV transcriptional activator Tat binds to the catalytic domain of SIRT1 and inhibits its activity, leading to RELA hyperacetylation, and favoring T cell activation and IL-2 production, conditions promoting viral infection [49]. The importance of the SIRT1-NF- κ B axis is also highlighted in several tissues and cell types, where a decline in SIRT1 level or activity is associated with exacerbated NF- κ B-dependent transactivation of inflammatory mediators. An inverse relationship between SIRT1 levels and the capacity to produce TNF in response to LPS has, for example, been reported in a study conducted in vitro using several cell lines [50]. Inhibition of SIRT1 expression and/or function led to accumulation of acetylated forms of RELA, increased NF- κ B activity, and augmented TNF release, clearly establishing a functional link between NF- κ B acetylation status and activity. Pharmacological inhibition of SIRT1 activity or siRNA-mediated SIRT1 knockdown similarly potentiated NF- κ B activation and proinflammatory cytokine release in several additional experimental models [51, 52]. Accordingly, genetic overexpression or functional activation of SIRT1 by pharmacological activators, such as resveratrol, SRT1720, and SRT2172, attenuated proinflammatory cytokine expression and lung inflammation [52, 53]. NF- κ B knockdown has been shown to attenuate the proinflammatory phenotype of *Sirt1*-knockout macrophages [54], whereas transgenic mice overexpressing SIRT1 under the control of the natural *Sirt1* promoter displayed reduced inflammation secondary to inhibited NF- κ B activity [55]. Finally, genetic deletion of a natural SIRT1 corepressor (deleted in breast cancer-1, or DBC1) has been shown to cause SIRT1 hyperactivation, reduced NF- κ B acetylation, whereas strongly attenuating proinflammatory cytokine secretion [56].

Of interest, SIRT1 has also been shown to deacetylate and inactivate PARP1, the founding member of the poly-ADP-ribosyltransferase family known to positively regulate NF- κ B activity, indicating a novel and indirect effect of SIRT1 on NF- κ B [57]. The same RELA lysine 310 residue is also the target of SIRT2. Deletion of SIRT2 strengthens NF- κ B-induced gene transcription upon TNF stimulation in vitro [58], although the extent of SIRT2 influence on inflammation is presumably reduced and has not been formally proven to occur in an in vivo setting.

NF- κ B is not the only transcription factor affecting immunity that is targeted by sirtuins. In a recent study, the beneficial effects of sirtuin activators in a model of chronic obstructive pulmonary disease were shown to be a result of the ability of SIRT1 to deacetylate and activate FOXO3, a transcription factor preventing cellular senescence [59].

AP-1 represents an additional transcription factor activated in response to immune stimuli, such as cytokines. AP-1 is a dimeric complex composed of JUN and FOS, both of which represent SIRT1 substrates. SIRT1-mediated deacetylation of AP-1 has been shown to inhibit its transcriptional activity, thus altering the expression of AP-1-controlled genes, such as COX-2, in macrophages [60]. In T cells, SIRT1 has been shown to down-regulate AP-1 activity, possibly contributing to control excessive T cell reactivity

and to promote peripheral tolerance, leading some authors to suggest that enhanced AP-1 activity may explain the predisposition of *Sirt1*-knockout mice to develop autoimmune diseases [61, 62].

In contrast to the previously described anti-inflammatory properties of SIRT1 (mostly explained by its ability to repress the activity of transcription factors often associated with a Th1-like inflammatory response), SIRT1 has also been shown to control the activity and function of other T cell subsets. SIRT1 affects Treg function and differentiation by binding and destabilizing FOXP3, a transcription factor whose expression is required for adequate Treg development and suppressive function [63]. Likewise, SIRT1 has also been shown to destabilize SMAD7, an important mediator of the signaling pathway of the suppressive cytokine TGF- β [64], implying a possible antagonistic role for SIRT1 in Treg development and function. It should be noted, however, that SIRT1 gene deletion does not affect CD4⁺FOXP3⁺ Treg number or function [62]. Lack of SIRT1 expression in DCs has been shown to reduce macrophage and eosinophilic lung infiltration in a murine model of antigen-induced airway allergy [65]. Accordingly, SIRT1 inhibitors led to reduced Th2 cell development and lung inflammation, highlighting an unexpected proinflammatory property of SIRT1 during Th2-like inflammatory responses. SIRT1 appeared to act by repressing PPAR- γ activity in DCs, thus promoting their pro-Th2 properties. Overexpression of SIRT1 in synovial fibroblasts and monocytes caused a modest but significant increase in the secretion of proinflammatory cytokines, such as TNF, whereas siRNA-mediated down-modulation of SIRT1 expression led to reduced cytokine secretion by the same cells [66]. Finally, a recent report has uncovered a novel mechanism by which SIRT1 may increase inflammatory responses. Overexpression of SIRT1 has been shown to increase TLR4 and TLR2 expression in cells extracted from periodontal fibers [67]. Given the important role of TLR4 in infectious and sterile inflammation (see ref. [68] for a review), these observations provide an interesting clue about how SIRT1 may, under some experimental settings, promote an inflammatory response.

SIRT6

Sirt6-knockout mice appear normal at birth but have a shortened lifespan and an aging-like phenotype characterized by a dramatic, systemic increase in lymphocyte apoptosis [69]. Lymphopenia could be rescued partially by RELA haplo-insufficiency, again suggesting a possible functional link between this sirtuin and NF- κ B-driven gene expression [70]. This hypothesis was further strengthened by the finding that SIRT1 deficiency in macrophages led to a compensatory elevated expression of SIRT6, acting at the level of NF- κ B-targeted promoters in response to TNF treatment [54]. Notably, however, SIRT6 regulates proinflammatory gene transcription by affecting chromatin structure, rather than directly deacetylating NF- κ B. Although SIRT6 physically associates with RELA, it modulates its transcriptional activity by deacetylating H3K9 on the promoter of selected NF- κ B target genes. Recently, 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 bone marrow-derived *Sirt6*-knockout macrophages were hypersensitive to LPS and displayed increased expression of MCP-1, IL-6, and TNF [71]. Therefore, even though acting at distinct levels, SIRT1 and SIRT6 negatively regulate the transcriptional activity of NF- κ B. Despite these findings, several reports concurred in demonstrating a proinflammatory role for SIRT6. Indeed, SIRT6 activity has been shown to promote expression of selected cytokines and chemokines, including IL-6, TNF, CXCL2, and IFN- γ , by a wide variety of immune cells [36, 37, 72]. In a particular study, SIRT6 has been found to positively regulate TNF secretion by increasing mRNA translational efficiency [36]. This dual and ambiguous role of SIRT6 has been exemplified further by a recent report examining the functional consequences of SIRT6 expression by pancreatic cancer cells. By modulating intracellular Ca²⁺ levels, SIRT6 has been shown to promote, rather than oppose, the secretion of IL-6, TNF, and of proangiogenic factors by tumor cells [73]. The ability of SIRT6 to promote cytokine secretion in some experimental settings may help to understand a series of independent studies showing that sirtuin inhibitors reduce proinflammatory cytokine secretion [36, 37, 40, 72, 73]. Notably, in the latter study, the pan-sirtuin inhibitors sirtinol and cambinol mitigated the inflammatory properties of macrophages, whereas the SIRT1-specific EX-527 compound displayed limited efficacy, in agreement with the idea that non-SIRT1 members of this enzyme family may operate as proinflammatory factors. Thus, whereas SIRT1 and SIRT6 appear to exert an anti-inflammatory action when acting at the transcriptional levels, scattered evidence seems to suggest a proimmune activity of SIRT6 by modulating distinct intracellular signaling events, such as Ca²⁺ homeostasis and mRNA translation efficiency.

SIRTUINS, METABOLISM, AND INFLAMMATION

A growing number of studies have demonstrated the important role of cell metabolism in regulating an innate and adaptive immune response. In particular, T lymphocytes and innate immune cells undergo a metabolic switch early after stimulation and during the resolving/memory generation phase of the response.

Metabolic shifts in activated immune cells

Naïve, quiescent T cells generate ATP via the tricarboxylic acid cycle and fatty acid β -oxidation [74, 75]. Upon antigen stimulation, T cells markedly increase their uptake of glucose and switch to a glycolytic mode to generate most of the intracellular ATP. Accordingly, pharmacological blocking of glycolysis reduces the differentiation of T cells into effector lymphocytes [76]. A similar metabolic shift has been demonstrated recently to occur after TLR stimulation of innate cells, in which glycolysis has been determined as the primary source of ATP. This glycolytic reprogramming occurs under normoxia and is highly reminiscent of the Warburg effect typical of many cancer cells [77].

The reason for switching to a less-efficient mode of generation of ATP in the early phase of an immune/inflammatory response is still unclear but may be related to the capacity of glycolytic intermediates to fuel anabolic reactions in activated cells [78]. Interestingly, a further switch to fatty acid oxidation is required for the differentiation of CD8⁺ memory T cells and the induction of the resolution/adaptation phase of an inflammatory response [79], confirming the notion that metabolic reprogramming plays an important role in innate and adaptive responses.

Sirtuins and the control of metabolic shifts

Although the identity and mode of action of the molecular actors at work in this intricate relationship between cellular bioenergetics and inflammation are still a matter of investigation, a series of recent evidence points to a role for SIRT1 and SIRT6 in linking immune cell responses to changes in metabolism [80]. SIRT6 is emerging as an important cell-autonomous regulator of glucose metabolism. *Sirt6* knockout mice die by severe hypoglycemia and persistent inflammation [81]. SIRT6 appears to strongly oppose glycolysis, by inhibiting the expression of many glycolytic genes by binding to their promoter, as demonstrated for lactate dehydrogenase, triose phosphate isomerase, aldolase, GLUT1, and the rate-limiting glycolytic enzyme phosphofructokinase [82]. HIF-1 α represents an important transcription factor known to activate glycolysis under low-oxygen conditions. SIRT6 has been shown to physically interact with HIF-1 α , counteracting its activity by multiple mechanisms including inhibition of HIF protein function and/or stability [81]. HIF-1 α has recently been shown to play an important role in the immune system by regulating the expression of several cytokines, such as IL-1 β [83, 84] and IL-22 [85] and by modulating T cell differentiation in normoxic conditions. In particular, HIF enhances the capacity of naive T lymphocytes to differentiate into Th17 cells, while concomitantly inhibiting Treg function by targeting the transcription factor FOXP3 for proteasomal degradation [86]. Collectively, these observations highlight the important and complex role of HIF-1 α in regulating an immune response and suggest a potentially broader immunomodulatory effect of SIRT6 when acting as a negative regulator of HIF-1 α .

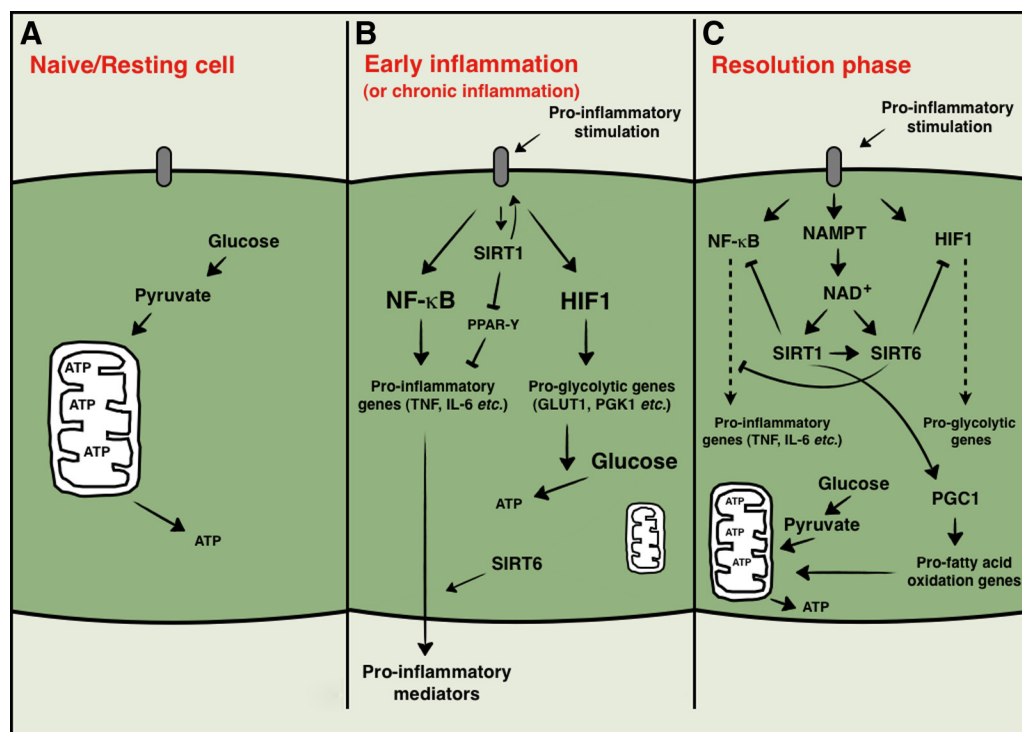
The switch toward fatty acid oxidation, characteristic of the resolution phase of an inflammatory response, and the differentiation of activated CD8⁺ lymphocytes into memory cells is known to be supported by the transcriptional coactivators PGC-1 α and PGC-1 β , both acting as master regulators of oxidative phosphorylation and fatty acid oxidation gene expression [87]. Reversible acetylation has emerged as a key post-translational modification affecting PGC-1 α and PGC-1 β activity. SIRT1-mediated deacetylation has been shown to increase the transcriptional activity of both PGC-1 cofactors [10, 88], suggesting a role for SIRT1 in promoting the resolution phase of an inflammatory response [10, 89]. Several observations are compatible with the notion that these important metabolic regulators may, in fact, also regulate an inflammatory response. The expression of TNF and

IL-6 is increased in tissues from *Pgc-1 α* knockout mice, and the serum level of IL-6 is enhanced in muscle-specific *Pgc-1 α* deficient animals [90]. Conversely, overexpression of PGC-1 α in C2C12 myotubes leads to reduced TNF and IL-6 mRNA expression. Similarly, a clear, negative correlation has been established between PGC-1 α and TNF or IL-6 mRNA expression in the skeletal muscles of human type 2 diabetics [90]. The closely related PGC-1 β reduces macrophage-mediated inflammation by supporting the activity of arginase and dampening production of IL-6 and IL-12 [87].

Homeostatic control of an inflammatory response by sirtuins

These observations contribute to the generation of a unifying concept (Fig. 2), integrating cell metabolism, sirtuin activity, and regulation of an inflammatory response [91, 92]. Following stimulation, quiescent cells, generating intracellular ATP from oxidative reactions, promptly shift to a glycolytic metabolism, a catabolic pathway representing a unique compromise between energy supply and provision of biosynthetic precursors important for cell division. Activation of immune cells leads to increased expression of NAMPT, the rate-limiting step in NAD⁺ biosynthesis [16, 23, 24, 93]. Increased and sustained NAD⁺ levels can therefore provide enough substrate to SIRT6 and SIRT1, whose activation appears instrumental in mediating the transition to the resolution phase of the response by, respectively, inhibiting the glycolytic pathway and activating fatty acid oxidation while negatively affecting NF- κ B activity. It should also be noted that SIRT1 has been shown to induce SIRT6, thus reinforcing the notion of a coordinated action of these sirtuins in terminating an inflammatory response. It is noteworthy that whereas mitochondria play a key role in energetic metabolism, no direct evidence has been reported yet for a role of mitochondrial sirtuins (SIRT3-5) during inflammation. Increased intracellular NAD⁺ levels have been shown to activate mitochondrial sirtuin activity [94–96]. SIRT3 has been shown to positively regulate mitochondrial activity via the deacetylation and activation of several components of the electron transport chain complex I and complex II [97, 98] and the acetyl-CoA synthase [99]. Complex I and complex II activity is lowered in SIRT3-deficient mice, in agreement with the observation that SIRT3-deficient mice produce up to 50% less ATP than WT animals (reviewed in ref. [100]). It is therefore tempting to speculate that increased SIRT3 activity may contribute to the reacquisition of an oxidative metabolism during the resolution/memory phase of an immune response [98, 101]. Although this chain of events remains largely speculative at this stage, chronic inflammation often appears to correlate with a defect in the NAD⁺-sensing network, as observed in pathological situation, such as diabetes, obesity with metabolic syndrome, and atherosclerosis [102–104]. The discovery of the intricate relationships between bioenergetics and inflammation will most probably pave the way to novel and promising new therapeutic approaches to the treatment of chronic inflammatory disorders.

Figure 2. A model of metabolic remodeling during inflammation. Metabolic reprogramming in inflammatory cells is characterized by two consecutive adjustments in energy metabolism, coordinated by multiple signaling pathways. Upon stimulation, naive cells (A) shift to a glycolytic mode (B) under the influence of the transcription factor HIF, promoting the transcription of genes involved in glycolysis. Activation also induces the NF- κ B pathway and the transcription of proinflammatory genes. SIRT1 and SIRT6 may exert proinflammatory properties at this stage by enhancing TLR4 expression, by activating gene transcription through PPAR- γ inhibition, and by promoting the translation of mRNA encoding for TNF (see text for references). PGK1, Phosphoglycerate kinase-1. (C) Stimulated cells display increased levels of NAMPT, leading to a delayed increase in intracellular NAD⁺ levels and subsequent increased activity of SIRT1 and SIRT6. Increased expression



and/or possible altered subcellular localization may induce a change in substrate preference for these sirtuins, possibly explaining their shift in functional properties from pro- to anti-inflammatory mediators. By deacetylating several nuclear substrates (including RELA and histones), these sirtuins decrease NF- κ B and other transcription factor activities, limiting proinflammatory cytokine secretion. SIRT1-mediated deacetylation will also activate PGC-1 factors, leading to increased fatty-acid metabolism, whereas SIRT6 will oppose the proglycolytic effects of HIF. Collectively, SIRT1 and SIRT6 appear to coordinately dampen proinflammatory secretion during the late phase of the response, while restoring an oxidative metabolism favoring the resolution of the inflammatory reaction. Dashed arrows indicate a reduction in the signaling flow leading to proinflammatory cytokine production.

THERAPEUTIC POTENTIAL OF SIRTUIN-INTERACTING DRUGS

The well-documented capacity of sirtuins to promote the resolution phase of an inflammatory response has led many research groups to focus on the identification and/or development of therapeutic strategies aiming at increasing or inhibiting their enzymatic activity in vivo, as summarized below.

Dietary supplementation with natural NAD⁺ precursors

As discussed previously, the enzymatic activity of sirtuins is closely linked to the intracellular concentration and bioavailability of NAD⁺, suggesting the possibility to alter sirtuin activity by manipulating NAD⁺ biosynthetic pathways. In vivo, Nam, a component of vitamin B3, is the major precursor for NAD⁺ biosynthesis [16]. NAD⁺ deficiency from poor diet causes pellagra, a clinical disorder with well-established inflammatory components, such as diarrhea and dermatitis. Restoring intracellular NAD⁺ levels using vitamin B3 reverses all pellagra symptoms, in keeping with the idea that low intracellular NAD⁺ levels may be functionally linked to an inflammatory status. In addition to acting as a NAD⁺ precursor, however, Nam, the end-product of all enzymatic reactions catalyzed by

NAD⁺-consuming enzymes, also acts as an endogenous, non-competitive sirtuin inhibitor [19]. At pharmacological, supra-physiological doses, Nam displays potent, anti-inflammatory properties in vitro and in vivo [36, 105–108], although not in human subjects [109]. It should be noted, however, that in the latter study, a lower dose (100 mg/kg) was used than in a similar study where 500 mg/kg was found to be effective in reducing cytokine production in mice in response to endotoxin [36]. The dual property of Nam as a NAD⁺ precursor and sirtuin inhibitor complicates the interpretation of these observations, and although in vivo data in humans do confirm that administration of Nam can cause a raise in intracellular NAD⁺ levels [23], it is presently difficult to conclude whether the observed anti-inflammatory properties of Nam can be ascribed to its ability to modulate sirtuin activity.

Use of alternative NAD⁺ precursors, unable to inhibit sirtuin activity, may provide an alternative strategy to increase intracellular NAD⁺ levels and thus, promote sirtuin activity. NR has been identified recently as an alternative NAD⁺ precursor. NR administration led to increased intracellular and mitochondrial NAD⁺ levels in mammalian cells and tissues, causing increased SIRT1 and SIRT3 enzymatic activity [110]. NR supplementation ameliorated metabolic and age-related disorders in mice, providing a proof-of-concept that

dietary manipulation of sirtuin activity may be of therapeutic value. NMN, the product of NAMPT, represents an additional candidate to manipulate NAD⁺ biosynthesis in vivo and was shown to be effective in the restoration of NAD⁺ functions in *Nampt*-deficient organs in mice [25, 42]. NMN was successfully used to treat the pathophysiology of HFD- and age-induced diabetes in mice. In this study, NMN antagonized the expression of genes, such as IL-1 β , whose expression was induced by a HFD [42].

Inhibition of NAD⁺-consuming enzymes

An alternative strategy to increase NAD⁺ bioavailability is to pharmacologically reduce the activity of NAD⁺-consuming enzymes, such as PARP1 or CD38, an ADP-ribosyl cyclase. PARP1 is an abundant nuclear protein whose overexpression is known to limit SIRT1 activity [111]. Inhibition or deletions of CD38 and PARP1 have been shown to activate SIRT1 activity by raising the intracellular NAD⁺ level [112, 113]. This strategy has been widely confirmed by others, indicating that PARP inhibitors preserve high intracellular NAD⁺ levels and consequently promote SIRT1 activity [114, 115]. Noteworthy, PARP inhibitors have been used extensively to suppress inflammation, based, in particular, on the known capacity of PARP1 to positively regulate the expression of several proinflammatory genes [116]. Based on the available evidence, it is reasonable to assume that some of the in vivo effects of these PARP inhibitors may reside in their indirect capacity to activate SIRT1 and SIRT6, thus actively promoting the resolution of an inflammatory response [117–119].

The sirtuin-activating compounds: small molecule sirtuin activators

Resveratrol is a phytoalexin polyphenol, naturally occurring in the grape *Vitis vinifera* [120] and thought to represent a potent SIRT1 activator. Although the mechanism by which resveratrol activates SIRT1 has been a matter of recent debate, in vivo administration of this compound has been shown to delay or prevent inflammatory conditions in mice by interfering with NF- κ B-dependent gene expression, IFN- γ signaling, chemokine secretion and activity, and leukocyte adhesion to the endothelium [121–126]. The therapeutic value of resveratrol and SRT501, a resveratrol formulation with improved bioavailability, has been confirmed in a wide range of animal models of inflammatory disorders, including chronic or relapsing-remitting experimental autoimmune encephalomyelitis [127, 128], colitis [123], acute pancreatitis [129], endotoxin-induced ocular uveitis [130], osteoarthritis [131], and tendinitis [132].

Structurally unrelated activators of SIRT1 with increased potency have been developed recently by Sirtris Pharmaceuticals, a GlaxoSmithKline company, and include SRT1460, SRT1720, SRT2104, SRT2183, and SRT2530 [133–135]. Other molecules with similar SIRT1-promoting activity have also been developed by others and include several oxazolo[4,5-b]pyridines, imidazo[1,2-b]thiazole derivatives, 1,4-dihydropyridine derivatives [136–138], inauhzin [139], and ammonium trichloro (di-oxoethylene-o,o') tellurate AS101 [140]. Anti-inflammatory

properties have been confirmed for many of these compounds, particularly for the very selective and highly potent SRT1720 and SRT2379 molecules [134, 141, 142]. Since these promising publications, several sirtuin activators have already entered clinical trials in the context of sepsis, psoriasis, or ulcerative colitis, for examples (<http://www.clinicaltrials.gov>), despite the fact that some of their in vivo effects may be SIRT1-independent [143, 144].

Alternative SIRT1 modulators

The aforementioned biological properties of SIRT1 may help explain some of the anti-inflammatory effects of natural compounds with poorly characterized modes of action. In vivo administration of chitosan, a natural linear polysaccharide, attenuates macrophage activation and proinflammatory cytokine release in a model of allergen-induced airway inflammation [145] and protects mice from LPS-induced sepsis [146]. Recently, chitosan has been shown to augment intracellular NAD⁺ and SIRT1 activity in vivo [147], thus suggesting a possible mechanism of action of this well-known biomedical agent. Administration of tyrosol, a phenolic compound, abundant in olive oil and white wine, induced the expression of SIRT1 and activated SIRT1 target proteins, such as FOXO3, AKT, and endothelial NOS, helping mice to overcome heart ischemic stress [148]. Although at this stage, most of these observations are merely correlative, they illustrate the growing interest of the medical community in nutraceuticals, a class of food-related compounds with pharmacological properties that are presently considered as candidates for anti-aging and anti-inflammatory molecules, a subset of which possibly acts in a sirtuin-dependent manner.

Use of sirtuin inhibitors

Finally and although contrasting with the previously cited literature, sirtuin inhibitors have been used successfully in animal models to treat inflammatory disorders [36, 37, 65, 72]. Even though in vivo studies using sirtuin inhibitors are of potential therapeutic value, most of the pharmacological compounds used to date display broad sirtuin specificity, impeding a precise identification of the targeted sirtuin and thus, an accurate interpretation of the data. Originally, the impetus for developing potent sirtuin inhibitors was related to the role of this family of enzymes in cell survival and proliferation. Cambinol has been first developed as a potential anticancer reagent [149] and later found to display anti-inflammatory properties in vivo [36, 65]. Conceptually, it is possible that by inhibiting cell survival and/or proliferation, sirtuin inhibitors can antagonize the development of immune responses. In any event, the development of novel, water-soluble sirtuin inhibitors (possibly with better-defined specificity) and further in vivo experimentation is required to evaluate the beneficial role of sirtuin inhibitors in inflammatory settings and to identify potential mechanisms at work.

CONCLUDING REMARKS

Cells and organisms have devised numerous strategies to respond to a changing environment. Energy supply, access to oxygen, infection, and cell injury represent sudden environmental changes, to which cells need to respond in a prompt and suitable fashion. In this context, sirtuins, present in virtually all cell compartments, have emerged as key sensors of the cellular metabolic status, controlling cell homeostasis via the modulation of numerous physiological processes. Inflammation induces major variations in tissue parameters, such as hypoxia, cell death, and oxidative stress. Sirtuins, especially SIRT1, have been shown to regulate many key cellular factors in the course of inflammation, such as RELA, HIF-1 α , and PGC1. Although the overall picture points to a role for sirtuins, and especially SIRT1, as a negative-feedback response, promoting resolution of an inflammatory response, several observations also suggest that under some circumstances, sirtuins may, in fact, promote some aspects of the inflammatory/immune response [36, 37, 65–67, 72, 73], a contention supported by several reports, illustrating the beneficial effect of sirtuin inhibitors in an inflammatory setting [36, 37, 65, 72]. Similarly, the anti-inflammatory properties of the NAMPT inhibitor FK866 are compatible with a positive mechanistic link between NAMPT activity and an inflammatory response. It is presently difficult to reconcile these apparent contradictory findings, and further work is required to better delineate the functional consequences of altered NAD⁺ metabolism and sirtuin activity during an immune response. Although these observations are difficult to reconcile, a recent report has offered an alternative view about how SIRT1 may down-regulate an inflammatory response [93]. With the use of an in vitro system, the authors demonstrate that SIRT1 inhibition only marginally increases TNF mRNA accumulation in the human THP-1 cell line in response to a primary LPS exposure. Following initial stimulation, THP-1 cells become “tolerant” to endotoxin, a state of profound unresponsiveness to LPS stimulation that is thought to represent an attempt to recover homeostasis [150]. Of interest, LPS-tolerant cells displayed high levels of NAMPT and SIRT1 proteins and recovered substantial TNF secretion capacities in response to SIRT1 inhibition. It is therefore tempting to speculate that the anti-inflammatory role of SIRT1, and very speculatively SIRT6, mostly operates during the late-resolution phase of an inflammatory response to dampen NF- κ B target gene transcriptions, while exerting their proinflammatory role by affecting alternative, non-NF- κ B targets during the early onset of the response (see also Fig. 2). Change in sirtuin function (from pro- to anti-inflammatory) may derive from a shift in substrate preference consecutively to the increased expression levels and/or altered subcellular localization. This model of a time-dependent shift in SIRT1 and SIRT6 function is difficult to address with genetically deficient animals but offers a plausible explanation to the apparent conflicting results published to date.

The promiscuous nature of sirtuins represents an additional feature that needs to be addressed when considering these

enzymes as targets for pharmacological intervention: (i) numerous substrates for SIRT1 have been recognized, placing this sirtuin member at the crossroads of a wide range of cellular functions and rendering the functional consequences of SIRT1 activation/inhibition difficult to predict at the organismal level; (ii) sirtuins may display overlapping specificities, e.g., SIRT1, SIRT2, and SIRT6 all deacetylate the same H3K56 [151, 152], and depending on experimental models and organisms considered, inhibition of a single sirtuin member can affect [152] or not [153] total H3K56 acetylation levels; (iii) substrates can be deacetylated by sirtuins and histone deacetylases [11, 154], indicating functional redundancy that could limit the efficacy of sirtuin-targeted pharmacological approaches.

Although further studies are warranted to better understand the complex biological role of sirtuins, these enzymes clearly represent promising targets for the development of novel strategies to treat metabolic and inflammatory disorders.

AUTHORSHIP

N.P. and O.L. wrote the paper.

ACKNOWLEDGMENTS

The authors acknowledge the support of the Belgian Program in Interuniversity Poles of Attraction initiated by Science Policy Programming, Belgium, the Research Concerted Action, and the “Fonds pour la formation à la Recherche dans l’Industrie et l’Agriculture” (F.R.I.A.) of the “Fédération Wallonie-Bruxelles”, the “Fonds David et Alice Van Buuren”, and the Walloon Region. We apologize to the authors whose work was not discussed directly as a result of the focus and space limitations of this review.

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KEY WORDS:

NAD · SIRT1 · SIRT6 · inflammation · NAMPT