Sirtuin-1 is a nutrient-dependent modulator of inflammation

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Inflammation accompanies obesity and its comorbidities—type 2 diabetes, non-alcoholic fatty liver disease and atherosclerosis, among others—and may contribute to their pathogenesis. Yet the cellular machinery that links nutrient sensing to inflammation remains incompletely characterized. The protein deacetylase sirtuin-1 (SirT1) is activated by energy depletion and plays a critical role in the mammalian response to fasting. More recently it has been implicated in the repression of inflammation. SirT1 mRNA and protein expression are suppressed in obese rodent and human white adipose tissue, while experimental reduction of SirT1 in adipocytes and macrophages causes low-grade inflammation that mimics that observed in obesity. Thus suppression of SirT1 during overnutrition may be critical to the development of obesity-associated inflammation. This effect is attributable to multiple actions of SirT1, including direct deacetylation of NFκB and chromatin remodeling at inflammatory gene promoters. In this work, we report that SirT1 is also suppressed by diet-induced obesity in macrophages, which are key contributors to the ontogeny of metabolic inflammation. Thus, SirT1 may be a common mechanism by which cells sense nutrient status and modulate inflammatory signaling networks in accordance with organismal energy availability.

Inflammation in Obesity

Obesity has become a public health crisis in the United States and much of the world. More than a billion people worldwide are estimated to be overweight, and at least 30% of these, or 300 million, are obese.1 Obesity itself would be of little concern if it were not associated with the “metabolic syndrome,” a constellation of metabolic abnormalities that portend the development of type 2 diabetes mellitus, atherosclerosis and non-alcoholic fatty liver disease.2 The list of diseases associated with obesity is ever growing. This inspires the question of how obesity triggers these complications and what can be done to intervene in the progression from obesity to disease.

Low-grade, chronic inflammation is now widely recognized to be a salient feature of obesity and many of its accompanying pathologies. Studies of critically ill humans and animals have demonstrated that inflammation can profoundly alter metabolic function. Investigators have postulated that obesity-associated inflammation may induce similar metabolic shifts. As in other unhealthy states, the teleological role of the immune system is likely to clear the insult and return the organism to a healthy, functional state.3,4 However, during obesity, this restoration of tissue function does not occur. Rather, the elaboration of cytokines and other inflammatory mediators seems to lead to a downward spiral of tissue dysfunction in metabolic organs. Many scientific investigators and clinicians have been interested in the possibility that suppressing inflammation may lead to improvement of metabolic parameters in obese patients. Thus, substantial effort has been placed on understanding how obesity incites inflammation, with the hope that such knowledge will lead to the development of novel and much needed therapeutics.5
The components of the generic inflammatory response can be conceptually divided into four major categories: inducers (LPS, PolyIC), sensors (TLRs, NLRs), mediators (cytokines, eicosinoids) and effectors (cells that respond to inflammatory mediators), which are connected in a simple circuit (Fig. 1). In obesity, elevated free fatty acids, “metabolic endotoxemia,” local hypoxia, products of adipocyte death and endoplasmic reticulum stress have all been implicated as potential inducers of inflammation.6 These are thought to activate sensors such as NLRP3, TLRs, HIF-1α and NFkB within adipocytes, hepatocytes and tissue macrophages, leading to the elaboration of mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 and the recruitment of inflammatory cells to the white adipose tissue (WAT). The best appreciated of these are CD11c+ macrophages, although nearly every cell of the immune system—conventional T cells, innate-like lymphocytes, B cells and mast cells—accumulates in obese WAT and has been implicated in the transition from healthy to inflamed fat. These cells release additional cytokines, which may act on adipocytes, myocytes or hepatocytes to induce insulin resistance and lipolysis.13 Conversely, a few cell types (Foxp3+CD4+ regulatory T cells and eosinophils, for example) may have a salutary influence on metabolic homeostasis in healthy WAT, and are reduced during obesity.

The inflammatory milieu of obesity is complex, featuring a panoply of elevated plasma and tissue cytokines, and infiltration of WAT with inflammatory cells. And while much progress has been made in identifying the inducers, sensors, mediators and effectors that participate in obesity-associated inflammation, many questions remain. One particularly enigmatic aspect of these investigations is that many of the reported inducers and sensors are present and active in physiologic states other than obesity, where they do not cause overt inflammation. Free fatty acids, for example, rise into the millimolar range during fasting, evidently without causing widespread activation of TLR4 or NLRP3. This suggests that something else—other than the simple presence or absence of a ligand for innate sensors—sets a context and contributes to the decision of whether or not to induce inflammation during obesity.

**Nutrient Sensing Modulates the Inflammatory Response**

The majority of studies examining the interaction between obesity and inflammation have concentrated on the pathophysiologic role of the immune system in metabolism. Activation of the immune system in the setting of overnutrition is often assumed to be an accident that occurs when there is an overabundance of potential immunologic ligands such as gut-derived endotoxin, fatty acids or ceramides. However, some investigators believe that the intimate association between energy metabolism and immunity is deliberate. For example, the receptivity of inflammatory networks to activation may be regulated by nutrient availability because immune activation—particularly the pyrogenic and acute phase responses—incurs a large energetic cost. In febrile humans, each 1°C increment in temperature raises basal metabolic rate (BMR) by 10–15%.14 As a result, during sepsis, BMR may be increased by 20–25%.15 When the bumblebee, *Bombus terrestris*, cannot prevent activation of the immune response under energy-limited conditions, high mortality ensues.16 Similarly, caloric restriction (CR) in mice leads to defective pathogen clearance and cytokine elaboration by peritoneal macrophages during infection, consequently reducing survival by 40%.17 Interestingly, the leptin-deficient *ob/ob* mouse, which lives in a state of simulated starvation, shows deficits in pathogen clearance similar to those of CR mice: an effect that can be rescued by exogenous leptin administration.18,19 Humans lacking leptin also exhibit lymphopenia and T-cell hyporesponsiveness.20

Opportunity costs are evident even at the level of individual tissues. Muscle protein wasting and the transcriptional suppression of many hepatic enzymes during sepsis provide amino acids and cellular machinery to support a dramatic increase in synthesis of acute phase proteins.21 Thus, the tight integration of metabolic and immune signaling seen in mammals may reflect an optimization process that reconciles the need for vigorous defense against pathogens with available energy supplies. For this reason, many organisms have evolved mechanisms to suppress the immune system during times of energy stress. As suggested above, soluble factors such as leptin may help communicate such signals between cells. Within cells, AMPK, mTOR and sirtuins have all been shown to participate in this communication. AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) sense energy depletion and repletion, respectively, and cooperate to permit an immune response only in the presence of adequate energy reserves. AICAR, a pharmacological activator of mTOR, suppresses LPS-mediated activation of NFκB while S6K1, a downstream target of mTOR, is required for leukotriene B4, TNF-α, IL-1β and IL-6 generation.20

**SirT1 Opposes Inflammation in Metabolic Tissues**

Sirtuin 1 (SirT1) is a NAD+-dependent protein deacetylase that coordinates the mammalian metabolic response to calorie restriction and fasting.22–25 During times of nutrient deficit, SirT1-mediated deacetylation of PGC1-α stimulates hepatic glucose production and fatty acid oxidation,22,26 while promoting metabolic efficiency through adiponectin production in adipose tissue.27

In addition to its metabolic effects, SirT1 can suppress inflammation.28,29
Overexpression of SirT1 (with Dnajc12) decreases hepatic expression of TNF-α and IL-6 in the setting of chronic high fat feeding, whereas liver-specific deletion of SirT1 increases hepatic NFKB activity. In 3T3-L1 adipocytes, reducing SirT1 levels with RNAi reduces inhibitory deacetylation of the NFKB subunit p65, and leads to increased NFKB activity at the TNF-α, IL-6, MCP-1, KC and IL-1β promoters. Yoshizaki et al. speculated that downregulation of SirT1 in adipose tissue of obese mice and humans “contributes to the heightened inflammatory state of adipose tissue in obesity.” We found that suppression of SirT1 expression in vivo causes WAT inflammation and elevation of circulating TNF-α and IL-1β, resulting in anorexia and lipolysis. As in obese animals, WAT of fat-specific SirT1 KO mice shows aberrant CD11c+ macrophage recruitment and production of proinflammatory cytokines. In contrast, inflammation is reduced in WAT of high fat diet fed SirT1 overexpressing mice. Moreover, in two distinct human cohorts, WAT SirT1 mRNA expression correlated negatively with indices of macrophage infiltration. In our studies, the effects of SirT1 on cytokine expression were attributable to chromatin remodeling; in the absence of SirT1 deacetylase activity, H3K9 was hyperacetylated, increasing the accessibility of inflammatory cytokine promoters to NFKB. These findings are supportive of elegant recent work demonstrating a critical role for SirT1 in maintaining silent, loci-specific facultative heterochromatin at the TNF-α and IL-1β promoters.

Macrophages Sense Nutritional Status through SirT1

Much argument still exists over where obesity-associated inflammation begins. A common view is that adipocytes initiate WAT cytokine production, and that macrophages simply propagate and amplify the original insult. However, tissue-resident macrophages, which abound in WAT, are specialized to act as sentinels for tissue pathology and are supremely sensitive to homeostatic threats. Moreover, macrophages, and in particular CD11c+ cells, are the primary producers of proinflammatory cytokines during metabolic disease. Thus, it is important to carefully assess the role of macrophages in initiating inflammation during metabolic stress.

In fact, recent work suggests that inflammation in macrophages is similarly influenced by SirT1 expression. Although we found that macrophages are not required for adipose tissue inflammation caused by SirT1 knockdown, we also found that suppression of SirT1 in peritoneal macrophages induces TNF-α mRNA expression. Similarly, RNAi targeting of SirT1 in RAW264.7 cells enhances LPS-elicited activation of the JNK and IKK pathways and increases NFKB hyperacetylation, and a heightened pro-inflammatory response to high fat feeding in liver and adipose tissue. Finally, the anti-inflammatory effects of AMPK activation in the presence of the inducers stearate and LPS require SirT1, specifically SirT1-mediated K310 deacetylation of NFKB p65. Thus, like adipocytes and hepatocytes, macrophages use SirT1 to determine immune responsiveness.

Building upon these observations, we sought to determine whether macrophages in vivo could also sense whole-body nutritional status using SirT1. To address this, we fed male mice high fat diet for 16 weeks, harvested peritoneal macrophages and isolated total RNA. Analysis of SirT1 expression by qPCR revealed a ~50% reduction in the high fat fed group, supporting the notion that decreases in SirT1 may also be involved in the pathogenesis of obesity-associated inflammation in macrophages in vivo (Fig. 2). These data raise the intriguing possibility that macrophages are autonomously capable of sensing whole body nutritional status, and can use this information to influence their propensity toward inflammation. Such a role could be important in determining the appropriateness of an immune response in cases of caloric deficit, in addition to calorie surfeit, such as we have discussed above.

Setting the Inflammatory Tone

Our studies and others suggest that SirT1 is a key participant in the inflammation that occurs in obesity through its actions in adipocytes and macrophages. Interestingly, unlike other proposed players in obesity-associated inflammation, SirT1 does not appear to act by providing novel ligands to stimulate immunologic receptors. Nor does it act as a sensor for an inflammatory ligand, a mediator to transduce inflammatory signals, or as a target for those mediators. Rather, SirT1 appears to act as a network regulator, which modifies the sensitivity of inflammatory circuits and thereby determines the outcome of circuit activation (Fig. 3). Network regulators like SirT1 may theoretically act at any point in the four-part model of inflammation. Given that microbial (e.g., LPS) and endogenous (i.e., fatty acid) inducers are ubiquitous, inflammation can thus result from increased sensitivity of sensors to inducers, increased elaboration of mediators in response to sensor activation, or increased response of effectors to mediators. This alteration of the circuit may be both quantitative and qualitative, changing not only the degree of inflammation (e.g., amount of cytokine produced), but also the type (e.g., ratio of “type 1” to “type 2” cytokines). Such a model could explain the ontology of metabolic inflammation.

SirT1 acts on many substrates, including histones, FoxO, NFKB and p53. How acetylation alters the function of these proteins remains incompletely understood. In the case of NFKB, acetylation appears to be an important determinant of transcriptional activity once evoked. Deacetylation of FoxO may contribute to both its likelihood of translocating to the nucleus, and the gene targets that it chooses to activate. At various sites, the

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(de)acetylation of histones may either open/close chromatin directly, or simply make it a more attractive target for transcriptional machinery. Thus, deacetylation of targets by SirT1 is, in general, a mechanism by which cells alter transcriptional activity both quantitatively and qualitatively in response to changing nutrient status.43

Phenomena such as LPS tolerance, wherein the response to LPS is qualitatively and quantitatively altered by prior exposure, have clearly demonstrated that inflammatory circuits can be modified depending on the context in which they occur.41-43 While such modulation could theoretically occur through many means—ligand scavenging, receptor antagonism or desensitization, inhibitors of transcriptional activity—epigenetic modification of target genes may be a common methodology. Such mechanisms may change the threshold for activation, the system gain, or the transcriptional targets in order that the same amount of inducer provoke a different degree or type of inflammatory response.

We believe the circuit-modifying role of SirT1 is further evidence that the immune system and nutrition have a hard-wired and adaptive association in physiology, as well as pathophysiology. As discussed, suppression of inflammation in the undernourished state may be critical to resource allocation. It remains unclear, however, whether the converse should also be true: whether inflammation should be disinhibited in an overnourished state. In this vein, one possibility we find interesting is that “parainflammation” might play an important physiological role in regulating metabolism in response to environmental stressors. In this formulation, the immune system is viewed more as a “general manager” of tissue homeostasis as opposed to specialized system for pathogen disposal. Such speculations are supported by accumulating observations that animals with altered immune function spontaneously develop disordered food intake, metabolic rate, substrate utilization and adipose tissue depot size.32,44-46 We anticipate many surprises in this vigorous field over the next several decades, ultimately leading to an integrative perspective on the link between metabolism and immunology, as well as new therapeutic avenues.

**Materials and Methods**

Male C57BL6 mice were fed high-fat diet (60% kcal from fat, D12492, Research Diets) or regular chow (2018s, Harlan Teklad) for 16 weeks. Macrophages were harvested by peritoneal lavage, RNA was extracted using the RNeasy Kit (Qiagen), reverse transcribed and gDNA removed with the QuantiTect Kit (Qiagen), and transcript abundance assessed by real-time PCR on a 7500 Fast Real-Time PCR System (Applied Biosystems) and analyzed by ΔΔCt method with SirT1 primer sequences F: 5′-CAC AAA TAC TGC CAA GAT GTG AAT-3′, R: 5′-TCC AAA ATA TTA CAC TCT CCC CAG TA-3′.
Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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