Sirt1 restrains lung inflammasome activation in a murine model of sepsis

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1Center for Research on Environmental Disease, University of Kentucky, Lexington, Kentucky; 2Graduate Center for Toxicology, College of Medicine, University of Kentucky, Lexington, Kentucky; 3The Second Hospital of Jilin University, Changchun, Jilin, People’s Republic of China; 4Center for Biomedical Research, University of Texas Health Science Center at Tyler, Tyler, Texas

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Gao R, Ma Z, Hu Y, Chen J, Shetty S, Fu J. Sirt1 restrains lung inflammasome activation in a murine model of sepsis. Am J Physiol Lung Cell Mol Physiol 308: L847–L853, 2015. First published February 6, 2015; doi:10.1152/ajplung.00274.2014.—Excessive inflammasome activation in sepsis is a major cause of organ damage during sepsis. The elderly are highly susceptible to sepsis-induced organ injury. Sirt1 expression is reduced during aging. In the present study, we investigated the role of Sirt1, a histone deacetylase, in controlling inflammatory responses in a murine sepsis model induced by cecal ligation and puncture (CLP). We examined lung inflammatory signaling in inducible Sirt1 knockout (Sirt1−/−) mice and wild-type littermates (Sirt1+/+) after CLP. Our results demonstrated that Sirt1 deficiency led to severe lung inflammatory injury. To further investigate molecular mechanisms of Sirt1 regulation of lung inflammatory responses in sepsis, we conducted a series of experiments to assess lung inflammasome activation after CLP. We detected increased lung inflammatory signaling including NF-κB, signal transducer and activator of transcription 3, and ERK1/2 activation in Sirt1−/− mice after CLP. Furthermore, inflammasome activity was increased in Sirt1−/− mice after CLP, as demonstrated by increased IL-1β and caspase-7 cleavage and activation. Aggravated inflammasome activation in Sirt1−/− mice was associated with the increased production of lung proinflammatory mediators, including ICAM-1 and high-mobility group box 1, and further disruption of tight junctions and adherens junctions, as demonstrated by dramatic reduction of lung claudin-1 and vascular endothelial-cadherin expression, which was associated with the upregulation of matrix metalloproteinase 9 expression. In summary, our results suggest that Sirt1 suppresses acute lung inflammation during sepsis by controlling inflammasome activation pathway.

Sepsis is a devastating disease with high mortality. The epidemiological studies from North America suggest a sepsis incidence of 3 cases per 1,000 persons annually (1). Because of its complexity and heterogeneity, sepsis remains a great challenge for scientists and clinicians. The lung and respiratory tract constitute a large surface of the body in direct contact with the outside environment, including inhaled microbes and particles (6). Pulmonary infection is the most common cause of sepsis, and meanwhile lung as the blood gas exchange site is the first line of sepsis-induced inflammation and tissue damage (48, 49). Molecular mechanisms of sepsis-induced lung inflammatory injury are yet to be determined. Tight regulation of the immune system appears to be critical to better maintain the balance between protective and tissue-damaging innate immune responses (9, 27).

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Innate immune system is the first line of defense against invading bacterial pathogens (5, 6, 39, 42). The innate immune receptors such as pattern recognition receptors recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (5, 6, 42). PAMP-induced Toll-like receptor signaling often results in the activation of MAPK cascades, including ERK1/2, c-JNK, and p38, and nuclear translocation of NF-κB, which consists of the first signal pathway of inflammasome activation (17, 19, 27, 35, 42, 44, 59). The nuclear translocation of NF-κB leads to the production of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, and chemokines, such as IL-8 (17, 19, 26, 27, 35, 42, 50). The inflammasome complex, a central player of the second signal pathway of inflammasome activation, comprises the nucleotide oligomerization domain-like receptor (NLR) protein such as NLRP3, the adapter apoptosis-associated speck-like protein containing caspase-recruitment domain (ASC), and pro-caspase-1 (30). The assembly and activation of the NLRP inflammasome complex cause caspase-1 and IL-1β cleavage and activation, IL-1β secretion (2, 46), and subsequent inflammatory responses (2, 30, 46).

Sirt1, a NAD+-dependent class III histone deacetylase, has been reported to exert its anti-inflammatory function by regulating the production of proinflammatory cytokines (4, 14, 16, 24, 40). However, the role of Sirt1 in sepsis-induced acute inflammatory injury and its function in modulating inflammasome activation pathway have yet to be determined. In the present study, we used a cecal ligation and puncture (CLP) sepsis model to investigate the role of Sirt1 in sepsis-induced acute lung inflammation by inducible deletion of Sirt1 in mice. Our studies demonstrated that Sirt1 knockout mice are highly susceptible to sepsis-induced inflammatory lung injury.

MATERIALS AND METHODS

Reagents. Tamoxifen was purchased from Sigma-Aldrich (St. Louis, MO). TNF-α and IL-6 ELISA kits were obtained from Biolegend (San Diego, CA). o-Dianisidine dihydrochloride and H2O2 were obtained from Sigma-Aldrich. ICAM-1, claudin-1, and vascular endothelial (VE)-cadherin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Phospho-signal transducer and activator of transcription 3 (STAT3) (Thr705), STAT3 (124H6), phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204), p44/42MAPK (ERK1/2), phosphorylated Ik-Bα, Ik-Bα, IL-1β, cleaved caspase-3, cleaved caspase-7, high-mobility group box 1 (HMG1B), and β-actin antibodies were obtained from Cell Signaling Technology (Beverly, MA). Matrix metalloproteinase 9 (MMP-9) antibody was obtained from R&D Systems (Minneapolis, MN).

Animal studies. Mice were housed in cages with free access to food and water in a temperature-controlled room with a 12-h:12-h dark/light cycle. All experiments and animal care procedures were approved by the Institutional Animal Care and Use Committee of the
induction. To assess the role of Sirt1 in sepsis-induced lung injury, we examined edema formation in the lungs of Sirt1−/− mice and Sirt1+/− littermates after the induction of sepsis. Lung wet/dry weight ratio was significantly increased in Sirt1−/− mice after CLP when compared with that of wild-type littermates (Fig. 1D).

Sirt1 deletion leads to increased IL-1β precursor and mature form production in the lung after sepsis induction. Inflammasome activation pathway contains two essential phases (2, 35, 43). The first is the activation of proinflammatory transcription factors, such as NF-κB (2, 35, 43, 62), which induces proinflammatory cytokine expression e.g., pro-IL-1β and IL-18 production. The second compulsory phase is to

RESULTS

Sirt1 deletion increases lung proinflammatory cytokine production and aggravates lung inflammation after sepsis induction. To assess lung inflammation in sepsis after Sirt1 deletion, lung MPO activity, an indicator of neutrophil infiltration was examined in Sirt1−/− mice and Sirt1+/− littermates (Fig. 1A). Furthermore, lung IL-6 and TNF-α (Fig. 1, B and C) levels were evaluated by ELISA. Proinflammatory cytokines IL-6 and TNF-α and MPO levels in the lung tissues were increased after CLP and much higher in Sirt1−/− mice, suggesting that Sirt1 plays a protective role against sepsis-induced lung inflammation. To explore molecular mechanisms of Sirt1 protection against sepsis-induced lung inflammation, we examined NF-κB activation. NF-κB activation is dependent on the phosphorylation and degradation of its inhibitory regulator Ik-Bα (43). We examined Ik-Bα phosphorylation and degradation. The increased Ik-Bα phosphorylation and degradation indicate that NF-κB was activated in the lung tissues of Sirt1−/− mice after CLP (Fig. 2, A–C). We also conducted experiments to assess the effects of Sirt1 deletion on other lung inflammatory signaling including STAT3 and ERK1/2 activation. Both STAT3 and ERK1/2 phosphorylation were increased in Sirt1−/− mice (Fig. 3, A–D).

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University of Texas Health Science Center at Tyler. The generation of inducible Sirt1 knockout mice was described previously (32, 60). Six to seven weeks after the birth, mice were given tamoxifen (100 mg/kg body wt in corn oil) by intraperitoneal (ip) injection daily for 5 days to induce nuclear translocation of Cre recombinase, as described previously (60). Twenty to twenty-eight weeks after Sirt1 deletion, age-matched female Sirt1−/− and Sirt1+/− littermates were used in the studies. CLP was performed as described by us previously (61). Sham animals underwent the same surgical procedure except for ligation and puncture of the cecum.

Lung MPO assay, ELISA, and immunoblotting assays. Neutrophil accumulation in the lung tissue was assessed by myeloperoxidase (MPO) activity as previously described (58). Briefly, 24 h after CLP surgery, lung tissue samples were frozen and homogenized in Hexadeoxytrimethylammonium bromide lysis buffer. MPO activity was examined. Six hour after CLP, mouse lung tissues were collected. Lung tissue IL-6 and TNF-α levels were determined by ELISA kits following the instruction of the commercial kits. For immunoblotting assay, 6 or 24 h after CLP surgery, lung tissues were obtained and homogenized in tissue lysis buffer. Immunoblotting assay was carried out as previously described (60).

Statistical analysis. Data were analyzed by two-way ANOVA with Bonferroni’s multiple-comparisons test using the GraphPad Prism version 6 (GraphPad Software, La Jolla, CA) and expressed as means ± SE. Statistical significance was assigned to P values <0.05.
generate the mature forms of the cytokines by inflammasome-mediated cleavage of the precursors (2, 12, 30, 42, 46). To assess the role of Sirt1 in this process, we examined both the precursor and mature forms of IL-1β production in the lung tissues after CLP. Our results showed that Sirt1 deletion led to increased pro-IL-1β and the mature form generation (Fig. 4, A–C), suggesting that downregulation of inflammasome activation pathway could be one of the mechanisms contributing to Sirt1 protection against lung inflammation in sepsis.

Sirt1 deletion leads to increased caspase-3 and caspase-7 activation in the lung after sepsis induction. Caspases are highly conserved cysteine-dependent aspartate-specific proteases, which can be grouped into inflammatory and apoptotic caspases (8, 37). Caspase-1, -3, and -7 can also mediate inflammatory responses, even though caspase-3 and caspase-7 are still considered major players in apoptosis or necrosis (8, 12, 37). Caspase-1 is a critical component of the inflammasome complex (11, 12, 37, 46). Caspase-7 is a substrate of caspase-1 (12, 37). To confirm the function of Sirt1 in inflammasome activation, we examined the production of cleaved caspase-3 and -7 in the lung tissues 6 h after CLP surgery. We observed that the activation of both caspase-3 and -7 was increased in Sirt1<sup>−/−</sup> mice after CLP with the level of caspase-7 cleavage much higher in the lungs of Sirt1<sup>−/−</sup> mice (Fig. 5, A–C). Our results indicate that Sirt1 regulation of caspase-1-mediated caspase-7 activation may also contribute to its protection against inflammatory signaling in sepsis.

Sirt1 deletion leads to increased lung ICAM-1 and HMGB1 expression after sepsis induction. Proinflammatory cytokines can regulate the expression of cell adhesion molecules such as ICAM-1 (45, 56). We examined the effects of Sirt1 deletion on ICAM-1 expression in lung tissues after CLP. Immunoblotting assays showed that Sirt1<sup>−/−</sup> mice exhibited significantly higher ICAM-1 expression after CLP (Fig. 6, A and C).
Additionally, PAMPs and DAMPs activate the double-stranded RNA-activated protein kinase R to trigger the inflammasome-dependent HMGB1 release (33). HMGB1 plays a role in mediating late inflammatory responses in sepsis (15, 25, 55, 63). Because it is released later than other proinflammatory cytokines, it became known as a “late mediator of sepsis” (41). We examined lung HMGB1 expression in Sirt1-deficient mice. Our results showed that Sirt1 deletion led to increased lung HMGB1 production (Fig. 6, B and D).

Sirt1 deletion increases lung MMP-9 production and decreases lung claudin-1/VE-cadherin levels after sepsis induction. Extracellular proteases, such as MMPs, have been reported to mediate inflammatory responses (51, 57) and induce endothelial/epithelial barrier disruption through cleavage and shedding of extracellular fragment of junction proteins (13, 34). We detected a significant increase of MMP-9 expression in the lung tissues of Sirt1−/− mice after CLP (Fig. 7, A and B). The increased extracellular protease activity may lead to degradation of cell-cell junction proteins in the lung. VE-cadherin and claudin are key components of cell-cell junctions (10, 18).

Here, we examined the effects of Sirt1 deletion on VE-cadherin and claudin-1 protein levels in the lung tissues after CLP. Consistent with increased MMP-9 production, Sirt1-deficient mice exhibited markedly reduced VE-cadherin and claudin-1 levels after CLP (Fig. 8, A–D).

DISCUSSION

Sepsis is a life-threatening disease with high morbidity and mortality (22, 23). The balance between pro- and anti-inflammatory pathways plays an important role in the fate of the patients (22). Up to now, no efficient therapeutics is available to treat this devastating disease (22, 23). A better understanding of the pathophysiological basis of the disorder is needed to develop novel strategies against sepsis.

It is now well established that early organ damage in sepsis is caused by overwhelming inflammation. However, both inflammatory organ injury and late-stage immune suppression, which leads to secondary infection, likely contribute to the high mortality in sepsis (20–22). Acute lung injury is usually associated with excessive lung inflammation (31, 35, 36). Immunosuppression, attributable to early exhaustion of immune response, is extremely common in the middle and late phases of sepsis and is associated with increased nosocomial infection and mortality (20–22). Therefore, early control of inflammatory responses is critical in the prognosis of sepsis. Sirt1, a member of histone deacetylase, has been reported to play a critical role in regulating metabolism, senescence, and aging-related pathways (3, 47). Our data demonstrate that sepsis-induced lung inflammatory injury in Sirt1−/− mice is aggravated, suggesting that Sirt1 plays a protective role against lung inflammatory injury in sepsis.

Sirt1 has been reported to control inflammatory cytokine production and activation through NF-κB acetylation (40, 64). Our studies demonstrate that Sirt1 regulates NF-κB activation in sepsis. Upregulation of proinflammatory cytokines by NF-κB activation induces the expression of cell adhesion molecules such as ICAM-1 to promote early leukocyte infiltration and lung inflammatory injury. HMGB1, a late mediator of inflammation in sepsis, is also increased subsequently (7, 52, 54). The increased lung ICAM-1 and HMGB1 expression in Sirt1-deficient mice is consistent with the aggravated lung injury after CLP. The increases of both the early and late
Inflammasome pathway has two main signal phases: transcription factor such as NF-κB-regulated gene expression of proinflammatory mediators, followed by mature cytokine production and release phase regulated by inflammasome complex activation (17, 19, 26, 27, 30, 35, 42, 46, 50). The first phase is triggered by the PAMPs or DAMPs, leading to the degradation and activation of the NF-κB inhibitor IκB (35). NF-κB activation leads to increased expression of proinflammatory mediators such as ICAM-1 and the precursor of proinflammatory cytokines such as IL-1β and IL-18, which are further processed by the inflammasome complex to generate the active forms (2, 30, 46). The inflammasome complex, which consists of pro-caspase-1, NLRP, and ASC, is required for IL-1β and IL-18 cleavage and release (2, 30, 46). Our data suggest that Sirt1 regulates both the first signal phase (NF-κB) and the second signal phase (inflammasome complex activation and precursor processing) in sepsis.

Members of the inflammatory caspases, also known as group I caspases, include caspase-1, -4, and -5 in human and caspase-1, -11, and -12 in mouse (29, 37). Caspase-1, an aspartate-specific cysteine protease, is a component of the inflammasome complex assembled upon pathogen recognition, which cleaves IL-1β and IL-18 to generate the mature and active forms (29, 37). Caspase-7 is also cleaved and activated by caspase-1 (12). Poly(ADP-ribose) polymerase-1 (PARP1), a target of caspase-7, has been reported to bind to the promoters of NF-κB target genes and to negatively regulate their gene expression (12). Caspase-7 activation by caspase-1 cleavage has been reported to modulate the expression of NF-κB target genes through PARP-1 cleavage (12). In our studies, caspase-7 activation was markedly increased in the Sirt1 knockout mice during sepsis, which may further induce NF-κB-regulated proinflammatory gene expression by PARP-1 cleavage.

The disruption of endothelial cell junctions leads to increased vascular permeability and edema formation during inflammatory lung injury (28, 38, 53). The VE-cadherin and claudins are key members of adherens junctions and tight junctions, respectively. VE-cadherin and claudins are targets of extracellular protease-mediated degradation (13, 18, 34). In our studies, we demonstrated that VE-cadherin and claudin-1 protein levels in the lung tissues of Sirt1-deficient mice were dramatically reduced during sepsis, which was associated with higher MMP-9 expression. Our results suggest that upregulated extracellular proteases such as MMP-9 activity in Sirt1-deficient mice may lead to the greater breakdown of the endothelial barrier function.

In summary, Sirt1 plays a protective role against inflammatory lung injury in CLP-induced sepsis. Sirt1 suppresses inflammasome activation pathway, which prevents the overproduction of proinflammatory mediators and associated tissue damage. Our studies indicate that Sirt1 is a potential therapeutic target to treat sepsis-induced lung inflammatory injury.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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