Acetate attenuates inflammasome activation through GPR43-mediated Ca\(^{2+}\)-dependent NLRP3 ubiquitination

Mengda Xu\(^1,2\), Zhengyu Jiang\(^1\), Changli Wang\(^1\), Na Li\(^1\), Lulong Bo\(^1\), Yanping Zha\(^1\), Jinjun Bian\(^1\), Yan Zhang\(^1\) and Xiaoming Deng\(^1\)

Abstract

Acetate has been indicated to be elevated and to regulate inflammation in inflammatory and metabolic diseases. The inflammasome serves as a key component of immune homeostasis, and its dysregulation can lead to various inflammatory disorders. However, little is known about the effects of acetate on inflammasome activation and the underlying mechanism. Here, we demonstrate that acetate attenuates inflammasome activation via GPR43 in a Ca\(^{2+}\)-dependent manner. Through binding to GPR43, acetate activates the G\(_{q/11}\) subunit and subsequent phospholipase C-IP\(_3\) signaling to decrease Ca\(^{2+}\) mobilization. In addition, acetate activates soluble adenylyl cyclase (sAC), promotes NLRP3 inflammasome ubiquitination by PKA, and ultimately induces NLRP3 degradation through autophagy. In vivo, acetate protects mice from NLRP3 inflammasome-dependent peritonitis and LPS-induced endotoxemia. Collectively, our research demonstrates that acetate regulates the NLRP3 inflammasome via GPR43 and Ca\(^{2+}\)-dependent mechanisms, which reveals the mechanism of metabolite-mediated NLRP3 inflammasome attenuation and highlights acetate as a possible therapeutic strategy for NLRP3 inflammasome-related diseases.

Introduction

The inflammasome is a series of multiprotein complexes that serve as a platform to promote interleukin (IL)-1\(\beta\) secretion and pyroptosis\(^1\). The NACHT, LRR, and PYD domain-containing protein 3 (NLRP3) inflammasome comprises the NOD-like receptor, the adaptor protein ASC and caspase-1 and is considered to play a crucial role in the immune response to pathogens\(^2\). However, dysregulation of NLRP3 inflammasome activation leads to excessive inflammation, which is linked to various inflammatory disorders, including type 2 diabetes, atherosclerosis, gout, and Alzheimer’s disease\(^3-5\).

Therefore, the regulation of NLRP3 inflammasome activation has emerged as a therapeutic target for inflammasome-related illnesses.

Recently, several metabolites, such as short-chain fatty acids, dopamine and bile acid, have been reported to be involved in NLRP3 inflammasome regulation\(^5\)-\(^7\). For example, by binding to transmembrane G-protein-coupled receptor-5 (TGR5) or dopamine receptor D1 (DRD1), metabolites can induce an increase in cyclic adenosine monophosphate (cAMP) and subsequently activate protein kinase A (PKA) and promote NLRP3 ubiquitination and degradation through autophagy or the proteasome\(^5,6\). cAMP generated by transmembrane or soluble adenylyl cyclase (tmAC or sAC, respectively) is considered to function as a brake for inflammasome activation\(^5,6,8\). However, it remains unknown whether other metabolites function in a similar manner.

Acetate is a short-chain fatty acid produced by the gut microbiota, and its effects\(^9-12\), when elevated in specific
conditions, are extensive and include modulation of tumor growth and regulation of the endocrine and cardiovascular systems. Gao et al.\(^9\) regard acetate to be an epigenetic modulator that promotes lipid synthesis in cancer cells. Another study showed that increased concentrations of acetate promoted metabolic syndrome in a parasympathetic signaling-dependent manner\(^10\). In most cases, the biological effect of acetate depends on its receptor\(^13\). G-protein-coupled receptor 43 (GPR43) is widely expressed on the surface of innate immune cells, including macrophages, and engages in immune activity. For example, in polymorphonuclear cells, GPR43 functions as a regulator that modulates the activity of neutrophils\(^14\). GPR43 is a natural receptor for acetate\(^15\), but most studies have focused on acetate in the context of the gut microbiota and the regulation of inflammatory homeostasis\(^13,16\). Despite the emerging importance of acetate in the regulation of inflammation, the specific function of acetate in inflammasome regulation and the underlying mechanism remain largely unknown.

Our investigation demonstrates that acetate regulates the NLRP3 inflammasome via GPR43-sAC-PKA signaling and attenuates its activation through ubiquitination and autophagy. Furthermore, acetate also shows anti-inflammatory effects on several peritonitis and endotoxemia models in vivo, indicating that acetate might represent an attractive strategy for treating inflammasome-related diseases.

**Materials and methods**

**Animal model and experiments**

Six- to 8-week-old male C57BL/6J mice were purchased from the Shanghai Laboratory Animal Center (SLAC) (China). All mice were housed at a temperature of 18–22 °C with a relative humidity of 50–60% and a 12-h light–dark cycle, with free access to water and food. Animal experiments were approved by the Scientific Investigation Board of Second Military Medical University. For the mouse peritonitis model, each mouse was pretreated with 40 µl of 2.5 M acetate (acetate solution, pH 5.2, Sigma-Aldrich, Lot. 3863) diluted in 400 µl of PBS. Thirty minutes later, the mice were injected with alum (2 mg/mouse; Thermo Scientific, China) or monosodium urate (MSU) (2 mg/mouse; Sigma, China) diluted in 50 µl of PBS or with 10 mg/kg lipopolysaccharide (LPS) (Escherichia coli O111:B4, Sigma, China) diluted in 300 µl of PBS; 6 h after the MSU or alum injection, peritoneal lavage was performed with 5 ml of normal saline (NS), and the lavage fluid was centrifuged.

**Cell preparation and stimulation**

For peritoneal macrophage (PM) generation, each mouse was injected with thioglycollate (BD, USA). Three days after the injection, peritoneal lavage was performed with 5 ml of NS. The cells were resuspended at 2–4 x 10^6 cells/ml and cultured in RPMI 1640 culture medium supplemented with 10% fetal bovine serum (FBS). For bone marrow-derived macrophage (BMDM) generation, mouse femoral tissue was isolated, and the bone marrow was flushed with 3 ml of NS. After red blood cell lysis, bone marrow cells were resuspended at 2–4 x 10^6 cells/ml in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS and 30 ng/ml GM-CSF (PeproTech, USA). The culture medium was changed every 2 days, and after 5–6 days of culture, the cells were subjected to further experiments. To activate the inflammasome in PMs or BMDMs, the cells were first primed with LPS (100 ng/ml) for 3 h, and ATP (5 mM), nigericin (20 µM), muramyl dipeptide (MDP) (200 ng/ml), flagellin (10 µM) or poly(dA:dT) (1 µg/ml) was added for 30 min. The supernatant was subjected to further analysis. Inhibitors (KH7, H89 and baflomycin A1 from Cayman Chemical, USA; MG-132 from Selleck, USA; and 3-MA from Merck, Germany) or agonists (GPR43 agonist, Millipore, USA) were added to the culture medium 30 min before acetate pretreatment and after LPS priming.

**Cytokine ELISA, lactate dehydrogenase (LDH) assay, and cAMP measurement**

IL-1β, IL-6, and tumor necrosis factor (TNF)-α were analyzed using an ELISA kit purchased from Invitrogen (USA), and IL-18 was analyzed using a kit from R&D Systems (USA). LDH release assays were performed using a Cytotoxicity Detection Kit purchased from Roche Life Science (USA). cAMP measurement was performed by using a cAMP Parameter Assay Kit (R&D Systems, USA).

**Immunoblotting and immunoprecipitation**

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (Beyotime, China), and protein concentrations were determined using a BCA assay (Thermo, China). The total protein samples (approximately 20 µg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride (PVDF) membranes (Merck, Germany), and blocked with 5% nonfat dry milk in phosphate-buffered saline with Tween (PBST), pH 7.5. The membranes were immunoblotted with primary antibodies for 4 h or overnight at 4 °C and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. The anti-ASC, anti-NLRP3, anti-p62, anti-LC3B, anti-β-actin, anti-K63, anti-K48, anti-pro-IL-1β, anti-phospho-IKKβ, anti-IKKβ, anti-phospho-p65, and anti-p65 antibodies were purchased from Cell Signaling Technology (MA, USA). Other antibodies used in the present study were as follows: anti-pro-caspase-1 (Adiopogen, USA), anti-GPR43 (Absin, China), anti-GPR41 (Thermo Fisher, USA), anti-MARCH7 (Bioso, China), and anti-sAC (FabGennix,
USA). The HRP-conjugated secondary antibodies were all purchased from Cell Signaling Technology (USA). Protein bands were detected with an enhanced chemiluminescence kit (Pierce, USA).

For the immunoprecipitation assay, briefly, the cells were collected and lysed using a mortar and pestle in a buffer containing 20 mM PIPES, pH 6.8, 1% Triton X-100, 150 mM NaCl, 150 mM sucrose, 0.2% sodium deoxycholate, 500 μM EDTA and protease inhibitors for 5 min on ice. After centrifugation, the supernatants were diluted to 2 μg/ml in dilution buffer containing 20 mM PIPES, pH 6.8, 1% Triton X-100, 150 mM NaCl, 150 mM sucrose, 2.5 mM MgCl₂ and 2.5 mM MnCl₂. Primary antibody-conjugated protein A beads were incubated with the lysates for 2 h at 4 °C before washing with dilution buffer. The subsequent immunoblot analysis was conducted using the abovementioned methods. The chemicals were purchased from Sigma.

Flow cytometry
Peritoneal lavage cells from individual mice were prepared in PBS and stained with antibodies at 4 °C for 15 min. After washing three times, the cells were resuspended in washing buffer and analyzed. Fluorescence data for 10⁵ events from each sample were acquired on a FACS LSR II (BD Bioscience, USA) and analyzed using FlowJo software (Tomy Digital Biology Co., Ltd., Japan). The antibodies used in these experiments (neutrophils: anti-mouse-CD11b and anti-mouse-GR-1; macrophages: anti-mouse-F4/80) were purchased from eBioscience or Invitrogen (USA).

Short-interfering RNA (siRNA) interference
Mouse PMs or BMDMs were cultured in half of the final total culture volume in FBS-free RPMI 1640 or DMEM and transfected with 3 ng/ml siRNA (si-GPR43, si-GPR41, si-MARCH7, si-sAC1, si-sAC2, or si-Gq) or control siRNA (GenePharma, China) by INTERFEREin (Invitrogen, USA) for 6 h according to the manufacturer’s instructions. Six hours later, the other half of the complete culture medium was added, and the cells were cultured for a total of 48 h. After 48 h of interference, the cells were subjected to further stimulation or experiments. The sequences of each siRNA were as follows: si-GPR43: GCUGGUACCUACCAAGAUAUdTdT; si-GPR41: GCUU CUUUUUUGGCAAUUAUdTdT; si-MARCH7: GGACUU AUGUAGAAUUGUdTdT; si-sAC1: AAUGUAUGGG CUUCAUGGAdTdT; si-sAC2: TCGGAGCATGATT GAAATCGA; and si-Gq: AAAUGACACUUUGUAAGU CAAAAGGG.

Cytosolic calcium analysis
Macrophages were primed with 100 ng/ml LPS (E. coli O111:B4, Sigma, China) for 3 h. After two washes, the cells were incubated in HBSS containing 2 μM Fluo 4-AM (Life Technologies, USA) for 30 min in the dark. The cells were then washed, treated with or without acetate (20 mM) for 30 min, treated with 2-APB (50 μM) (Sigma-Aldrich, USA) for 15 min, resuspended in HBSS and added to black 96-well plates (Corning Costar, USA). After 10 min of incubation, the cells were stimulated with nigericin (20 μM) (Sigma-Aldrich, USA). A DMI6000 inverted fluorescence microscope (Leica, Germany) with an X-Cite® 200DC fluorescent light source (Lumen Dynamic, Canada) was used for fluorescence analysis. Fluorescence emission at 520 nm and excitation at 485 nm were used. ΔFluorescence was calculated as $F_{\text{max}}$ (fluorescence of the calcium-saturated indicator) minus $F_0$ (fluorescence in nonstimulated cells in HBSS).

Statistical analysis
Data were expressed as the mean ± SEM. Student’s t-test was conducted for comparisons between two groups, and one-way ANOVA was performed for comparisons among several groups. A P-value < 0.05 was considered to be statistically significant.

Results
Acetate attenuates NLRP3-mediated inflammasome activation in vitro and in vivo
To explore the anti-inflammatory effect of acetate on NLRP3 inflammasome activation, LPS-primed BMDMs were treated with acetate before nigericin stimulation. The concentrations of IL-1β and IL-18 in the supernatant were tested. We found that acetate suppressed the ATP- or nigericin-induced production of IL-1β and IL-18 in a dose-dependent manner (Fig. 1a–d). In addition, the attenuation of cytokine production could be observed when acetate was added after LPS and nigericin activation (Fig. 1e). The acetate treatment had little effect on LDH production (Supplemental Fig. 1), which suggests that the inhibitory effect was not due to damage to the cells.

We further performed comprehensive experiments investigating the role of acetate in suppressing inflammation and the inflammasome. We found that acetate suppressed IL-1β and IL-18 production in response to pretreatment, simultaneous treatment or post treatment with LPS and nigericin (Fig. 2a, b). Moreover, acetate decreased TNF-α and IL-6 production by LPS-primed BMDMs (Fig. 2c, d) and partly attenuated NF-κB signaling activation accordingly (Supplemental Fig. 2). In the further analysis of NLRP3 inflammasome activation in response to acetate pretreatment, we found that acetate could decrease the level of cleaved caspase-1 (p20) in the supernatant and cell lysate of BMDMs and NLRP3 in cell lysates (Fig. 2e), indicating the inhibitory effect of acetate on NLRP3 inflammasome activation.
We also determined that acetic acid, rather than hydrogen ions, exerts these inhibitory effects because sodium acetate had an inhibitory effect similar to that of acetate (Fig. 1f). These results indicated that the suppression mediated by acetate was independent of the treatment period, which suggests that acetate exerted its inhibitory effect on the inflammasome during the activation stage.

Different inflammasomes respond to different signals and trigger different responses. To determine whether acetate impacts other types of inflammasomes, we tested the impact of acetate on the Absent in melanoma 2...
(AIM2), NLR family CARD domain-containing protein 4 (NLRC4) and NACHT, LRR and PYD domain-containing protein 3 (NALP3) in inflammasomes after activation by poly(dA:dT), flagellin or MDP. Acetate showed no detectable inhibitory effect (NLRC4 and NALP3) or relatively limited attenuation (AIM2) on these inflammasomes (Fig. 2f, g). Taken together, our results show that acetate suppresses IL-1β production mediated by the NLRP3 inflammasome.

We further evaluated the anti-inflammatory effect of acetate in vivo. To adapt MSU- and alum-induced peritonitis, we pretreated mice with acetate intraperitoneally to investigate its anti-inflammatory effects. Peritoneal lavage fluid (PLF), peritoneal exudate cells (PECs) and serum were harvested for further assessments. As shown in Fig. 3 and Supplemental Fig. 3, compared with those of the control mice, IL-1β levels and the numbers of PECs, neutrophils and macrophages were all decreased in acetate-treated mice during MSU- or alum-induced peritonitis (Fig. 3a–h). Moreover, acetate also suppressed the secretion of IL-6 and TNF-α in serum and IL-1β in serum and PLF during LPS-induced peritonitis (Fig. 3i–l). Collectively, these results demonstrated that acetate could suppress inflammation in vivo and attenuate NLRP3-mediated inflammasome activation in vitro and in vivo.
Acetate-induced attenuation of the NLRP3 inflammasome is mediated by GPR43-Gq/11-Ca\(^{2+}\)

We next analyzed the possible mechanism by which acetate mediates inflammasome suppression. Acetate has been proven to be capable of activating short-chain fatty acid receptors, including GPR41 and GPR43\(^{15}\). Previous studies have reported the anti-inflammatory effects of GPR43/41 in several biological processes, and among short-chain fatty acids, acetate is one of the strongest agonists of GPR43\(^{15}\). Therefore, we hypothesized that acetate exerts its inhibitory effect by activating a short-chain fatty acid receptor. To validate this hypothesis, we knocked down Gpr43 or Gpr41 in BMDMs (Supplemental Fig. 4A, B) and treated the cells with acetate before nigericin stimulation. The results showed that the knockdown of Gpr43 reversed the effect of acetate on the inflammasome, while the knockdown of GPR41 had no effect (Fig. 4a). We further used an agonist of GPR43 in BMDMs to validate the suppression of IL-1\(\beta\) and IL-18 in the supernatant. We found that the GPR43 agonist had an inhibitory effect similar to that of acetate (Fig. 4b), suggesting that acetate inhibited the inflammasome via GPR43 signaling.

Then, we asked why GPR43, but not GPR41, was required because acetate has an affinity for both receptors. Previous studies discovered that GPR41 couples to the GI/O subunit while GPR43 couples to either the GI/O or Gq/11 subunit\(^{16}\). We hypothesized that different G-protein subunits may enable GPR43 to perform distinct biological functions. Therefore, we used pharmacological and genetic methods to determine which subunit was involved in the inhibitory effect of acetate on the NLRP3 inflammasome. We found that treating BMDMs with pertussis toxin (PTX), a GI/O subunit inhibitor, did not alter the inhibitory effect of acetate, whereas knockdown of the Gq/11 subunit (Supplemental Fig. 4C) abolished the suppression of IL-1\(\beta\) secretion, suggesting that the Gq/11 subunit is essential for the effect of acetate (Fig. 4c, d).

Previous research reported that the Gq/11 subunit could couple with phospholipase C (PLC)\(^{19,20}\). This interaction
Fig. 4 (See legend on next page.)
may enable GRP43 to engage phosphatidylinositol signals when bound to acetate and subsequently lead to the hydrolysis of phosphatidylinositol bisphosphate (PIP2) to inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG); these products activate protein kinase C (PKC) and InsP3R, increasing the concentration of intracellular calcium and eventually promoting NLRP3 inflammasome activation. Therefore, we determined whether sAC and tmAC inhibitor 5,6,28, reversed the inhibitory effect of acetate on IL-1β production (n = 3). b Antagonist levels measured by ELISA were decreased by acetate in lysate- and Nig-treated BMDMs (n = 3). n = biological replicates. Data are presented as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001, Student’s t-test compared with the control group.

Acetate induces NLRP3 inflammasome polyubiquitination and autophagy

The relationship between ubiquitination and GPCR signaling has been investigated previously, and growing evidence suggests that ubiquitination links GPCR signaling and inflammasome regulation. Thus, to determine the role of GPCR signaling-driven ubiquitination in our study, we assessed the ubiquitination level of the NLRP3 inflammasome in BMDMs after treatment with acetate. We found that acetate promoted polyubiquitination of the NLRP3 inflammasome, which contained a mix of K48 and K63 ubiquitin chains (Fig. 5d). In addition, we found that RNA interference of MARCH7 (Supplemental Fig. 4E), a reported E3 ligase that promotes NLRP3 ubiquitination, led to the reversal of the acetate-induced IL-1β reduction (Fig. 5e). These results indicate that NLRP3 inflammasome polyubiquitination could be an essential event for the acetate-mediated suppression of NLRP3 inflammasome activation.

We further investigated whether autophagy or the proteasome participated in ubiquitination and protein degradation of the NLRP3 inflammasome. The results showed that both 3-MA and bafilomycin A could abolish the preventive effect of acetate on IL-1β secretion, while MG-132 could not (Fig. 6a–c), suggesting that autophagy, rather than proteasome-mediated proteolysis, was involved in acetate-induced NLRP3 inflammasome inhibition. We further measured NLRP3 and ASC in LPS-primed macrophages treated with acetate. We found that...
acetate promoted NLRP3 degradation but did not affect
ASC, and this alteration was consistent with the changes
in LC3B and p62 (Fig. 6c, d). In addition, after treatment
with bafilomycin A1, which inhibits autophagy, we found
that acetate increased the accumulation of LC3B, which
indicates that acetate increased the formation of

Fig. 5 Acetate-induced suppression of the NLRP3 inflammasome occurs via the sAC-PKA axis and promotes polyubiquitination of NLRP3.

- ELISA of IL-1β indicated that acetate (20 mM) suppression in BMDMs was dependent on sAC. Inhibition of sAC (KH7) (a) or siRNA interference for two types of sAC (b) reversed the suppressive effects of acetate (n = 6). 
- ELISA of IL-1β indicated that the inhibition of PKA (H89) reversed the suppressive effects of acetate in BMDMs (n = 3). 
- Immunoprecipitation of NLRP3 polyubiquitination. The K48 and K63 ubiquitin chains were enhanced after acetate treatment (n = 3). 
- ELISA of IL-1β indicated that siRNA interference of MARCH7, an E3 ligase involved in NLRP3 ubiquitination, reversed the suppressive effects of acetate in BMDMs (n = 6). 

n = biological replicates. Data are presented as the mean ± SEM. *

\[ P < 0.05 \] and ** \[ P < 0.01 \], Student’s t-test compared with the control group.
Fig. 6 Acetate promotes NLRP3 degradation through autophagy. a–c ELISA of IL-1β indicated that acetate (25, 30, and 35 mM) mediated suppression of the NLRP3 inflammasome in a manner dependent on autophagy but not the proteasome. Inhibition of autophagy (3-MA and bafilomycin A1) reversed the suppression of IL-1β (a, b), whereas inhibition of the proteasome induced few alterations (c) in BMDMs (n = 3).

c, d Western blot of NLRP3, ASC, p62, and LC3B. The degradation of NLRP3 was accompanied by increased NLRP3 expression and decreased p62 expression in BMDMs (n = 3). n = biological replicates. Data are presented as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001, Student’s t-test compared with the control group (a–c) or one-way ANOVA for comparisons of differences (d).
autophagosomes in a dose-dependent manner (Fig. 7a, b). These results suggest that acetate promotes degradation of the NLRP3 inflammasome by ubiquitination and subsequent autophagy.

**Discussion**

Free fatty acids (FFAs), a diverse class of aliphatic hydrocarbon chains with a carboxyl group at one end, have been reported to participate in numerous biological processes, including immune regulation. Different FFAs play distinct roles; for example, palmitate promotes IL-1β and IL-18 secretion in a dose-dependent manner, whereas omega-3 fatty acids can inhibit the NLRP3 inflammasome via GPR120 and GPR40. Very recently, the role of acetate in type 2 diabetes, heart failure and tumors has also been highlighted. Acetate is present at a relatively low concentration in the blood, but under specific conditions, acetate concentrations can increase to millimolar levels and exert a number of effects. In vitro studies focusing on the effects of acetate at the cellular and molecular levels have generally applied a 10–20 mM concentration to mimic the effects of acetate in the microenvironment. In the present study, we found that the anti-inflammatory effects of acetate were dose-dependent with no significant cytotoxicity, which is consistent with the results of previous research.

Acetate shows a similar affinity for GPR41 and GPR43. The present study showed that the acetate-mediated suppression of NLRP3 inflammasome activation relied on GPR43. We believe this effect may be due to the coupling GPRs to different signaling subunits. GPR43 exhibits dual coupling to the Gq/11 subunit but not to the Gi subunit. Specific activation of GPR43 subsequently influences cAMP and Ca2+, and thus activates downstream signaling, and the Gq/11 subunit is capable of activating PLC and IP3 to activate downstream signaling pathways. Consistent with these conclusions, our results proved that acetate could decrease Ca2+ levels after stimulation and that this process required IP3R participation, as the blockade of IP3R reversed the Ca2+ decrease after acetate treatment. Moreover, although acetate decreased Ca2+ levels, the signal still required Ca2+ participation because the chelation of calcium also abolished the acetate-mediated attenuation of IL-1β. These results indicate that Gq/11-Ca2+ signaling is a possible mechanism for the acetate-mediated attenuation of the NLRP3 inflammasome and highlight the dual role of Ca2+ in signal transduction.

However, studies of calcium-sensing receptor (CaSR) found that increased Ca2+, accompanied by decreased cAMP, could further promote NLRP3 inflammasome activation, which is inconsistent with the present finding. To address this issue, we focused on the crucial role of cAMP in inflammasome regulation. In recent years, several works found that cAMP could serve as a brake for the NLRP3 inflammasome. Dopamine and bile acid suppress the NLRP3 inflammasome via DR1 signaling or TGR5 signaling and consequently enhance the production of cAMP. The enhanced cAMP can further bind to NLRP3 directly to induce the ubiquitination of NLRP3 through PKA and thus suppress inflammasome activation. Thus, the balance of Ca2+ and cAMP signaling determines the exact outcome of inflammasome activation. CAMP is generated by two types of adenylyl cyclases that are present in different cellular compartments: the transmembrane adenylyl cyclase (tmAC) within the plasmalemma, and the soluble adenylyl cyclase (sAC) in the cytosol and within distinct organelles. Unlike tmAC, sAC can be activated by Ca2+, generates cAMP and may mediate NLRP3 inflammasome attenuation. Indeed, although cAMP concentrations remain relatively low during acetate treatment, we still found an essential role for sAC in the acetate-mediated attenuation of IL-1β production, as both the KH7 inhibitor and siRNA interference of sAC reversed acetate-induced IL-1β attenuation. Hence, the present finding may raise the question of how the cell coordinates the convergence between two different sources of cAMP in regulating the ubiquitination of the NLRP3 inflammasome. Previous studies focused on the regulatory effect of cAMP produced by tmAC on NLRP3 degradation, but few studies have focused on cAMP synthesized by sAC. In fact, the cAMP signal generated by tmAC is...
thought to be confined to defined compartments by PDE activity, which makes it difficult for the cAMP signal to be transmitted throughout the cytosol. Considering the location of the NLRP3 receptor, sAC may take advantage of its location in interacting with the NLRP3 inflammasome. Thus, the present study indicates that compartmentalized cAMP, rather than total cAMP in the cytoplasm, may serve as a crucial factor in acetate-mediated attenuation of the NLRP3 inflammasome, supplementing the current theory of Ca^{2+}/cAMP-mediated NLRP3 inflammasome attenuation.

Post translational modification has emerged as an endogenous regulatory mechanism of the inflammasome, and several investigations have reported that ubiquitination acts as a negative regulator of NLRP3 inflammasome activation in a cAMP-PKA-dependent manner. In this study, a similar pathway of PKA was also proven to be essential in acetate-induced ubiquitination of the NLRP3 inflammasome, and acetate subsequently induced inflammasome degradation through autophagy, rather than the proteasome. Thus, the present study suggests that acetate may bind to GPR43 and signal through sAC-PKA to attenuate the inflammasome.

Our data also showed that acetate plays a protective role in vivo. A previous study found that suppressing the NLRP3 inflammasome by blocking PKM2-dependent glycolysis protects mice from endotoxemia and polymicrobial sepsis. This protective effect of acetate may be consistent. On the one hand, NLRP3 is reported to be involved in endotoxemia and acetate attenuates NLRP3 activation. On the other hand, our data also suggest that acetate somewhat attenuates LPS-induced NF-κB signaling activation (Fig. S2). Thus, by showing the protective effects of acetate in LPS-induced endotoxemia and the NLRP3 inflammasome-dependent peritonitis model, the present study may serve as the basis for more extensive research on acetate in sepsis and other inflammatory diseases.

In summary, in the present study, we demonstrate that acetate and GPR43 signaling attenuate NLRP3 inflammasome activation, and we report a novel mechanism of inflammasome regulation by metabolites. Additionally, our results reveal a critical role for sAC in NLRP3 inflammasome activation, which supplements the present mechanism of NLRP3 inflammasome regulation. The regulatory effect of acetate on inflammation provides an example of the mechanisms by which metabolites regulate immune responses and may offer a new therapeutic strategy for the clinical management of inflammatory diseases.

Acknowledgements

This research was funded by the National Natural Science Foundation of China (81772105 to M.D., 31570886 and 81771697 to Y.Z., 81875179 and 81671939 to J.J.B., 81600948 to Y.P.Z., and 81671887 to L.L.B.), the Shanghai Young Outstanding Youth Medical Professionals Training Program (No. 2017YQ015 to L.L.B.) and the Scientific Research Foundation of Shanghai Municipal Commission of Health and Family Planning (201740050 to N.L.). We would like to thank Mr. Jun Wang and Mrs. Kangqing Fang for technical support and kind help during the experiments. We appreciate the careful animal care work of Mrs. Guijing Ji.

Authors’ contributions

M.D. performed most of the experiments. Z.Y.J. performed some of the experiments and prepared the manuscript. C.L.W. and N.L. offered technical support and helped with some experiments. L.L.B., Y.P.Z. and J.J.B. provided constructive suggestions and discussions during the study. Y.Z. and X.M.D. designed the entire study and secured funding for the research.

Author details

1Faculty of Anesthesiology, Changhai Hospital, Second Military Medical University, 200433 Shanghai, China. 2Department of Anesthesiology, Wuhan General Hospital, PLA, 430070 Wuhan, Hubei Province, China.

Conflict of interest

The authors declare that they have no conflict of interest.

Publisher’s note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information

accompanies this paper at https://doi.org/10.1038/s41227-019-0276-5.

Received: 6 October 2018 Revised: 16 January 2019 Accepted: 11 February 2019

Published online: 23 July 2019

References

1. Martino, F., Burns, K. & Tschopp, J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of pro–beta. Nat. Cell. Biol. 10, 417–426 (2008).
2. Wen, H., Miao, E. A. & Ting, J. P. Mechanisms of NOD-like receptor-associated inflammasome activation. Immunity 39, 432–441 (2013).
3. Guarda, G. et al. Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity 34, 213–223 (2011).
4. Thorburn, A. N., Macia, L. & Mackay, C. R. Diet, metabolites, and “western-lifestyle” inflammatory diseases. Immunity 40, 833–842 (2014).
5. Guo, C. et al. Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. Immunity 45, 802–816 (2016).
6. Yan, Y. et al. Dopamine controls systemic inflammation through inhibition of NLRP3 inflammasome. Cell 160, 62–73 (2015).
7. Wen, H. et al. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat. Immunol. 12, 408–415 (2011).
8. Lee, G. S. et al. The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca^{2+} and cAMP. Nature 492, 123–127 (2012).
9. Gao, X. et al. Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. Nat. Commun. 7, 11960 (2016).
10. Perry, R. J. et al. Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. Nature 534, 213–217 (2016).
11. Bolduc, J. F., Hany, L., Barat, C., Ouellet, M. & Tremblay, M. J. Epigenetic metabolite acetate inhibits class I/II histone deacetylases, promotes histone acetylation, and increases HIF-1 integration in CD4(+) T cells. J. Virol. 91, pii: e01943–16 (2017).
12. Marques, F. Z. et al. High-fiber diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in hypertensive mice. Circulation 135, 964–977 (2017).
13. Macia, L. et al. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fiber-induced gut homeostasis through regulation of the inflammasome. Nat. Commun. 6, 6373 (2015).
28. Bitterman, J. L., Ramos-Espiritu, L., Diaz, A., Levin, L. R. & Buck, J. Pharmacological distinction between soluble and transmembrane adenylyl cyclases. J. Pharm. Exp. Ther. 347, 589–598 (2013).

29. Wojcikiewicz, R. J. Regulated ubiquitination of proteins in GPCR-initiated signaling pathways. Trends Pharmac. Sci. 25, 35–41 (2004).

30. Alvarez-Curto, E. & Milligan, G. Metabolism meets immunity: the role of free fatty acid receptors in the immune system. Biochem. Pharmacol. 114, 3–13 (2016).

31. Yan, Y. et al. Omega-3 fatty acids prevent inflammation and metabolic disorder through inhibition of NLRP3 inflammasome activation. Immunity 38, 1154–1163 (2013).

32. Wölin, M. J. & Miller, T. L. Interactions of microbial populations in cellulose fermentation. Fed. Proc. 42, 109–113 (1983).

33. Qian, J. et al. Agonist-induced activation of human FFA1 receptor signals to extracellular signal-regulated kinase 1 and 2 through Gq- and Gi-coupled signaling cascades. Cell Mol. Biol. Lett. 22, 13 (2017).

34. Zulfiker, A. H., Hashimi, S. M., Good, D. A., Giroi, I. D. & Wei, M. Q. Cane toad skin extract-induced upregulation and increased interaction of serotonin 2A and D2 receptors via Gq/11 signaling pathway in CLU213 cells. J. Cell Biochem. 118, 979–993 (2017).

35. Hong, T. Calcium signaling and mitochondrial destabilization in the triggering of the NLRP3 inflammasome. Trends Immunol. 35, 253–261 (2014).

36. Brosseau, M., Levin, L. R. & Buck, J. Intracellular cAMP signaling by soluble adenylyl cyclase. Kidney Int. 79, 1277–1288 (2011).

37. Schmid, A., Meili, D. & Salathe, M. Soluble adenylyl cyclase in health and disease. Biochim. Biophys. Acta 1842, 2584–2592 (2014).

38. Valsecchi, F., Konrad, C. & Manfredi, G. Role of soluble adenylyl cyclase in mitochondria. Biochim. Biophys. Acta 1842, 2555–2560 (2014).

39. Laidov, Y. & Appukuttan, A. Role of soluble adenylyl cyclase in cell death and growth. Biochim. Biophys. Acta 1842, 2646–2655 (2014).

40. Zippin, J. H. et al. CO2/HCO3– and calcium-regulated soluble adenylyl cyclase as a physiological ATP sensor. J. Biol. Chem. 288, 33283–33291 (2013).

41. Han, S. et al. Lipopolysaccharide primes the NALP3 inflammasome by inhibiting its ubiquitination and degradation mediated by the SCFFBXL2 E3 ligase. J. Biol. Chem. 290, 1181–11813 (2015).

42. Yang, J., Liu, Z. & Xiao, T. S. Post-translational regulation of inflammasomes. Cell Mol. Immunol. 14, 65–79 (2017).

43. Xie, M. et al. PKM2-dependent glycolysis promotes NLRP3 and AIM2 inflammasome activation. Nat. Commun. 7, 13280 (2016).

44. Lammelier, M. et al. Inflammasome-dependent release of thealarmin HMGGB1 in endotoxemia. J. Immunol. 185, 4388–4392 (2010).