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Review

Mitochondrial ACOD1/IRG1 in infection and sterile inflammation☆

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ABSTRACT

Immunometabolism is a dynamic process involving the interplay of metabolism and immune response in health and diseases. Increasing evidence suggests that impaired immunometabolism contributes to infectious and inflammatory diseases. In particular, the mitochondrial enzyme aconitate decarboxylase 1 (ACOD1, best known as immunoresponsive gene 1 [IRG1]) is upregulated under various inflammatory conditions and serves as a pivotal regulator of immunometabolism involved in itaconate production, macrophage polarization, inflammasome activation, and oxidative stress. Consequently, the activation of the ACOD1 pathway is implicated in regulating the pathogenic process of sepsis and septic shock, which are part of a clinical syndrome of life-threatening organ failure caused by a dysregulated host response to pathogen infection. In this review, we discuss the latest research advances in ACOD1 expression and function, with particular attention to how the ACOD1-itaconate pathway affects infection and sterile inflammation diseases. These new insights may give us a deeper understanding of the role of immunometabolism in innate immunity.

Introduction

The innate immune system is comprised of different components, including various immune cells (e.g., macrophages, monocytes, dendritic cells [DCs], and neutrophils), which can rapidly recognize invading pathogens through pattern recognition receptors (PRRs). The innate immune response is not only the first line of defense against invading pathogens, it also triggers an inflammatory response through the production of various immune mediators, especially cytokines.[1–3] A cytokine storm is a pathological hallmark of various critical illnesses caused by sepsis and septic shock.[4–7] For example, the current coronavirus disease 2019 (COVID-19), caused by the virus designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is associated with an excessive activation of innate immune response and a cytokine storm.[8,9]. Therefore, understanding the mechanism and modulation of inflammatory responses during infection is important for the prevention and treatment of critical diseases.

Under inflammatory conditions, immune cell activation is characterized by significant metabolic changes, including altered cell-intrinsic metabolite levels, increased aerobic glycolysis, a remodeled tricarboxylic acid (TCA) cycle, and impaired mitochondria respiration.[10–15] These metabolic reprogramming processes are mediated by the upregulation of specific enzymes, which usually have low expression or activity under normal conditions.[10,16] The production of metabolites in the process of metabolic reprogramming can affect the function of immune cells through different mechanisms, such as signal transduction, protein activity modification, and gene expression regulation.[17–19] Excessive cytokine production further aggravates the reprogramming of the metabolic pathways of immune cells. The study of immunometabolism, namely the interaction between metabolism and immune response, has recently been greatly expanded, leading to new therapeutic targets for translational medicine.

Aconitate decarboxylase 1 (ACOD1, also known as immunoresponsive gene 1 [IRG1]) is a key regulator of immunometabolism during infection and inflammation.[20–22] It was first discovered in 1992 as a lipopolysaccharide (LPS)-inducible gene in murine macrophages.[23] Later, metabolomics profiling analyses revealed that ACOD1 is responsible for ita-

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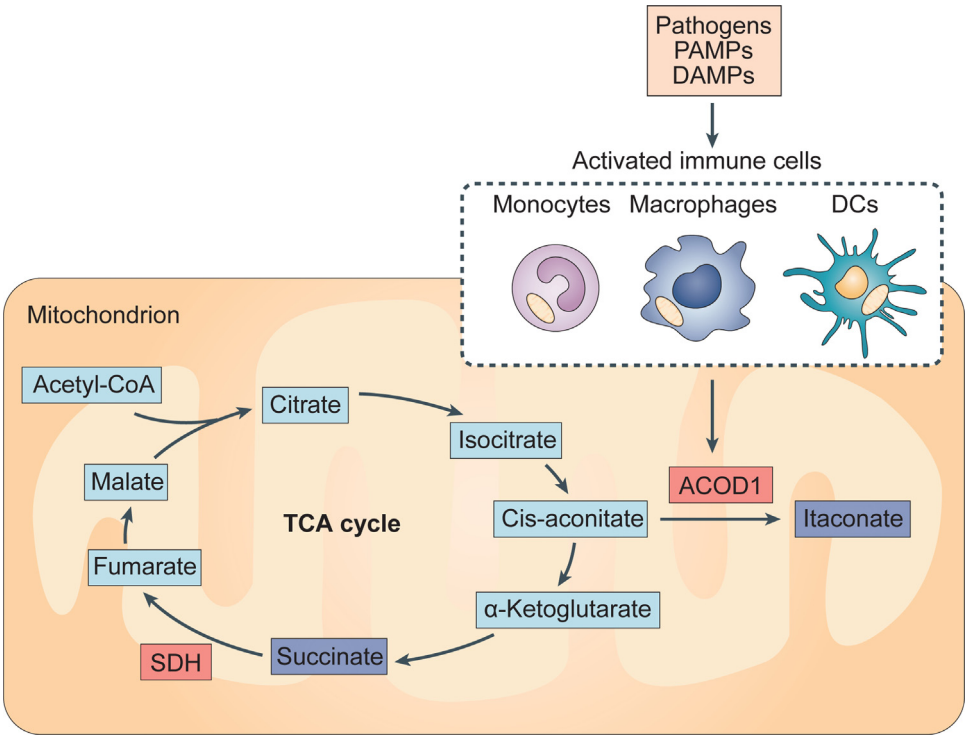

Figure 1. Metabolism of itaconate. Multiple stimuli, such as live pathogens, PAMPs, and DAMPs, trigger the activation of innate immune cells and induce the expression of ACOD1 in mitochondria. Activated immune cells (such as monocytes, macrophages, and DCs) undergo metabolic reprogramming and alter the TCA cycle to produce high levels of itaconic acid through ACOD1.
ACOD1: Aconitate decarboxylase 1; DAMPs: Damage-associated molecular patterns; DCs: Dendritic cells; PAMPs: Pathogen-associated molecular patterns; SDH: Succinate dehydrogenase; TCA: Tricarboxylic acid.

conate production in macrophages during an inflammatory response[24] [Figure 1]. Accumulated evidence in different disease models shows that ACOD1-dependent itaconate production can either promote or inhibit inflammation. In this review, we not only explain the expression and function of ACOD1 in innate immunity but also discuss the potential of manipulating ACOD1 as a treatment for infectious and inflammatory diseases.

The Structure and Localization of ACOD1

Human ACOD1 contains 481 amino acids and is highly conserved across species, including in chimpanzees, rats, mice, cattle, dogs, chickens, zebrafish, *Xenopus* species (frogs), and mussels.[25–28] Crystal structure analysis showed that human ACOD1 is a homodimer, and eight active site residues (Asp93, Thr97, His103, His159, Lys207, Lys272, His277, and Tyr318) are required for its catalytic activity.[26] In *Bacillus subtilis, cis*-aconitic acid decarboxylase activity requires His102 (a residue of His103 in humans) instead of His277 and Tyr318, indicating that certain protein structures of ACOD1 are not conserved.[29] Cell-staining studies have shown the co-localization of ectopically overexpressed ACOD1 and MitoTracker, revealing that ACOD1 is a mitochondrial protein.[21,30] However, the mitochondrial localization signal or mitochondrial targeting sequence of ACOD1 remains unknown.

The Expression and Upregulation of ACOD1

The expression of ACOD1 is cell- and tissue-specific. Under normal conditions, the expression of ACOD1 is very low. Under stress conditions, especially inflammatory stimulation, the expression of ACOD1 is upregulated by macrophages, monocytes, and DCs in the innate immunity system. Pathogen-associated molecular patterns (PAMPs), such as LPS, lipoteichoic acid, poly I:C, and CpG-DNA, are structural components or products of microorganisms, which can strongly upregulate ACOD1 expression by combining different PRRs in macrophages. In addition, live pathogens (e.g., bacteria, viruses, fungi, protozoa, spirochetes, and *Chlamydia*), cytokines (e.g., interferon beta 1 [IFNB1], interferon-gamma [IFNG], tumor necrosis factor [TNF], and interleukin-1β [IL1B]), and small molecule drugs (e.g., cycloheximide, carbon monoxide releasing molecule-2, cobalt protoporphyrin IX, or chemical inducer of heme oxygenase-1 [HMOX1]) can stimulate the expression of ACOD1 in immune cells in a context-dependent manner.[22,31,32] These findings make inducible ACOD1 a biomarker of an activated innate immune response.

In addition to immune cells, the expression of ACOD1 is also upregulated in the tissue under infection, such as nervous tissue infected by West Nile virus (WNV) or Zika virus (ZIKV),[33] lung tissues infected by influenza A virus (IAV)[34] or respiratory syncytial virus (RSV) or *Chlamydia pneumoniae*,[35,36] splenic tissues infected by *Leishmania donovani*,[37] and pouch membrane tissues exposed to monosodium urate (MSU).[38] Overall, these findings highlight that the upregulation of ACOD1 may be a universal genetic event in infection and inflammation.

Although the precise mechanism of ACOD1 upregulation is poorly understood, the activation of several immune-related transcription factors contributes to ACOD1 expression.[39,40] The upregulated ACOD1 also acts as a feedback mechanism to regulate the activation of transcription factors. For example, lipid A induces *Acod1* mRNA upregulation by activating

two transcription factors, namely nuclear factor kappa B subunit 1 (NFKB1) and interferon regulatory factor 3 (IRF3),[41] while the increased expression of ACOD1 inhibits NFKB1 and IRF3, leading to LPS tolerance.[42] This negative feedback mechanism between ACOD1 and NFKB1 can be mediated by the deubiquitinase TNF alpha-induced protein 3 (TNFAIP3, also known as A20), which inhibits NFKB1 activation and subsequent ACOD1 expression in myeloid cells in response to LPS, TNF, or carbon monoxide (CO).[31,42–44] Increased ACOD1 expression limits NFKB1 activation by sustaining the expression of TNFAIP3. In addition, ACOD1-mediated itaconate production leads to the expression of activating transcription factor 3 (ATF3), thereby inhibiting the translation of the NFKB inhibitor zeta (NFKBIZ) and subsequent interleukin-6 (IL6) expression.[45] Unlike NFKB, the transcriptional factor signal transducer and activator of transcription 1 (STAT1), STAT3, or CCAAT enhancer-binding protein beta (CEBPB) contributes to ACOD1 upregulation in activated macrophages caused by infection with *Mycobacterium tuberculosis* (*Mtb*) or *Salmonella*,[30,46] but it is unclear whether ACOD1 affects the activity of these transcriptional factors. In conclusion, the mechanism of inducible ACOD1 upregulation involves multiple transcription factors, which produce complex regulatory feedback for regulating the immune response.

The Immunological Activities of ACOD1

ACOD1 is identified as the enzyme that catalyzes the production of itaconate through the decarboxylation of *cis*-aconitate[24] [Figure 1]. ACOD1-dependent itaconate production is increased in activated macrophages *in vitro* and *in vivo*, which contributes to a broad range of immunological activities.[24,47,48] In addition to limiting inflammation, the activation of the ACOD1 pathway also promotes an inflammation response. Moreover, the reciprocal regulation between ACOD1 and other inflammatory mediators has been found to shape the immune response. Along with the feedback effect between ACOD1 and pro-inflammatory transcription factors (e.g., NFKB1) discussed earlier, we will now review the dual role of ACOD1 in immune regulation.

Metabolic rewiring

Succinate dehydrogenase (SDH) is the enzyme involved in the citric acid cycle and electron transport chain.[49] Itaconate acts as an endogenous inhibitor of SDH [Figure 2]. Due to the structural similarity of itaconate to succinate, itaconate competitively limits the activity of SDH during inflammation.[50] For example, ACOD1-dependent itaconate production can impair SDH activity, reduce succinate oxidation, and promote succinate accumulation in LPS-activated macrophages, finally resulting in a decrease in the oxygen consumption rate and inhibiting the production of mitochondrial reactive oxygen species (mROS).[48,51,52] In contrast, the loss of itaconate production in *Acod1*-deficient macrophages decreases the level of succinate, leading to increased expression of hypoxia-inducible factor 1 subunit alpha (HIF1A), IL1B, and other inflammatory cytokines in response to LPS.[51] ACOD1 and itaconate also restrict ZIKV replication in mouse neurons by inhibiting SDH activity, supporting that itaconate exerts an anti-inflammatory effect in a succinate-dependent manner.[33]

Of note, ACOD1-dependent itaconate production drives the immune paralysis of human monocytes, and β-glucan (a fungal cell wall component) reverses this effect by reducing ACOD1 expression and restoring SDH expression.[53] Accumulated succinate caused by the ACOD1-itaconate pathway favors the metabolic adaptation of bacteria *Pseudomonas aeruginosa* in tissue-resident macrophages and epithelial cells from infected mice.[54] These findings indicate that an appropriate level of itaconate is important for initiating an immune response.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is an enzyme involved in breaking down glucose to obtain energy through glycolysis.[55] The 4-octyl itaconate (4-OI)-induced alkylation of cysteine residue (Cys) 22 on the protein GAPDH inhibits its enzymatic activity, thereby downregulating aerobic glycolysis and subsequent production of IL1B, NOS2, and TNF in activated macrophages.[56] The anti-inflammatory effect of itaconate can be counteracted by over-expressing wild-type GAPDH, rather than cys-22 mutant GAPDH.[56] Moreover, the activity of GAPDH-mediated glycolysis is enhanced in *Acod1*-deficient macrophages following LPS exposure.[56] These findings indicate that itaconate inhibits glycolysis through the alkylation of GAPDH.

Itaconate also regulates other metabolic rewiring pathways, including prenylation, mitochondria substrate-level phosphorylation, and the glyoxylate shunt. Prenylation, a modification of proteins by isoprenoid lipids, aids viruses in anchoring to cell membranes.[57] ACOD1 induced by the vesicular stomatitis virus (VSV) and itaconate upregulation enhance the synthesis of geranylgeranyl diphosphate and the prenylation processes that follow, facilitating the entry and replication of viruses in macrophages.[58] The inhibitory effect on viral infection caused by *Acod1* deficiency can be abrogated by the addition of 4-OI or dimethyl itaconate (DMI) in murine bone marrow-derived macrophages (BMDMs).[58] Mitochondrial substrate-level phosphorylation is a process by which adenosine triphosphate (ATP) is generated in the absence of oxidative phosphorylation.[59] The inhibition of *Acod1* by siRNA reverses LPS-induced mitochondrial substrate-level phosphorylation impairment, showing an elevated oxygen consumption rate. Macrophages treated with itaconate or overexpressed with *Acod1* lose the capacity of mitochondrial substrate-level phosphorylation to mount an immune defense, although this observation requires more evidence from *in vivo* research.[60] Despite affecting the metabolic pathway in host immune cells, itaconate also inhibits the activity of isocitrate lyase, a critical enzyme of glyoxylate shunt in *Mtb*, thereby exhibiting an antimicrobial effect by limiting bacterial growth.[24] Overall, the contribution of itaconate to inflammation-related metabolic rewiring is context-dependent.

Oxidative stress

Reactive oxygen species (ROS) plays a dual role in inflammation regulation and pathogen clearance.[61,62] LPS or IFNB1 triggers ACOD1-dependent ROS production and activates STAT1 and STAT3, thereby promoting the expression of transporter 1 ATP binding cassette subfamily B (TAP1) and proteasome 20S subunit beta 9 (PSMB9), which are transporters involved in antigen processing.[63] Similarly, increased ACOD1 in LPS-treated or LPS-tolerized macrophages promotes ROS-mediated TNFAIP3

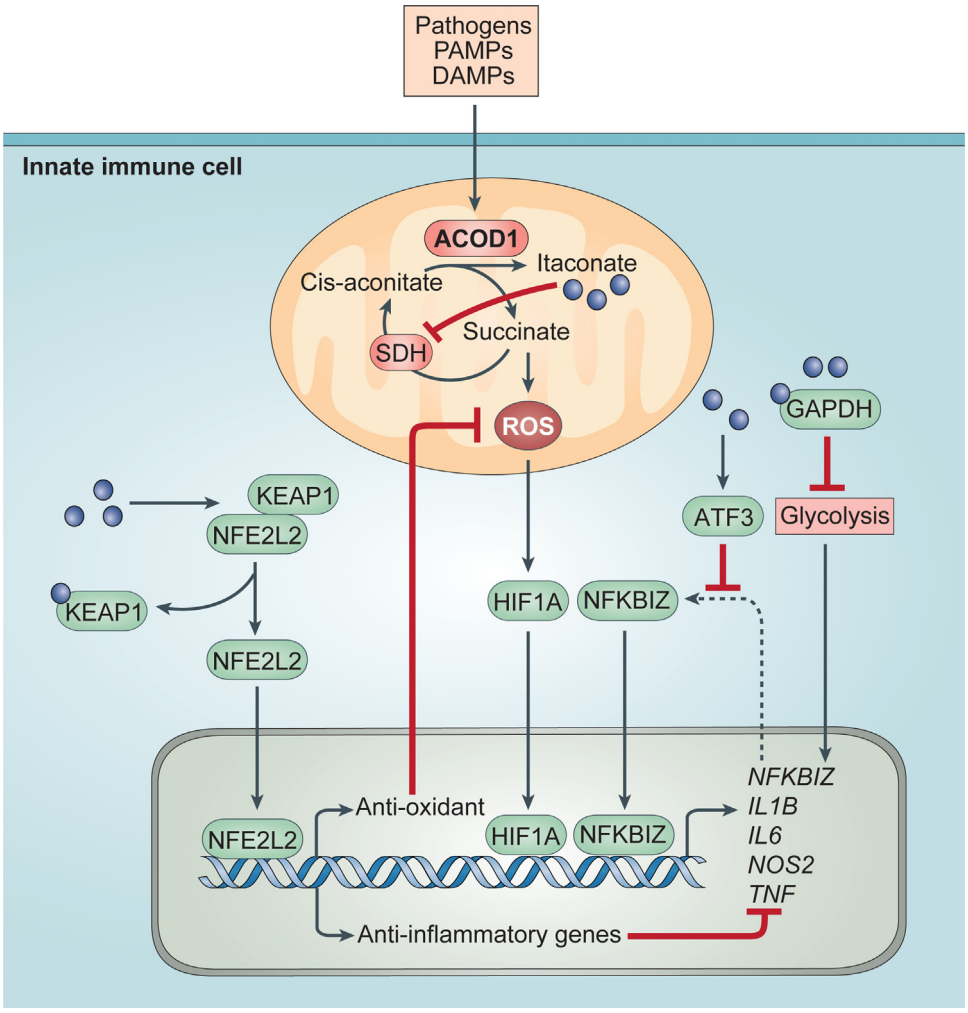

Figure 2. Role of ACOD1-dependent itaconate production in signal transduction. ACOD1-mediated itaconate production impairs SDH activity, decreases succinate oxidation, and promotes succinate accumulation, thereby suppressing the inflammatory processes mediated by succinate, such as ROS production, HIF1A activity, and IL1B expression. Itaconate alkylates the Cyss on KEPA1 or GAPDH, leading to increased NFE2L2 activation or inhibited glycolysis, thereby limiting the expression of inflammatory genes (e.g., IL1B, IL6, TNF, and NOS2) or ROS production. In addition, itaconate-mediated ATF3 expression inhibits the NFKBIZ pathway.
ACOD1: Aconitate decarboxylase 1; ATF3: Activating transcription factor 3; Cys: Cysteine residue; GAPDH: Glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase; HIF1A: Hypoxia-inducible factor 1 subunit alpha; IL6: Interleukin 6; IL1B: Interleukin-1β; KEAP1: Kelch-like ECH-associated protein 1; NFE2L2: Nuclear factor erythroid 2-like 2; NFKBIZ: NFKB inhibitor zeta; NOS2: Nitric oxide synthase 2; SDH: Succinate dehydrogenase; ROS: Reactive oxygen species; TNF: Tumor necrosis factor.

transcription.[42,43] As a negative regulator of NFKB1 signaling, the upregulation of TNFAIP3 induced by ACOD1 inhibits the activation of NFKB1 and IRF3, thereby reducing the release of IL1B, IL6, TNF, and IFNB1.[42,43] In *Acod1*-overexpressing macrophages, ROS scavengers abrogate the upregulation of TAP1, PSMB9, and TNFAIP3, indicating that ROS plays an indispensable role in ACOD1-driven inflammatory gene expression[42,63] [Figure 3]. Consistent with the view that ACOD1-mediated ROS production promotes inflammation, ACOD1 favors ROS-dependent lung inflammation and lung tissue damage during RSV infection.[35] Nonetheless, whether ACOD1-mediated ROS production depends on itaconate production remains to be investigated.

Immune cells also use the production of ROS to fight pathogens. When exposed to LPS, elevated ACOD1 enhances the production of ROS in the mitochondria by promoting the utilization of fatty acids during oxidative phosphorylation.[30] The bactericidal activity of ROS is reduced after the depletion of *Acod1* in a zebrafish infection model.[30] Thus, ACOD1-mediated ROS production plays dual roles in modulating the immune response.

Nuclear factor erythroid 2-like 2 (NFE2L2, also known as NRF2) is the master transcription factor that activates antioxidant genes to prevent oxidative damage during inflammation.[64] Under normal circumstances, NFE2L2 is mainly degraded by a proteasome pathway mediated by kelch-like ECH-associated protein 1 (KEAP1). In contrast, cell-permeable itaconate derivatives 4-OI and DMI can inhibit the activity of KEAP1 by alkylating the cysteine residues (cys-151, cys-257, cys-288, cys-273, and cys-297) on KEAP1, thereby increasing the stability of the NFE2L2 protein. This process does not rely on itaconate-mediated SDH inhibition.[65] Consequently, ACOD1-dependent itaconate production and subsequent activation of NFE2L2 limit the production of ROS and inflammatory cytokines in LPS models[45,64,65] and in sterile inflammation

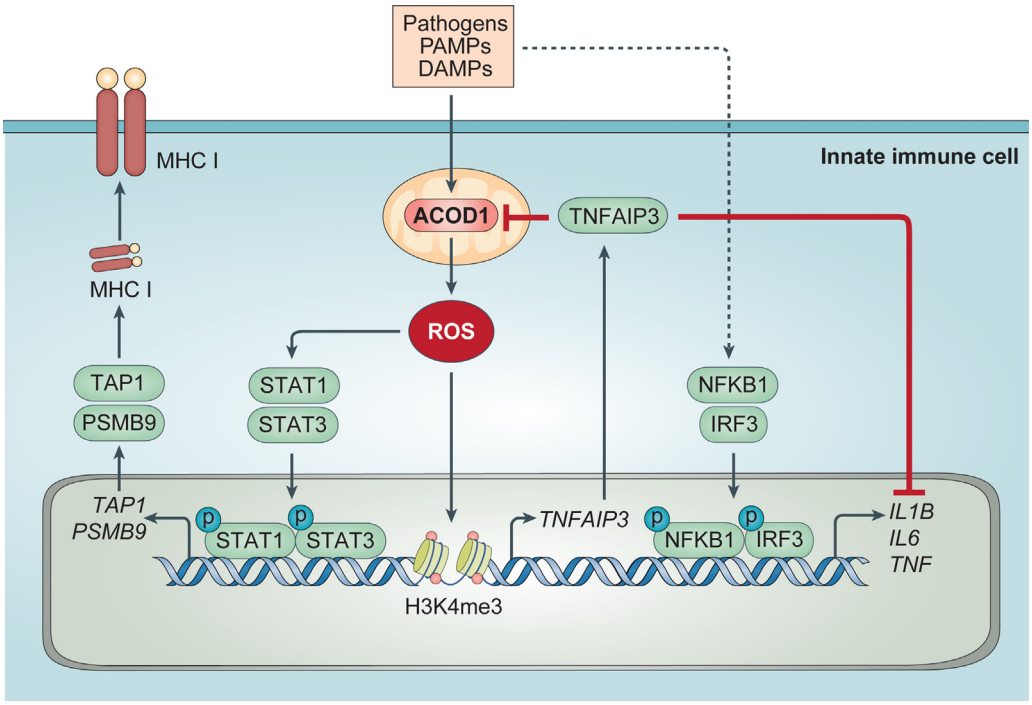

Figure 3. Role of ACOD1-dependent ROS production in signal transduction. ACOD1-dependent ROS production activates STAT1 and STAT3, thereby increasing the expression of TAP1 and PSMB9, which are transporter proteins for promoting the presentation of MHC-I on the cell surface. ACOD1-dependent ROS production also promotes the expression of TNFAIP3 by increasing the methylation of H3K4. TNFAIP3 acts as a negative regulator of NFKB1 signaling to inhibit NFKB1 and IRF3 activation, subsequently reducing the release of IL1B, IL6, TNF, and IFNB1. Increased TNFAIP3 inhibits ACOD1 transcription.
ACOD1: Aconitate decarboxylase 1; ATP: Adenosine triphosphate; H3K4me3: H3K4 methylation; IFNB1: Interferon beta 1; IL6: Interleukin 6; IL1B: Interleukin-1β; IRF3: Interferon regulatory factor 3; MHC-I: Class I major histocompatibility complex; NFKB1: Nuclear factor-kappa B subunit 1; PSMB9: Proteasome 20S subunit beta 9; ROS: Reactive oxygen species; STAT1: Signal transducer and activator of transcription 1; STAT3: Signal transducer and activator of transcription 3; TAP1: Transporter 1 ATP binding cassette subfamily B; TNF: Tumor necrosis factor; TNFAIP3: TNF alpha-induced protein 3.

models, such as those for acute kidney injury, liver ischemia–reperfusion injury, and abdominal aortic aneurysms.[66–68] However, it is possible that the activation of the NFE2L2 pathway is due to the stress response caused by ACOD1-mediated ROS production.

Macrophage polarization

Depending on the stimuli, macrophages are extremely plastic cells that can have two distinct functional phenotypes via classical M1 activation or alternative M2 activation.[69] The M1-like phenotype is characterized by an increased pro-inflammatory state, while M2-type macrophages have an anti-inflammatory phenotype.[69] The dysregulated state of macrophage M1–M2 polarization leads to inflammatory conditions or diseases by changing the production profile of pro-inflammatory or anti-inflammatory cytokines.[70] Metabolic reprogramming, including itaconate production, plays a significant role in the modulation of macrophage polarization. One study showed that under normal and hypoxic conditions, the overexpression of *Acod1* in macrophages leads to the upregulation of the M1 marker nitric oxide synthase 2 (NOS2),[71] highlighting a molecular link between hypoxia and inflammation mediated by ACOD1. In contrast, the suppression of *Acod1* in mouse macrophages reduces itaconate production, resulting in an increased expression of the M2 marker arginase 1 (ARG1).[72] These findings demonstrate the potential role of the activation of the ACOD1–itaconate axis in maintaining inflammation by M1 polarization.

Inflammasome inhibition

Inflammasomes are intracellular multiprotein complexes that respond to various PAMPs and damage-associated molecular patterns (DAMPs).[73,74] Activated inflammasomes trigger the cleavage of pro-caspase-1 (pro-CASP1) or pro-caspase-11 (pro-CASP11) into active CASP1 or CASP11, thereby leading to maturation and secretion of interleukin 1 cytokines (e.g., IL1B and interleukin-18 [IL18]), cleaved gasdermin D (GSDMD)-mediated pyroptosis, or the release of coagulation factor and DAMPs (e.g., high mobility group box 1 [HMGB1] and sequestosome 1 [SQSTM1]).[75–85] The NOD-like receptor (NLR) family pyrin domain containing 3 (NLRP3) inflammasome is one of the best-characterized inflammasomes, consisting of its sensor NLRP3, the adaptor apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and the effector zymogen (pro-CASP1).[86] The activation of NLRP3 inflammasomes requires a priming signal (e.g., toll-like receptor ligands) and the subsequent second signal (e.g., ATP, nigericin, and MSU).[87] DMI inhibits the expression of LPS-induced genes (e.g., *Il1b, Il18, Casp1*, and *Asc*) that are associated with inflammasome activation and function in BMDMs,[51] indicating that DMI inhibits inflammasome activation at the priming phase. Consistently, 4-OI shares similar properties with DMI that blocks pro-IL1B induction and IL1B secretion, resulting in an immunosuppressive phenotype of macrophages. In contrast, natural itaconate suppresses IL1B release, rather than pro-IL1B induction, suggesting that natural itaconate-mediated inhibition of inflam-

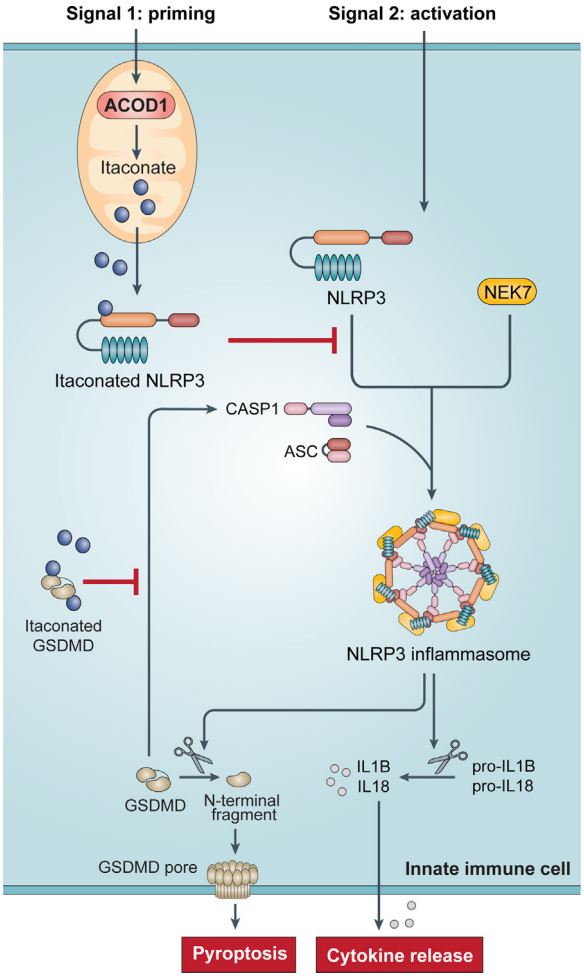

Figure 4. Role of ACOD1 in inflammasomes. In response to signal 1 and signal 2, the sensor NLRP3, the partner NEK7, the adaptor ASC, and the effector CASP1 assemble activated NLLRP3 inflammasomes, which trigger the maturation and secretion of cytokines (e.g., IL1B and IL18) or cleaved GSDMD-mediated pyroptosis. ACOD1 induced by signal 1 promotes the production of itaconate, which modifies Cys548 on NLRP3 and Cys77 on GSDMD. This process is called itaconation. Itaconated NLRP3 fails to interact with NEK7 in response to signal 2, whereas itaconated GSDMD loses the ability to trigger pyroptosis.
ACOD1: Aconitate decarboxylase 1; ASC: Apoptosis-associated speck-like protein containing a caspase recruitment domain; CASP1: Caspase 1; Cys: Cysteine residue; GSDMD: Gasdermin D; IL18: Interleukin 18; IL1B: Interleukin-1β; NEK7: NIMA-related kinase 7; NLRP3: NLR family pyrin domain containing 3.

masome function is due to a defective second signal.[52] These different inhibitory effects between modified and unmodified itaconate may attribute to the stronger electrophilic properties of itaconate derivatives.[52]

Mechanistically, itaconate inhibits the activation of NLRP3 inflammasomes by modifying the Cys-of NLRP3 or GSDMD, and this process of protein posttranslational modification is called itaconation. Cys 548 on NLRP3 is itaconated by 4-OI, thereby blocking the interaction between NLRP3 and NIMA-related kinase 7 (NEK7), a key step in the activation process.[88] In the late stage of macrophage activation, itaconate modifies the Cys 77 on GSDMD, which reduces the sustained CASP1 activation and GSDMD cleavage, and decreases IL1B and pyroptosis.[89] Conversely, macrophages from *Acod1*-deficient mice exhibit depleted itaconate and increased NLRP3 inflammasome activation, while increased ACOD1 expression in HEK293T cells has the opposite effect.[88,89] Thus, both endogenous and exogenous itaconate regulates the activation of NLRP3 inflammasomes [Figure 4]. Overall, these findings establish an anti-inflammatory role for ACOD1 in blocking inflammasome activation, mainly through itaconation.

ACOD1 in Infectious and Inflammatory Diseases

ACOD1 plays a significant role at the intersection of the host's inflammatory response to infectious and sterile threats. Infectious diseases are disorders caused by microorganisms (especially bacteria and viruses), while sterile inflammatory occurs in the absence of microorganisms. Typical sterile inflammatory diseases include peripheral arterial diseases, ischemia–reperfusion injury diseases, and neuroinflammatory diseases. These inflammatory reactions contribute to the progress of pathophysiology. In this section, we discuss the complex role of ACOD1 in immune-related pathological conditions and diseases [Figure 5].

ACOD1 in infectious diseases

Sepsis is a severe clinical syndrome characterized by a dysregulated host response to infection (e.g., from bacteria and viruses), which can lead to hypotension, tissue damage, immunocoagulation, multiple organ failure, and even death.[90–93] Preclinical studies using animal models of acute endotoxemia confirmed the protective effect of ACOD1 in limiting lethal inflammation in sepsis.[24] The administration of 4-OI prolongs survival, reduces inflammatory cytokine release (e.g., IL1B, TNF, and IL6), and reduces lactate production in mice exposed to lethal endotoxemia.[56,65] In mouse models, the treatment of endotoxemia with CO and HMOX1 inducers reduces the production of TNF in liver tissue and serum by inducing the expression of ACOD1.[31] Clinically, although there is acute inflammation in the early stage of sepsis, the immunosuppression in the late stage is the main cause of patient death. Thus, ACOD1-mediated immune paralysis may lead to life-threatening secondary infections during sepsis.[42,53] In contrast, β-glucan treatment inhibits LPS-induced ACOD1 expression and restores the immune activity of macrophages[42,53] which provides a potential strategy to restore immune responsiveness in patients with sepsis.

In addition to the endotoxemia model, the role of ACOD1 in sepsis has also been studied in bacterial or viral infection models *in vitro* and *in vivo*. ACOD1 mainly exhibits bactericidal effects during acute bacterial infections. For example, *Mtb* infection, during the etiology of tuberculosis, promotes the expression of ACOD1 and subsequent itaconate production in mouse macrophages and DCs.[46] The loss of *Acod1* in macrophages increases the intracellular replication of *Mtb*.[24,94,95] Consistently, *Acod1*-deficient mice are more susceptible to *Mtb* infection, leading to increased mycobacterial burden and lethal inflammation in the lungs.[94,95] This phenotype is reversed by itaconate,[94] indicating that ACOD1-mediated itaconate production can prevent *Mtb* infection.

Another frequent cause of severe pneumonia in humans is *Legionella pneumophila* infection. IFNB1 or IFNG limits the intracellular growth of L. *pneumophila* by inducing the expression of ACOD1 and itaconate production *in vitro* and *in vivo*.[37,96] Infection from *Salmonella enterica* serovar *Typhimurium* (*S. Ty-*

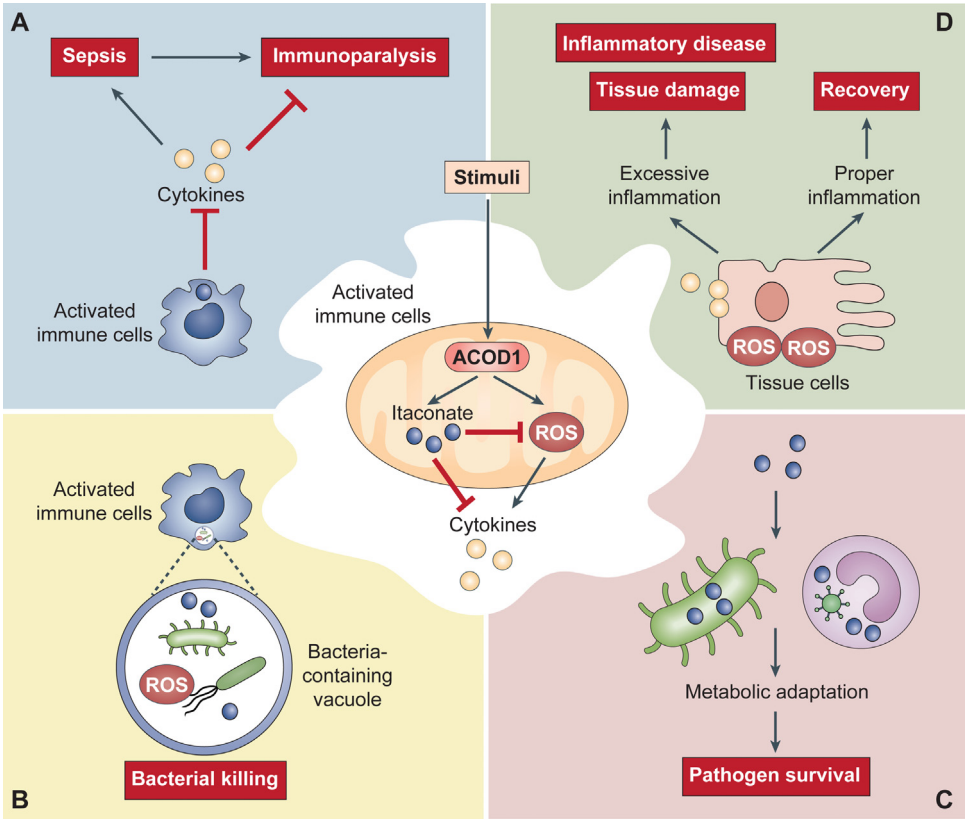

Figure 5. ACOD1 in immune-related diseases. A: Itaconate protects mice against experimental sepsis by reducing inflammatory cytokine production and release (e.g., IL1B, TNF, and IL6) in the early stage. The upregulation of ACOD1 also leads to immune paralysis at the late stage. B: ACOD1-induced itaconate and ROS are delivered to pathogen-containing vacuoles, thereby contributing to bacterial killing during infection. C: ACOD1-induced itaconate production triggers bacteria or virus-infected cells to undergo metabolic reprogramming, thereby favoring the survival of pathogens. D: ACOD1-related immune response in disease. ACOD1 plays a context-dependent role in inflammatory diseases. Generally, excessive inflammation leads to tissue injury and multiple diseases, whereas appropriate inflammation is beneficial for the recovery from diseases.
ACOD1: Aconitate decarboxylase 1; IL6: Interleukin 6; IL1B: Interleukin-1β; ROS: Reactive oxygen species; TNF: Tumor necrosis factor.

phimurium) or *Mycobacterium avium (M. avium)* is also restricted by the induction of ACOD1 expression or itaconate production.[24,30,40,43,97] In contrast, the deletion of *Acod1* in mice or zebrafish larvae leads to enhanced susceptibility to *S. Typhimurium* or *M. avium* infection. Mechanistically, enhanced tethering between mitochondria and bacteria-containing vacuoles facilitates the subsequent delivery of itaconate into the vacuoles, leading to reduced replication of *S. Typhimurium* or *M. avium*.[40,97] Alernatively, ACOD1-dependent mROS production delivers itaconate to bacteria-containing phagosomes, thereby contributing to bacterial killing during *S. Typhimurium* infection.[30,98] Furthermore, ACOD1 is upregulated in *Staphylococcus aureus*- or *Escherichia coli*-infected human blood cells,[99] *Listeria monocytogenes*-infected primary murine macrophages and splenic tissue,[21] *Burkholderia pseudomallei*-infected J774A.1 cells (a murine macrophage cell line),[100] and *Brucella melitensis*-infected BMDMs,[101] indicating a wide role for ACOD1 in the defense against bacterial infections.

Unlike in bacterial infections, ACOD1 plays a dual role in viral infections. On the one hand, the antiviral effect of ACOD1 has been observed during WNV, ZIKV, and hepatitis B virus (HBV) infections.[33,102,103] *Acod1*-deficient mice show higher mortality, increased brain viral load, and worsening neurological diseases after ZIKV infection, which is rescued by 4-OI treatment.[33] On the other hand, ACOD1 favors viral infection under certain conditions. For example, RSV infection induces ACOD1 expression and subsequent ROS production in A549 cells (a human alveolar epithelial cell line) and lung tissues of RSV-infected mice, leading to increased inflammatory cell infiltration, oxidative damage, and lung injury *in vivo*.[35] Similarly, VSV-induced ACOD1 expression and itaconate production promote geranylgeranyl diphosphate synthesis and prenylation, thereby facilitating virus entry and intracellular replication.[58] Consequently, the suppression of ACOD1 impairs viral overgrowth and protects mice from RSV and VSV infection-induced lung injury.[35,58] Further understanding the different mechanisms of ACOD1 in viral infection may help clarify the selectivity of viral immunity.

In addition to the previously mentioned bacterial and viral infections, the immune function of ACOD1 is confirmed in other pathogen infections, including *Chlamydia* and spirochete and protozoan infections. For example, *Chlamydia pneumoniae*-induced *Acod1* mRNA expression in lung tissue is associated with reduced pulmonary inflammatory infiltration in an MYD88 innate immune signal transduction adaptor (MYD88)-dependent manner.[36] ACOD1 is related to the pro-inflammatory response of *Borrelia burgdorferi* spirochete infection, the cause of Lyme disease.[20] The upregulation of ACOD1 contributes to the susceptibility of splenic macrophages to the parasite L. *donovani*,[104] but limits neurotoxicity in brain macrophages (mi-

croglia) that is mediated by the parasite *Toxoplasma gondii*.[105] Moreover, microglia that fail to induce ACOD1 expression during lymphocytic choriomeningitis virus (LCMV) infection develop into a neuroprotective phenotype, supporting the neurotoxic effect of ACOD1.[105] Taken together, these findings confirm a context-dependent role of ACOD1 during pathogen infections.

ACOD1 in sterile inflammatory diseases

In addition to pathogen infections, inflammation can also be triggered by tissue damage and stress, leading to various diseases. The abnormal expression of ACOD1 is also related to the development of sterile inflammatory diseases. Below, we highlight some examples of how ACOD1 is implicated in those diseases.

Peripheral artery disease

Peripheral artery disease is a circulatory problem in which occlusions in arteries to organs cause tissue ischemia. Severe peripheral artery disease often results in tissue necrosis and limb amputation.[106] In peripheral artery disease, macrophages are recruited into the occluded vessels to induce a potent M1 polarization, leading to decreased angiogenesis and arteriogenesis.[107,108] The ACOD1–itaconate pathway mediates M1 polarization, leading to impaired perfusion recovery in experimental peripheral artery disease.[72] The suppression of ACOD1 by miR93 mimic or *Acod1* knockout in macrophages induces an M2-like phenotype, which is beneficial for the recovery of ischemic muscle.[72] Studies of these topics are advancing our understanding of the mechanism of ACOD1-mediated macrophage polarization in peripheral artery disease.

Ischemia–reperfusion injury disease

In addition to ischemic injury, re-established blood supply may increase local inflammation and ROS production, leading to secondary injury in ischemic tissue, called ischemia–reperfusion injury.[109] Despite the upregulation of *Acod1* being involved in ligands (soluble CD74 and recombinant migration inhibitory factor [MIF]) that induced necroptosis of cardiac myofibroblasts,[110] itaconate exerts an anti-inflammatory effect and reduces the myocardial infarction size in a mice model of ischemia–reperfusion injury.[51] A similar protective effect for ACOD1 and itaconate has been validated in experimental models of ischemia–reperfusion injury of the liver, kidneys, and brain.[32,66,67] It is unclear whether and to what extent different immune cells confer increased itaconate production resulting in decreased ischemia–reperfusion injury. Additional investigations are required to determine the specific effect of ACOD1 in ischemia–reperfusion injury, especially investigations that use genetic models of ACOD1 inhibition in different cells or tissues.

Neuroinflammatory disorders

Activated microglia (macrophages of the brain and spinal cord) mediate dysregulated neuroinflammatory processes, which contributes to the pathogenesis of neurological diseases.[111] In addition to promoting LCMV infection in microglia, the pathological effects of ACOD1 have also been observed in mice exposed to a high-fat diet, leading to chronic neuroinflammation and ultimately cognitive impairment.[71] However, the administration of DMI in mice with experimental autoimmune encephalomyelitis or ischemic stroke suppresses neuroinflammation and ameliorates disease severity,[32,112] indicating that ACOD1 and itaconate may have non-overlapping functions. In addition, α-synuclein increases the expression of ACOD1 in microglia, indicating an inflammatory state in Parkinson's disease.[113] These findings highlight the functional role of ACOD1 in highly specialized macrophages in tissue.

Pulmonary fibrotic diseases

A dysregulated inflammatory response in lung tissue is a crucial factor in pulmonary fibrotic diseases, including cystic fibrosis and idiopathic pulmonary fibrosis,[114,115] but the contribution of ACOD1 in these diseases are different. In the cystic fibrosis airway, ACOD1-dependent itaconate production helps bacteria to undergo metabolic reprogramming, thereby promoting their survival, lung colonization, and persistent infection in the airways.[54,116] Idiopathic pulmonary fibrosis is featured by a deposition of excessive extracellular matrix in the lung parenchyma, and this process is regulated by airway macrophages.[117,118] In a bleomycin-induced pulmonary fibrosis model, the loss of *Acod1* results in increased expression of profibrotic genes (e.g., *Cebpb*, transforming growth factor 2 [*Tgf2*], and Smad family member 7 [*Smad7*]) in airway macrophages and persistent fibrosis in mice. The application of itaconate decreases the fibrotic activity of lung fibroblasts and ameliorates the severity of pulmonary fibrosis *in vivo*.[119] Thus, although itaconate shows therapeutic potential in idiopathic pulmonary fibrosis, researchers should carefully consider its application to avoid unexpected bacterial infection favored by itaconate.

Rheumatoid arthritis

Rheumatoid arthritis is an autoimmune disease that can cause joint pain and damage. ERG240 is an inhibitor of branched-chain amino acid transaminase 1 (BCAT1), which inhibits LPS-induced ACOD1 expression and subsequent itaconic production in macrophages.[120] The administration of ERG240 reduces the severity of rheumatoid arthritis and crescentic glomerulonephritis in murine models.[120] Through microarray analysis, investigators have identified highly upregulated genes, including *Acod1*, in synovial macrophages in mice with collagen antibody-induced arthritis or in air pouch membranes stimulated by MSU crystals.[38,121] Collectively, these findings indicate the association of ACOD1 with collagen- or crystal-induced inflammation or inflammatory arthropathies (such as rheumatoid arthritis or gout), but additional studies are needed to precisely determine the function of ACOD1 in these conditions.

Other diseases

In an animal model of psoriasis induced by imiquimod, NFKBIZ-driven skin inflammation was inhibited by DMI and increased by *Acod1* depletion.[45,89] In a mouse model of abdominal aortic aneurysm induced by angiotensinogen, 4-OI suppressed the formation of abdominal aortic aneurysms in apolipoprotein E (*Apoe*)-deficient mice by inhibiting NFE2L2-mediated vascular inflammation, while *Acod1* deficiency exerted the opposite effect.[68] In a dextran sodium sulfate (DSS)-induced ulcerative colitis mouse model, DMI inhibited the inflammatory response by decreasing the secretion of cytokines

(IL1B and C-C motif chemokine ligand 2 [CCL2]) from intestinal epithelial cells, thereby reducing macrophage infiltration.[122] Researchers have found that 4-OI not only protects against urate-induced peritonitis *in vivo* by inhibiting NLRP3-dependent inflammasome activation[88] but also suppresses the inflammation response of peripheral blood mononuclear cells (PBMCs) from cryopyrin-associated periodic syndrome patients with NLRP3 mutations.[88,123] These studies support a beneficial effect of ACOD1-mediated itaconate in alleviating excessive inflammation in immunological disorders.

Conclusions and Perspective

Emerging evidence on inducing ACOD1 expression in immunometabolism supports it as a therapeutic approach, especially for infection and inflammatory diseases.[22] To date, the immunoregulatory role of ACOD1 has been revealed mostly in processes of innate immunity, including metabolism reprogramming, oxidative stress, macrophage polarization, inflammasome inhibition, and inflammatory gene regulation. The function of ACOD1 in adaptive immune cells (e.g., T cells and B cells) or adaptive immunity has not been well-defined, although the expression of ACOD1 in macrophages may affect antigen presentation and the function of T cells in infection or antitumor immunity.[124] Another characteristic of ACOD1's immune function is closely related to its production of itaconate. The anti-inflammatory activity of itaconate makes it a promising therapeutic strategy to limit the pathological consequences of immune-mediated diseases in multiple preclinical models. However, the induction of ACOD1 or the use of itaconate also has detrimental effects, such as promoting virus replication, aggravating tissue damage, inducing immune paralysis, or causing cell death (e.g., ferroptosis).[35,53,125,126] Nevertheless, identifying itaconate-independent functions of ACOD1 may facilitate the development of more precise treatments to overcome the side effects of itaconate.

In addition to ACOD1, oxoglutarate dehydrogenase (OGDH) also contributes to the production of itaconate.[127] Thus, it is important to investigate the signaling, mechanism, and regulation of OGDH-dependent itaconate production in the context of inflammation and immune response. Since ACOD1 is mainly induced and expressed in myeloid cells, OGDH-dependent itaconate production may play a wide range of roles in physiological processes. Given that itaconate derivatives and endogenous itaconate have opposite immunological effects due to their distinct electrophilic properties, an appropriate form of itaconate to select should be considered in future experiments.[52] More importantly, whether itaconate is useful in clinical situations needs further verification.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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