Canadian Journal of Chemistry

Itaconate: an antimicrobial metabolite of macrophages

Journal:	<i>Canadian Journal of Chemistry</i>
Manuscript ID	cjc-2021-0117.R1
Manuscript Type:	Mini Review
Date Submitted by the Author:	15-Jun-2021
Complete List of Authors:	Duncan, Dustin; McGill University Auclair, Karine; McGill University
Is the invited manuscript for consideration in a Special Issue? :	McGill
Keyword:	

SCHOLARONE™
Manuscripts

Itaconate: an antimicrobial metabolite of macrophages

Dustin Duncan and Karine Auclair*

Department of Chemistry, McGill University, Montreal, Quebec, Canada

*Corresponding author:

Karine Auclair

Chemistry Department, McGill University

801 Sherbrooke Street West, Montreal, Quebec, Canada, H3A 0B8

+1-514-398-2822; karine.auclair@mcgill.ca

Draft

Abstract

Itaconate is a conjugated 1,4-dicarboxylate produced by macrophages. This small molecule has recently received increasing attention due to its role in modulating the immune response of macrophages upon exposure to pathogens. Itaconate has also been proposed to play an antimicrobial function; however, this has not been explored as intensively. Consistent with the latter, itaconate is known to show antibacterial activity *in vitro* and was reported to inhibit isocitrate lyase, an enzyme required for survival of bacterial pathogens in mammalian systems. Recent studies have revealed bacterial growth inhibition under biologically relevant conditions. In addition, an antimicrobial role for itaconate is substantiated by the high concentration of itaconate found in bacteria-containing vacuoles, and by the production of itaconate-degrading enzymes in pathogens such as *Salmonella enterica* ser. Typhimurium, *Pseudomonas aeruginosa*, and *Yersinia pestis*. This review describes the current state of literature in understanding the role of itaconate as an antimicrobial agent in host-pathogen interactions.

Keywords: antimicrobial; antimicrobial resistance; isocitrate lyase; itaconic acid; glyoxylate cycle; macrophages.

Introduction

Itaconate (or methylenesuccinate, Figure 1, in red) was first isolated from fungi like *Aspergillus* sp.¹⁻² Since its first isolation from natural sources, this conjugate dicarboxylate has been of great interest. Early work investigating its toxicity in mammals already suggested a link between itaconate and the citric acid cycle.³⁻⁴ For several decades, however, itaconate was of interest mostly as a building block in the synthesis of renewable polymers with applications ranging from superabsorbent microspheres⁵ to trace metal detection,⁶ and pH-sensitive drug delivery.⁷ This led to the exploration, the bioengineering, and even the discovery of different itaconate-producing organisms such as other *Aspergillus* species, *Ustilago* species, and *Escherichia coli*, thereby improving its economic viability as a polymer starting material.^{2, 8-9} The recent discovery that macrophages also produce itaconate has reinvigorated and broadened interest for this molecule.¹⁰⁻¹¹ Rapidly, this small metabolite was found to play a critical role in immunomodulation and in the innate immune response to infection.¹² This review focuses on the current state of literature with respect to the antimicrobial activity of itaconate.

Isocitrate lyase inhibition

Itaconate is a known inhibitor of isocitrate lyase (Icl), a critical enzyme in the glyoxylate cycle.¹³⁻¹⁴ This pathway comprises part of the citric acid cycle but diverts isocitrate from isocitrate dehydrogenase (Figure 1). The glyoxylate cycle serves to conserve carbon atoms by avoiding two decarboxylation events (catalyzed by isocitrate dehydrogenase and α -ketoglutarate dehydrogenase) and therefore preserving all carbon atoms of isocitrate (Figure 1). This pathway allows for adaptability of bacteria to numerous types of environments, such as that found within macrophages. Indeed, an intact glyoxylate cycle is often required for bacterial survival and pathogenesis.¹⁵⁻²⁰ As expected, different organisms have different mechanisms for regulating the glyoxylate cycle, as reviewed by Dolan and Welch.²¹ Since the glyoxylate cycle is conditionally essential, it has been the target of numerous antibacterial development efforts.²²⁻⁴⁵

Figure 1: Intersection between the glyoxylate and the citric acid cycles. The glyoxylate cycle (in black) circumvents the use of isocitrate dehydrogenase and α -ketoglutarate dehydrogenase (in grey) of the citric acid cycle (full circle). In red, itaconate inhibits isocitrate lyase.

Itaconate has been shown to inhibit various Icl isoforms (Table 1). In particular, bacterial Icl enzymes shown to be inhibited by itaconate include the *Pseudomonas indigofera* isoform with a K_i of $0.9 \mu\text{M}$,¹⁴ the two *Mycobacterium tuberculosis* isoforms (Icl1 and Icl2, the latter of which is also known as AceA) with K_i values of $120 \mu\text{M}$ and $220 \mu\text{M}$ respectively,⁴⁶ and the *Corynebacterium glutamicum* isoform with a K_i of $5.05 \mu\text{M}$.

Table 1: Inhibition constants of itaconate towards various Icl isoforms.

Isoform	K_i (μM)	Ref
Bacteria		
<i>Pseudomonas indigofera</i>	0.9	¹⁴
<i>Mycobacterium tuberculosis</i>	Icl1: 120 AceA/Icl2: 220	⁴⁶
<i>Corynebacterium glutamicum</i>	5.05	⁴⁷
Eukaryota		
<i>Leishmania amazonensis</i>	4500	⁴⁸
<i>Ashbya gossypii</i>	170	⁴⁹
<i>Fomitopsis palustris</i>	68	⁵⁰
<i>Ricinus communis</i> L. cv. Zanzibariensis	11.9	⁵¹
<i>Tetrahymena pyriformis</i>	3.5	⁵²
<i>Linum usitatissimum</i> L.	17	⁵³
<i>Pinus densiflora</i> Sieb et Zucc	2.8	⁵⁴
<i>Caenorhabditis elegans</i>	19	⁵⁵
<i>Ascaris suum</i>	7.3	⁵⁵
<i>Aspergillus nidulans</i>	40	⁵⁶

Although there are numerous crystal structures reported for Icl in complex with inhibitors such as 3-nitropropionate (PDB: 6C4C,⁵⁷ 6C4A,⁵⁷ and 1F8I⁵⁸) and bromopyruvate (PDB: 1F8M),⁵⁸ there is only one structure of an itaconate-bound Icl (PDB: 6XPP).⁵⁹ This complex of *M. tuberculosis* Icl1 reveals the formation of a covalent adduct between the enzyme and the inhibitor, as a result of a Michael-addition between a conserved Cys191 and itaconate (Figure 2).⁵⁹

Figure 2: Binding of itaconate (red) to *Mt*Icl1 (cpk colors, PDB 6XPP). Cys191 of the enzyme is covalently linked to C4 of itaconate (in red, 2.36 Å) to form an adduct between the enzyme and the inhibitor. C1 of itaconate (one of the carboxylates) forms hydrogen-bonds with Arg228 and the backbone amide of Cys191, and also interacts with a Mg^{2+} ion. C5 of itaconate (the other carboxylate) interacts with Asn313, Ser 315, Ser317, and Thr347 via hydrogen bonds.

Despite being a stronger Michael acceptor, the dimethyl ester of itaconate shows 22 – 25-fold weaker inhibition for *M. tuberculosis* Icl1 ($\text{IC}_{50} = 420 \pm 20 \mu\text{M}$ for itaconate, $>10,000 \mu\text{M}$ for dimethyl itaconate).⁵⁹ This suggests that the interactions of the carboxylate groups with the active site of Icl (Figure 2, involving Arg 228, Asn313, Ser 315, Ser 317, and Thr347) are central to itaconate binding. Furthermore, adding substituents at C-1' (see numbering in Figure 1) of itaconate greatly reduces the inhibitory effect,⁵⁹ likely as a result of steric hindrance. The authors reported that Mg^{2+} was necessary for formation of the Icl1-itaconate adduct, and that complete covalent bond formation required approximately 5 hours.⁵⁹ It is perhaps unsurprising that the adduct is slow to form since α,β -unsaturated carboxylates are very weak Michael acceptors. Furthermore, itaconate was reported to have similar affinity for the Cys191Ser mutant of Icl1 ($K_D = 112 \pm 11 \mu\text{M}$ for wild type versus $155 \pm 29 \mu\text{M}$ for mutant)⁵⁹ which does not undergo adduct formation, suggesting that the covalent bond is not a major contributor to inhibition. This contrasts with 3-nitropropionate and bromopyruvate, which both depend upon Cys191 for significant

inhibition to be observed.⁵⁷⁻⁵⁸ In summary, given that 1) cysteine is a conserved residue in Icl, that 2) recent findings by Kwai *et al.* suggest a covalent adduct formation between Icl and itaconate, and that 3) the binding affinity of itaconate for the Cys191Ser mutant of Icl is similar to that of the wild type enzyme, itaconate may initially behave as a competitive inhibitor, before acting as an affinity label (potentially also reversible but at a slower rate). The time-dependence of these interactions may explain the large variations observed in measured K_i values.

Antimicrobial activity of itaconate

The minimal inhibitory concentration (MIC) of itaconate has been measured in numerous bacterial species, but with widely varying results (Table 2). Notably, many of these MICs were measured without controlling the pH, which very likely biases the results.⁶⁰ Indeed, the lack of consideration toward the change in pH of these experiments conflates the effects of itaconate and pH, and limits the usability of these data. A detailed description of the relationship between pH and itaconate is described below.

The bacterial target of itaconate remains to be confirmed. Itaconate has been found to inhibit bacterial enzymes other than Icl,^{13-14, 46} including propionyl-coenzyme A decarboxylase (*Rhodospirillum rubrum*),⁶¹ and methylmalonyl-coenzyme A mutase (*Mycobacterium tuberculosis*).⁶² This (mostly *in vitro*) information is insufficient to settle on a mechanism of antibacterial activity for itaconate.

If the antimicrobial activity of itaconate proceeds via Icl inhibition, growth media containing only carbon sources metabolised via the glyoxylate cycle are needed to reveal the full activity. The growth media used to determine reported MIC values vary between reports. Further complicating MIC determination is the high acidity of itaconate, which may considerably affect the pH of the medium.

Table 2: MIC values of itaconic acid (or sodium itaconate) in various bacterial species

Species	Experimental Condition	MIC	Ref
<i>Acinetobacter baumannii</i>	Itaconic acid resuspended in PBS for killing assays after growth in AYE broth	10 mM	63
	Itaconic acid in M9A	10 mM	60
	Sodium itaconate (pH 7.2) in M9A	>40 mM	60
<i>Enterobacter faecium</i>	Itaconic acid in M9A	10 mM	60
	Sodium itaconate (pH 7.2) in M9A	>40 mM	60
<i>Escherichia coli</i>	Itaconic acid in M9A	1 mM	64
	Itaconic acid in M9A	5 mM	60
	Sodium itaconate (pH 6.4) in M9A	0.37 mM	60
	Sodium itaconate (pH 7.2) in M9	80 mM	60
	Itaconate (salt and pH unspecified)	10 mM	65
<i>Klebsiella pneumoniae</i>	Itaconic acid MIC	10 mM	60
	Sodium itaconate (pH 7.2) in M9	>40 mM	60
<i>Legionella pneumoniae</i>	Itaconic acid resuspended in PBS for killing assays after growth in AYE broth	10 mM	63
<i>Mycobacterium tuberculosis</i>	Itaconic acid in M9A with Tyloxapol detergent	1 mM	60
	Sodium itaconate (pH 7.2) in M9A with Tyloxapol detergent used	4 mM	60
	Itaconic acid MIC in acetate supplemented 7H9	25–50 mM	66
<i>Pseudomonas spp.</i>	<i>Pseudomonas</i> MA, itaconate (salt and pH unspecified)	10 mM	65
	<i>Pseudomonas aeruginosa</i> TAU5, itaconate (salt and pH unspecified)	10 mM	67
	Itaconic acid in M9A	20 mM	60
	Sodium itaconate (pH 7.2) in M9A	>40 mM	60
	<i>Pseudomonas indigofera</i> M1, sodium itaconate (pH 7.0) with ethanol or sodium butyrate carbon sources	20 mM	14
<i>Salmonella enterica</i> spp. Typhimurium	Itaconic acid in acetate-containing media	10 mM	66
	Itaconic acid in M9A	20 mM	60, 64
	Sodium itaconate (pH 6.0) in M9A	3.7 mM	60
	Sodium itaconate (pH 7.2) in M9A	400 mM	60
<i>Staphylococcus aureus</i> (MRSA)	Itaconic acid resuspended in PBS for killing assays after growth in AYE broth	10 mM	63
<i>Yersinia pestis</i>	Sodium itaconate (pH 7.0)	75 mM	68

It is well established that small organic acids are capable of inhibiting bacterial growth at low pH values, potentially via a “proton shuttle” effect.⁶⁹⁻⁷¹ With addition of an organic acid to growth medium, the pH decreases, favoring the protonated (uncharged) form of the acid, which

may cross bacterial membranes more easily (Figure 3). Once inside the (less acidic) bacterial cytosol, the organic acid releases a proton, thereby reducing the cytosolic pH and causing toxicity. However, organic acids have different toxicities that do not always correlate well with their respective pKa values,⁷²⁻⁷⁵ implying that the exact molecular structure of the organic acid may lead to specific toxicity effects. Itaconate is known to have more potent antimicrobial activity at lower pH values,^{60, 71, 76} and this has recently been investigated in more detail.⁶⁰ By comparing the MIC of itaconic acid with that of its sodium salt, Duncan *et al.* demonstrated that the decrease in pH caused by itaconic acid addition to the growth medium increases the antimicrobial activity of itaconate against numerous bacterial species.⁶⁰ The authors also evaluated the MIC of itaconate in *E. coli* and *S. Typhimurium* (itaconate-sensitive and itaconate-resistance bacterial strains, respectively) at controlled pH values, which allowed them to rule out a proton-shuttle mechanism, and establish that itaconate and acidity show synergistic antibacterial activity.⁶⁰ It is not clear what the mechanism for this phenomenon is. The authors observed that *S. Typhimurium* displayed a rapidly increasing sensitivity to itaconate as the pH was lowered, with MIC values ranging from >200 mM at pH 6.3, to 11 mM at pH 6.2, and 3.7 mM at pH 6.0. Similarly, in *E. coli* the MIC of itaconate was found to be 80 mM at pH 7.2 and 0.37 mM at pH 6.4.⁶⁰ Considering that the concentration of itaconate in *Salmonella*-containing vesicles (pH ~5.0)⁷⁷ is about 5 – 6 mM,⁷⁸ these observations suggest that the concentration of itaconate encountered by bacteria in macrophages is likely sufficient to inhibit their growth. Therefore, despite the relatively high MIC values reported (Table 2), the synergistic behaviour of pH on itaconate activity coupled with the acidic environments encountered by bacteria within macrophages suggests that itaconate may indeed behave as an antibacterial compound *in vivo*.

Figure 3: The proton shuttle mechanism. Under neutral conditions, small organic acids are ionized and may not transverse the membrane effectively. Under acidic conditions, however, most of them are protonated, and therefore neutral, thereby more likely to traverse the membrane. Once in the cytosol (mostly neutral), the organic acids are expected to ionize, liberating protons and decreasing the pH, thus effectively “shuttling” protons from the acidic medium into the cell.

Macrophages and the production of itaconate

Although it has been known since 1939 that fungi generate itaconate,¹ the production of itaconate by macrophages was only reported in 2011.¹⁰⁻¹¹ Soon after this discovery, the gene responsible for itaconate production was identified as the immunoresponsive gene 1 (*irg1*),⁶⁶ which is highly upregulated upon infection.⁷⁹⁻⁸⁴ This gene encodes a *cis*-aconitate decarboxylase (known as IRG1, CAD, or ACOD1).⁶⁶ Human IRG1 was found to have high sequence identity to the corresponding isoform in *Aspergillus terreus* (a fungus used for the industrial production of itaconate).^{2, 85} Next, a connection was rapidly made between the macrophage response to LPS and this small metabolite, which led to many studies on the immunomodulatory role of itaconate, as reviewed elsewhere.^{12, 86-99}

Itaconate production from the citric acid cycle intermediate *cis*-aconitate is catalysed by IRG1, which is associated with the mitochondria,⁸² from which the small molecule is transported to extra- and intracellular locations. Transport into bacteria-containing vesicles, such as *Salmonella*-containing vacuoles, is believed to occur through a Rab32-BLOC3-mediated

mechanism, potentially involving IRG1.⁷⁸ The concentration of itaconate in macrophages varies from 3-8 mM in mice to 60 μ M in humans,^{11, 66} although these numbers must be used with caution because they may vary largely with the location, the cell type and the activation level. Furthermore, besides *Salmonella*-containing vesicles (known to contain 5 – 6 mM itaconate),⁷⁸ the local concentration of itaconate in various organelles of mammalian cells is unknown.

Whereas the link between LPS-stimulation and itaconate production is well established, the mechanism of action of itaconate with respect to the immune response is complex and remains incompletely understood. For example, the inhibition of succinate dehydrogenase is well-established,¹⁰⁰ but *irg1*^{-/-} mice are susceptible to *Δicl1 M. tuberculosis* infections, implying that itaconate is required beyond the inhibition of Icl in the invasive pathogen.¹⁰¹ Similarly, although several lines of evidence suggest a harmful effect of itaconate on bacteria themselves, more studies are warranted to confirm the target(s).

Itaconate-resistant bacteria

Further corroborating an antimicrobial role for itaconate is the presence of an itaconate degradation pathway in several pathogenic bacteria and fungi.¹⁰²⁻¹⁰⁸ Itaconate-producing organisms, such as mammals and certain fungi,^{104, 107, 109-110} are expected to metabolize itaconate, but this would not be expected for non-producers unless there is significant exposure. In mammals, the itaconate degradation pathway serves to regulate the immunological response associated with itaconate, whereas in pathogens, itaconate degradation may be necessary to survive and proliferate within the host. Considering that itaconate inhibits the enzyme Icl, which is essential for bacterial pathogenesis,¹⁵⁻²⁰ it is not surprising that bacteria such as *P. aeruginosa*, *S. enterica*, *Y. pestis*, *Burkholderia* species and *Mycobacterium* species,^{111, 112} have developed resistance to itaconate. These bacteria can transform itaconate to pyruvate and acetyl coenzyme A (AcCoA) using enzymes encoded by the *rip* (required for intracellular proliferation) operon, comprising the *ripA*, *ripB*, and *ripC* coding genes.¹¹¹ The RipA protein (also known as itaconate CoA transferase or Ict) first produces itaconyl-CoA from itaconate. RipB (itaconyl-CoA hydratase or Ich) next isomerizes and hydrates the double bond of itaconyl-CoA to generate (*S*)-citramalyl-CoA. This product is finally cleaved by RipC (citramalyl-CoA lyase or Ccl) into AcCoA and pyruvate (Figure 4). Interestingly, Sasikaran *et al.*¹¹¹ have reported little homology between the itaconate-degradation enzymes of *Y. pestis* and those of *P. aeruginosa*, implying that the itaconate-degrading enzymes

of these pathogens likely arose through convergent evolution. Herein, the enzymes that comprise the itaconate-degradation pathway will be referred to as Ict, Ich, and Ccl to better describe their function, since the pathway may be genetically dissimilar among different organisms.¹¹¹

Figure 4: The itaconate degradation pathway. Itaconate CoA transferase (Ict) condenses itaconate and CoA. Itaconyl-CoA hydratase (Ich) transforms itaconyl-CoA to (S)-citramalyl-CoA. (S)-Citramalyl-CoA lyase (Ccl) cleaves (S)-citramalyl-CoA into AcCoA and pyruvate.

Crystal structures have been reported for *Y. pestis* Ict,¹¹²⁻¹¹³ although the protein is catalogued as a coenzyme A transferase (PDB: 3QLI, 3QLK, 3S8D)¹¹² or as a 4-hydroxybutyrate CoA-transferase (PDB: 4N8H, 4N8I, 4N8J, 4N8K, 4N8L) in the PDB.¹¹³ The reaction has been suggested to proceed via two double displacements (two Ping Pong reactions, Figure 5). First, the acetyl group of AcCoA is transferred to the catalytic Glu249 of Ict to form an acetyl anhydride, which undergoes nucleophilic attack by the thiol of CoA to form a CoA-enzyme adduct. This thioester is then attacked by one of the carboxylate groups of itaconate to produce an itaconate-enzyme adduct, before nucleophilic attack of the thiol of CoA to yield itaconyl-CoA and regenerate Glu429.¹¹³

Figure 5: Proposed mechanism of *Y. pestis* Ict.¹¹³

There are no crystal structures published for any Ich isoforms, whereas crystal structures have been reported for Ccl from different pathogenic species, including *Y. pestis* RipC (PDB 3QLL),¹¹⁴ *Homo sapiens* CLYBL (PDB 5VXO, 5VXC, 5VXS),¹¹⁵ and *M. tuberculosis* Rv4589c (annotated as CitE; PDB 6AQ4, 1U5H),^{108, 116} as well as the non-pathogenic bacteria *Deinococcus radiodurans* (PDB: ISG1), *Burkholderia xenovorans* (PDB: 3R4I), and *Ralstonia eutropha* (3QQW).¹¹⁴ These enzymes are structurally similar to each other, forming a homotrimer that adopts a $\beta_8\alpha_8$ -TIM barrel fold.¹¹⁴⁻¹¹⁶ The putative active site residues of the *Y. pestis* Ccl isoform include Glu39, Asp40, Arg71, Glu129, Asp156, and Pro192, which have significant overlap with the analogous enzyme *Haloferax volcanii* malate synthase H.¹¹⁴ Based on the overlay presented by Torres *et al.*, we propose a mechanism in which Asp40 sits near where the hydroxyl group of (S)-citramalyl-CoA is expected to be and may facilitate its deprotonation, while Glu129 and Asp 156 may lie close to the carboxylate group, likely orienting the small molecule (Figure 6).¹¹⁴ As the deprotonated alcohol forms a carbonyl, the C-C bond breaks, resulting in pyruvate and the AcCoA anion, which promptly picks up a proton. This mechanism is analogous to what has been proposed for another lyase.¹¹⁷

Figure 6: Proposed mechanism of *Y. pestis* Ccl.

In *S. Typhimurium*, itaconate binds to the regulator protein RipR, which then forms a complex with the promoter of the *rip* operon (also known as itaconate response operon or IRO in this species).⁷¹ This induces transcription of *ict*, *ich*, and *ccl*. Induction of the *rip* operon appears to be dependent only upon itaconate (or structural analogues such as mesaconate, citramalate, and methylsuccinate, albeit at a much lower level of induction), whether the growth medium is glucose-deprived or rich (*e.g.* Lysogeny broth or glucose-containing minimal media).⁷¹ It is not currently known if this regulatory mechanism of the itaconate degradation pathway is conserved across other itaconate-degrading organisms.

The therapeutic potential of itaconate

Given that itaconate is an antimicrobial produced by the immune system, there may be therapeutic value to altering the pathways in which it is involved. Little is known about the secretion and uptake mechanisms of itaconate in mammalian cells,⁹⁶ let alone its bioavailability and distribution. In bacteria, this small diacid may be transported into cells by dicarboxylate transporters. Considering that many enzymes are known that bind both succinate and itaconate such as isocitrate lyase,¹¹⁸ succinate dehydrogenase,¹⁰⁰ and succinate-CoA ligase,¹¹⁹ it is possible that the succinate transporter DcuB may be involved in the uptake of itaconate in *S. Typhimurium*,¹²⁰ while it has been determined that itaconate transport is not dependent on the succinate transporter DctA.⁷¹

The inhibition of itaconate degradation has recently been explored.⁶⁴ This is an intriguing approach to treat bacterial infections since the lack of antimicrobial activity of the active agent would minimize resistance selection. Hammerer *et al.*⁶⁴ have synthesized a series of compounds

designed to inhibit the itaconate-degradation pathway in *S. Typhimurium*. The molecules are prodrugs, which once activated are structural analogues of itaconyl-CoA (the product of Ict and substrate of Ich). These pantothenamide derivatives (e.g. compound **1**) were designed to be selectively activated in bacteria by enzymes of the CoA-biosynthetic pathway (Figure 7), a prodrug activation strategy that has recently been reviewed by Duncan and Auclair.¹²¹ Although deprived of antimicrobial activity, compound **1** reduced the MIC of itaconate in *S. Typhimurium* from 20 mM to approximately 1.1 mM,⁶⁴ i.e. below the concentration of itaconate found within the *Salmonella*-containing vacuoles of macrophages.⁷⁸ This compound has also shown effectiveness in clearing *S. Typhimurium* in macrophage cells.¹²² Compound **1** cannot be used *in vivo*, however, because it is rapidly cleaved by serum proteins called pantetheinases. This vulnerability has long plagued the development of pantothenamides, but there has been recent progress in identifying pantothenamide analogues that are pantetheinase-resistant while retaining biological activity.¹²³⁻

126

This itaconate-resensitization approach is also promising to target *P. aeruginosa*. In the lungs, this bacterium metabolises succinate and itaconate, leading to an increased activity of the glyoxylate cycle and biofilm production.¹²⁷ Itaconate triggers a defence response associated with electrophilic stress,¹²⁸⁻¹³¹ and appears to suppress glucose consumption at the expense of itaconate.¹³⁰ This dependence on itaconate as a carbon source could be exploited to decrease virulence and proliferation, wherein the inhibition of itaconate-degradation would promote starvation of the pathogen, in addition to allowing itaconate to inhibit glycolysis and Icl.

Figure 7: Bioactivation of compound 1. This involves the microbial enzymes pantothenate kinase (PanK), phosphopantetheine adenylyltransferase (PPAT), and dephospho-CoA kinase (DPCK).

Interestingly, in *Burkholderia pseudomallei*, itaconate-degrading enzymes¹¹¹ might be involved in the persistence phenotype (*i.e.* where a population of bacteria is slow-growing or non-proliferating¹³²). Upon infection with *B. pseudomallei*, a persister population arises in the host, but inhibition of Icl was reported to reactivate *B. pseudomallei*, which then proliferates rapidly and may kill the host if the Icl inhibitor is not combined with an antimicrobial.²⁰ This suggests the possibility of using itaconate-degradation inhibitors as antibiotic resensitizers against this bacterium. In support of this hypothesis, the combination of itaconate and ceftazidime was found to reduce the bacterial load of infected rats more effectively than ceftazidime alone or itaconate alone.²⁰ A similar result has been observed for the related *Burkholderia cepacia* complex; itaconate pre-treatment of the bacteria reduced the persister population, sensitizing them to tobramycin.¹³³

Unanswered questions

Itaconate is ubiquitous in nature, as revealed by its production in fungi,¹ mussels,¹³⁴ shrimp,¹³⁵ mammals,¹⁰⁻¹¹ and even some bacteria (e.g. *Bacillus subtilis*).¹³⁶ Nevertheless, its biological roles remain largely unknown. In addition to an immunomodulatory function of itaconate in mammals, several lines of evidence are supporting an antibacterial role in macrophages, including: 1) the known antibacterial activity of itaconate; 2) the reported itaconate inhibition of Icl, an enzyme essential for the survival of many pathogens in mammals; 3) the active transport of itaconate in *Salmonella*-containing vesicles; and 4) the expression of itaconate degradation enzymes in several pathogens. Questions remaining to be answered are however still numerous. Is itaconate resistance universal among intracellular pathogens? How diversified are itaconate resistance mechanisms? How does itaconate enter bacterial and mammalian cells? What is/are its bacterial target(s) *in vivo*? Does it form covalent adducts with them (as observed in mammalian cells)?¹³⁷ Answers to these questions will likely reveal several new drug targets, not only for antimicrobials but also for disorders of the immune system.^{115, 138-139}

Acknowledgements

Research in this area in the Auclair lab was supported by research grants from the Canadian Institute of Health Research (CIHR) and the Fonds de recherche du Quebec (FRQ).

References

1. Calam, C. T.; Oxford, A. E.; Raistrick, H., Studies in the biochemistry of micro-organisms: Itaconic acid, a metabolic product of a strain of *Aspergillus terreus* Thom. *Biochem J* **1939**, 33 (9), 1488-95.
2. Cunha da Cruz, J.; Machado de Castro, A.; Camporese Sérvulo, E. F., World market and biotechnological production of itaconic acid. *3 Biotech* **2018**, 8 (3), 138.
3. Finkelstein, M.; Gold, H.; Paterno, C. A., Pharmacology of itaconic acid and its sodium, magnesium, and calcium salts. *J Am Pharm Assoc Am Pharm Assoc* **1947**, 36 (6), 173-9.
4. Booth, A. N.; Taylor, J.; Wilson, R. H.; Deeds, F., The inhibitory effects of itaconic acid in vitro and in vivo. *J Biol Chem* **1952**, 195 (2), 697-702.
5. Wu, X.; Huang, X.; Zhu, Y.; Li, J.; Hoffmann, M. R., Synthesis and application of superabsorbent polymer microspheres for rapid concentration and quantification of microbial pathogens in ambient water. *Sep Purif Technol* **2020**, 239, 116540.
6. Qin, J.; Su, Z.; Mao, Y.; Liu, C.; Qi, B.; Fang, G.; Wang, S., Carboxyl-functionalized hollow polymer microspheres for detection of trace metal elements in complex food matrixes by ICP-MS assisted with solid-phase extraction. *Ecotoxicol Environ Saf* **2021**, 208, 111729.

7. Tila, D.; Yazdani-Arazi, S. N.; Ghanbarzadeh, S.; Arami, S.; Pourmoazzen, Z., pH-sensitive, polymer modified, plasma stable niosomes: promising carriers for anti-cancer drugs. *EXCLI J* **2015**, *14*, 21-32.
8. Becker, J.; Tehrani, H. H.; Ernst, P.; Blank, L. M.; Wierckx, N., An Optimized *Ustilago maydis* for Itaconic Acid Production at Maximal Theoretical Yield. *J Fungi (Basel)* **2020**, *7* (1).
9. Tran, K. T.; Somasundaram, S.; Eom, G. T.; Hong, S. H., Efficient Itaconic acid production via protein-protein scaffold introduction between GltA, AcnA, and CadA in recombinant *Escherichia coli*. *Biotechnol Prog* **2019**, *35* (3), e2799.
10. Sugimoto, M.; Sakagami, H.; Yokote, Y.; Onuma, H.; Kaneko, M.; Mori, M.; Sakaguchi, Y.; Soga, T.; Tomita, M., Non-targeted metabolite profiling in activated macrophage secretion. *Metabolomics* **2012**, *8* (4), 624-633.
11. Strelko, C. L.; Lu, W.; Dufort, F. J.; Seyfried, T. N.; Chiles, T. C.; Rabinowitz, J. D.; Roberts, M. F., Itaconic acid is a mammalian metabolite induced during macrophage activation. *J Am Chem Soc* **2011**, *133* (41), 16386-9.
12. O'Neill, L. A. J.; Artyomov, M. N., Itaconate: the poster child of metabolic reprogramming in macrophage function. *Nat Rev Immunol* **2019**.
13. Rittenhouse, J. W.; McFadden, B. A., Inhibition of isocitrate lyase from *Pseudomonas indigofera* by itaconate. *Arch Biochem Biophys* **1974**, *163* (1), 79-86.
14. McFadden, B. A.; Purohit, S., Itaconate, an isocitrate lyase-directed inhibitor in *Pseudomonas indigofera*. *J Bacteriol* **1977**, *131* (1), 136-44.
15. McKinney, J. D.; Honer zu Bentrup, K.; Munoz-Elias, E. J.; Miczak, A.; Chen, B.; Chan, W. T.; Swenson, D.; Sacchettini, J. C.; Jacobs, W. R., Jr.; Russell, D. G., Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature* **2000**, *406* (6797), 735-8.
16. Fang, F. C.; Libby, S. J.; Castor, M. E.; Fung, A. M., Isocitrate lyase (AceA) is required for *Salmonella* persistence but not for acute lethal infection in mice. *Infect Immun* **2005**, *73* (4), 2547-2549.
17. Lindsey, T. L.; Hagins, J. M.; Sokol, P. A.; Silo-Suh, L. A., Virulence determinants from a cystic fibrosis isolate of *Pseudomonas aeruginosa* include isocitrate lyase. *Microbiology (Reading)* **2008**, *154* (Pt 6), 1616-1627.
18. Wall, D. M.; Duffy, P. S.; Dupont, C.; Prescott, J. F.; Meijer, W. G., Isocitrate lyase activity is required for virulence of the intracellular pathogen *Rhodococcus equi*. *Infect Immun* **2005**, *73* (10), 6736-41.
19. Abdou, E.; Jiménez de Bagüés, M. P.; Martínez-Abadía, I.; Ouahrani-Bettache, S.; Pantesco, V.; Occhialini, A.; Al Dahouk, S.; Köhler, S.; Jubier-Maurin, V., RegA Plays a Key Role in Oxygen-Dependent Establishment of Persistence and in Isocitrate Lyase Activity, a Critical Determinant of In vivo *Brucella suis* Pathogenicity. *Front Cell Infect Microbiol* **2017**, *7*, 186.
20. van Schaik, E. J.; Tom, M.; Woods, D. E., *Burkholderia pseudomallei* isocitrate lyase is a persistence factor in pulmonary melioidosis: implications for the development of isocitrate lyase inhibitors as novel antimicrobials. *Infect Immun* **2009**, *77* (10), 4275-83.
21. Dolan, S. K.; Welch, M., The Glyoxylate Shunt, 60 Years On. *Annu Rev Microbiol* **2018**, *72*, 309-330.
22. Novotná, E.; Waisser, K.; Kuneš, J.; Palát, K.; Skálová, L.; Szotáková, B.; Buchta, V.; Stolaříková, J.; Ulmann, V.; Pávová, M.; Weber, J.; Komrsková, J.; Hašková, P.; Vokřál, I.; Wsól, V., Design, Synthesis, and Biological Evaluation of Isothiosemicarbazones with Antimycobacterial Activity. *Arch Pharm (Weinheim)* **2017**, *350* (8).
23. Krátký, M.; Vinšová, J., Advances in mycobacterial isocitrate lyase targeting and inhibitors. *Curr Med Chem* **2012**, *19* (36), 6126-37.

24. Krátký, M.; Vinšová, J.; Novotná, E.; Mandíková, J.; Wsól, V.; Trejtnar, F.; Ulmann, V.; Stolaříková, J.; Fernandes, S.; Bhat, S.; Liu, J. O., Salicylanilide derivatives block Mycobacterium tuberculosis through inhibition of isocitrate lyase and methionine aminopeptidase. *Tuberculosis (Edinb)* **2012**, *92* (5), 434-9.
25. Sriram, D.; Yogeewari, P.; Senthilkumar, P.; Sangaraju, D.; Nelli, R.; Banerjee, D.; Bhat, P.; Manjashetty, T. H., Synthesis and antimycobacterial evaluation of novel Phthalazin-4-ylacetamides against log- and starved phase cultures. *Chem Biol Drug Des* **2010**, *75* (4), 381-91.
26. Hautzel, R.; Anke, H.; Sheldrick, W. S., Mycenon, a new metabolite from a Mycena species TA 87202 (basidiomycetes) as an inhibitor of isocitrate lyase. *J Antibiot (Tokyo)* **1990**, *43* (10), 1240-4.
27. Shingnapurkar, D.; Dandawate, P.; Anson, C. E.; Powell, A. K.; Afrasiabi, Z.; Sinn, E.; Pandit, S.; Venkateswara Swamy, K.; Franzblau, S.; Padhye, S., Synthesis and characterization of pyruvate-isoniazid analogs and their copper complexes as potential ICL inhibitors. *Bioorg Med Chem Lett* **2012**, *22* (9), 3172-6.
28. Sriram, D.; Yogeewari, P.; Senthilkumar, P.; Naidu, G.; Bhat, P., 5-Nitro-2,6-dioxohexahydro-4-pyrimidinecarboxamides: synthesis, in vitro antimycobacterial activity, cytotoxicity, and isocitrate lyase inhibition studies. *J Enzyme Inhib Med Chem* **2010**, *25* (6), 765-72.
29. Krátký, M.; Volková, M.; Novotná, E.; Trejtnar, F.; Stolaříková, J.; Vinšová, J., Synthesis and biological activity of new salicylanilide N,N-disubstituted carbamates and thiocarbamates. *Bioorg Med Chem* **2014**, *22* (15), 4073-82.
30. Bae, J.; Cho, E.; Park, J. S.; Won, T. H.; Seo, S. Y.; Oh, D. C.; Oh, K. B.; Shin, J., Isocadiolides A-H: Polybrominated Aromatics from a Synoicum sp. Ascidian. *J Nat Prod* **2020**, *83* (2), 429-437.
31. Ji, L.; Long, Q.; Yang, D.; Xie, J., Identification of mannich base as a novel inhibitor of Mycobacterium tuberculosis isocitrate by high-throughput screening. *Int J Biol Sci* **2011**, *7* (3), 376-82.
32. Krátký, M.; Vinšová, J.; Novotná, E.; Stolaříková, J., Salicylanilide pyrazinoates inhibit in vitro multidrug-resistant Mycobacterium tuberculosis strains, atypical mycobacteria and isocitrate lyase. *Eur J Pharm Sci* **2014**, *53*, 1-9.
33. Kozic, J.; Novotná, E.; Volková, M.; Stolaříková, J.; Trejtnar, F.; Wsól, V.; Vinšová, J., Synthesis and in vitro antimycobacterial and isocitrate lyase inhibition properties of novel 2-methoxy-2'-hydroxybenzanilides, their thioxo analogues and benzoxazoles. *Eur J Med Chem* **2012**, *56*, 108-19.
34. Banerjee, D.; Yogeewari, P.; Bhat, P.; Thomas, A.; Srividya, M.; Sriram, D., Novel isatinyl thiosemicarbazones derivatives as potential molecule to combat HIV-TB co-infection. *Eur J Med Chem* **2011**, *46* (1), 106-21.
35. Tiwari, A.; Kumar, A.; Srivastava, G.; Sharma, A., Screening of Anti-mycobacterial Phytochemical Compounds for Potential Inhibitors against Mycobacterium Tuberculosis Isocitrate Lyase. *Curr Top Med Chem* **2019**, *19* (8), 600-608.
36. Sriram, D.; Yogeewari, P.; Methuku, S.; Vyas, D. R.; Senthilkumar, P.; Alvala, M.; Jeankumar, V. U., Synthesis of various 3-nitropropionamides as Mycobacterium tuberculosis isocitrate lyase inhibitor. *Bioorg Med Chem Lett* **2011**, *21* (18), 5149-54.
37. Krátký, M.; Jand'ourek, O.; Baranyai, Z.; Novotná, E.; Stolaříková, J.; Bősze, S.; Vinšová, J., Phenolic N-monosubstituted carbamates: Antitubercular and toxicity evaluation of multi-targeting compounds. *Eur J Med Chem* **2019**, *181*, 111578.
38. Sriram, D.; Yogeewari, P.; Vyas, D. R.; Senthilkumar, P.; Bhat, P.; Srividya, M., 5-Nitro-2-furoic acid hydrazones: design, synthesis and in vitro antimycobacterial evaluation against log and starved phase cultures. *Bioorg Med Chem Lett* **2010**, *20* (15), 4313-6.
39. McVey, A. C.; Bartlett, S.; Kajbaf, M.; Pellacani, A.; Gatta, V.; Tammela, P.; Spring, D. R.; Welch, M., 2-Aminopyridine Analogs Inhibit Both Enzymes of the Glyoxylate Shunt in Pseudomonas aeruginosa. *Int J Mol Sci* **2020**, *21* (7).
40. Sriram, D.; Yogeewari, P.; Senthilkumar, P.; Dewakar, S.; Rohit, N.; Debjani, B.; Bhat, P.; Veugopal, B.; Pavan, V. V.; Thimmappa, H. M., Novel phthalazinyl derivatives: synthesis,

antimycobacterial activities, and inhibition of Mycobacterium tuberculosis isocitrate lyase enzyme. *Med Chem* **2009**, *5* (5), 422-33.

41. Shukla, R.; Shukla, H.; Sonkar, A.; Pandey, T.; Tripathi, T., Structure-based screening and molecular dynamics simulations offer novel natural compounds as potential inhibitors of Mycobacterium tuberculosis isocitrate lyase. *J Biomol Struct Dyn* **2018**, *36* (8), 2045-2057.
42. Liu, Y.; Zhou, S.; Deng, Q.; Li, X.; Meng, J.; Guan, Y.; Li, C.; Xiao, C., Identification of a novel inhibitor of isocitrate lyase as a potent antitubercular agent against both active and non-replicating Mycobacterium tuberculosis. *Tuberculosis (Edinb)* **2016**, *97*, 38-46.
43. Shukla, R.; Shukla, H.; Tripathi, T., Structural and energetic understanding of novel natural inhibitors of Mycobacterium tuberculosis malate synthase. *J Cell Biochem* **2018**.
44. Krieger, I. V.; Freundlich, J. S.; Gawandi, V. B.; Roberts, J. P.; Gawandi, V. B.; Sun, Q.; Owen, J. L.; Fraile, M. T.; Huss, S. I.; Lavandera, J. L.; Ioerger, T. R.; Sacchettini, J. C., Structure-guided discovery of phenyl-diketo acids as potent inhibitors of M. tuberculosis malate synthase. *Chem Biol* **2012**, *19* (12), 1556-67.
45. Shukla, R.; Shukla, H.; Tripathi, T., Structure-based discovery of phenyl-diketo acids derivatives as Mycobacterium tuberculosis malate synthase inhibitors. *J Biomol Struct Dyn* **2020**, 1-14.
46. Honer Zu Bentrup, K.; Miczak, A.; Swenson, D. L.; Russell, D. G., Characterization of activity and expression of isocitrate lyase in Mycobacterium avium and Mycobacterium tuberculosis. *J Bacteriol* **1999**, *181* (23), 7161-7.
47. Reinscheid, D. J.; Eikmanns, B. J.; Sahm, H., Characterization of the isocitrate lyase gene from Corynebacterium glutamicum and biochemical analysis of the enzyme. *J Bacteriol* **1994**, *176* (12), 3474-83.
48. Hernández-Chinea, C.; Maimone, L.; Campos, Y.; Mosca, W.; Romero, P. J., Apparent isocitrate lyase activity in Leishmania amazonensis. *Acta Parasitol* **2017**, *62* (4), 701-707.
49. Schmidt, G.; Stahmann, K.-P.; Sahm, H., Inhibition of purified isocitrate lyase identified itaconate and oxalate as potential antimetabolites for the riboflavin overproducer Ashbya gossypii. *Microbiology (Reading, England)* **1996**, *142* (2), 411-417.
50. Munir, E.; Hattori, T.; Shimada, M., Purification and characterization of isocitrate lyase from the wood-destroying basidiomycete Fomitopsis palustris grown on glucose. *Arch Biochem Biophys* **2002**, *399* (2), 225-31.
51. Runquist, M.; Kruger, N. J., Control of gluconeogenesis by isocitrate lyase in endosperm of germinating castor bean seedlings. *Plant J* **1999**, *19* (4), 423-431.
52. Dang, A. Q.; Cook, D. E., Itaconic acid: An inhibitor of isocitrate lyase in Tetrahymena pyriformis in vitro and in vivo. *Biochim Biophys Acta Gen Subj* **1981**, *678* (3), 316-321.
53. Khan, F. R.; McFadden, B. A., Enzyme Profiles in Seedling Development and the Effect of Itaconate, an Isocitrate Lyase-directed Reagent. *Plant Physiol* **1979**, *64* (2), 228-231.
54. Tsukamoto, C.; Ejiri, S.-i.; Katsumata, T., Purification and Some Properties of Isocitrate Lyase from the Pollen of Pinus densiflora Sieb, et Zucc. *Agr Biol Chem* **1986**, *50* (2), 409-416.
55. Patel, T. R.; McFadden, B. A., Caenorhabditis elegans and Ascaris suum: inhibition of isocitrate lyase by itaconate. *Exp Parasitol* **1978**, *44* (2), 262-8.
56. De Lucas, J. R.; Amor, C.; Díaz, M.; Turner, G.; Laborda, F., Purification and properties of isocitrate lyase from Aspergillus nidulans, a model enzyme to study catabolite inactivation in filamentous fungi. *Mycol Res* **1997**, *101* (4), 410-414.
57. Ray, S.; Kreidler, D. F.; Gulick, A. M.; Murkin, A. S., The Nitro Group as a Masked Electrophile in Covalent Enzyme Inhibition. *ACS Chem Biol* **2018**, *13* (6), 1470-1473.
58. Sharma, V.; Sharma, S.; Hoener zu Bentrup, K.; McKinney, J. D.; Russell, D. G.; Jacobs, W. R., Jr.; Sacchettini, J. C., Structure of isocitrate lyase, a persistence factor of Mycobacterium tuberculosis. *Nat Struct Biol* **2000**, *7* (8), 663-8.

59. Kwai, B. X. C.; Collins, A. J.; Middleditch, M. J.; Sperry, J.; Bashiri, G.; Leung, I. K. H., Itaconate is a covalent inhibitor of the Mycobacterium tuberculosis isocitrate lyase. *RSC Med Chem* **2021**.
60. Duncan, D.; Lupien, A.; Behr, M. A.; Auclair, K., Effect of pH on the antimicrobial activity of the macrophage metabolite itaconate. *Microbiology (Reading)* **2021**, 167 (5).
61. Berg, I. A.; Filatova, L. V.; Ivanovsky, R. N., Inhibition of acetate and propionate assimilation by itaconate via propionyl-CoA carboxylase in isocitrate lyase-negative purple bacterium *Rhodospirillum rubrum*. *FEMS Microbiol Lett* **2002**, 216 (1), 49-54.
62. Ruetz, M.; Campanello, G. C.; Purchal, M.; Shen, H.; McDevitt, L.; Gouda, H.; Wakabayashi, S.; Zhu, J.; Rubin, E. J.; Warncke, K.; Mootha, V. K.; Koutmos, M.; Banerjee, R., Itaconyl-CoA forms a stable biradical in methylmalonyl-CoA mutase and derails its activity and repair. *Science* **2019**, 366 (6465), 589-593.
63. Naujoks, J.; Tabeling, C.; Dill, B. D.; Hoffmann, C.; Brown, A. S.; Kunze, M.; Kempa, S.; Peter, A.; Mollenkopf, H. J.; Dorhoi, A.; Kershaw, O.; Gruber, A. D.; Sander, L. E.; Witzernath, M.; Herold, S.; Nerlich, A.; Hocke, A. C.; van Driel, I.; Suttorp, N.; Bedoui, S.; Hilbi, H.; Trost, M.; Opitz, B., IFNs Modify the Proteome of Legionella-Containing Vacuoles and Restrict Infection Via IRG1-Derived Itaconic Acid. *PLoS Pathog* **2016**, 12 (2), e1005408.
64. Hammerer, F.; Chang, J. H.; Duncan, D.; Castaneda Ruiz, A.; Auclair, K., Small Molecule Restores Itaconate Sensitivity in Salmonella enterica: A Potential New Approach to Treating Bacterial Infections. *ChemBioChem* **2016**, 17 (16), 1513-7.
65. Bellion, E.; Kelley, R. L., Inhibition by itaconate of growth of methylotrophic bacteria. *J Bacteriol* **1979**, 138 (2), 519-22.
66. Michelucci, A.; Cordes, T.; Ghelfi, J.; Pailot, A.; Reiling, N.; Goldmann, O.; Binz, T.; Wegner, A.; Tallam, A.; Rausell, A.; Buttini, M.; Linster, C. L.; Medina, E.; Balling, R.; Hiller, K., Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. *Proc Natl Acad Sci U S A* **2013**, 110 (19), 7820-5.
67. Shimamoto, G.; Berk, R. S., Taurine catabolism. III. Evidence for the participation of the glyoxylate cycle. *Biochim Biophys Acta* **1980**, 632 (3), 399-407.
68. Hillier, S.; Charnetzky, W. T., Glyoxylate bypass enzymes in Yersinia species and multiple forms of isocitrate lyase in Yersinia pestis. *J Bacteriol* **1981**, 145 (1), 452-8.
69. Stratford, M.; Anslow, P. A., Evidence that sorbic acid does not inhibit yeast as a classic 'weak acid preservative'. *Lett Appl Microbiol* **1998**, 27 (4), 203-6.
70. Lee, I. Y.; Gruber, T. D.; Samuels, A.; Yun, M.; Nam, B.; Kang, M.; Crowley, K.; Winterroth, B.; Boshoff, H. I.; Barry, C. E., 3rd, Structure-activity relationships of antitubercular salicylanilides consistent with disruption of the proton gradient via proton shuttling. *Bioorg Med Chem* **2013**, 21 (1), 114-26.
71. Hersch, S. J.; Navarre, W. W., The Salmonella LysR family regulator, RipR, activates the SPI-13 encoded itaconate degradation cluster. *Infect Immun* **2020**.
72. van Beilen, J. W.; Teixeira de Mattos, M. J.; Hellingwerf, K. J.; Brul, S., Distinct effects of sorbic acid and acetic acid on the electrophysiology and metabolism of *Bacillus subtilis*. *Appl Environ Microbiol* **2014**, 80 (19), 5918-26.
73. Krebs, H. A.; Wiggins, D.; Stubbs, M.; Sols, A.; Bedoya, F., Studies on the mechanism of the antifungal action of benzoate. *Biochem J* **1983**, 214 (3), 657-63.
74. Piper, P. W., Yeast superoxide dismutase mutants reveal a pro-oxidant action of weak organic acid food preservatives. *Free Radic Biol Med* **1999**, 27 (11-12), 1219-27.
75. Romick, T. L.; Fleming, H. P., Acetoin production as an indicator of growth and metabolic inhibition of *Listeria monocytogenes*. *J Appl Microbiol* **1998**, 84 (1), 18-24.
76. Zhu, X.; Lei, H.; Wu, J.; Li, J. V.; Tang, H.; Wang, Y., Systemic responses of BALB/c mice to Salmonella typhimurium infection. *J Proteome Res* **2014**, 13 (10), 4436-45.

77. Alpuche Aranda, C. M.; Swanson, J. A.; Loomis, W. P.; Miller, S. I., *Salmonella typhimurium* activates virulence gene transcription within acidified macrophage phagosomes. *Proc Natl Acad Sci U S A* **1992**, *89* (21), 10079-83.
78. Chen, M.; Sun, H.; Boot, M.; Shao, L.; Chang, S. J.; Wang, W.; Lam, T. T.; Lara-Tejero, M.; Rego, E. H.; Galán, J. E., Itaconate is an effector of a Rab GTPase cell-autonomous host defense pathway against *Salmonella*. *Science* **2020**, *369* (6502), 450-455.
79. Lee, C. G.; Jenkins, N. A.; Gilbert, D. J.; Copeland, N. G.; O'Brien, W. E., Cloning and analysis of gene regulation of a novel LPS-inducible cDNA. *Immunogenetics* **1995**, *41* (5), 263-70.
80. Thomas, D. M.; Francescutti-Verbeem, D. M.; Kuhn, D. M., Gene expression profile of activated microglia under conditions associated with dopamine neuronal damage. *FASEB J* **2006**, *20* (3), 515-7.
81. Basler, T.; Jeckstadt, S.; Valentin-Weigand, P.; Goethe, R., *Mycobacterium paratuberculosis*, *Mycobacterium smegmatis*, and lipopolysaccharide induce different transcriptional and post-transcriptional regulation of the IRG1 gene in murine macrophages. *J Leukoc Biol* **2006**, *79* (3), 628-38.
82. Degrandi, D.; Hoffmann, R.; Beuter-Gunia, C.; Pfeiffer, K., The proinflammatory cytokine-induced IRG1 protein associates with mitochondria. *J Interferon Cytokine Res* **2009**, *29* (1), 55-67.
83. Tangsudjai, S.; Pudla, M.; Limposuwan, K.; Woods, D. E.; Sirisinha, S.; Utaisincharoen, P., Involvement of the MyD88-independent pathway in controlling the intracellular fate of *Burkholderia pseudomallei* infection in the mouse macrophage cell line RAW 264.7. *Microbiol Immunol* **2010**, *54* (5), 282-90.
84. Gautam, A.; Dixit, S.; Philipp, M. T.; Singh, S. R.; Morici, L. A.; Kaushal, D.; Dennis, V. A., Interleukin-10 alters effector functions of multiple genes induced by *Borrelia burgdorferi* in macrophages to regulate Lyme disease inflammation. *Infect Immun* **2011**, *79* (12), 4876-92.
85. Bentley, R.; Thiessen, C. P., Biosynthesis of itaconic acid in *Aspergillus terreus*. I. Tracer studies with C14-labeled substrates. *J Biol Chem* **1957**, *226* (2), 673-87.
86. Cordes, T.; Michelucci, A.; Hiller, K., Itaconic Acid: The Surprising Role of an Industrial Compound as a Mammalian Antimicrobial Metabolite. *Annu Rev Nutr* **2015**, *35*, 451-73.
87. Luan, H. H.; Medzhitov, R., Food Fight: Role of Itaconate and Other Metabolites in Antimicrobial Defense. *Cell Metab* **2016**, *24* (3), 379-387.
88. Ryan, D. G.; O'Neill, L. A. J., Krebs cycle rewired for macrophage and dendritic cell effector functions. *FEBS Lett* **2017**, *591* (19), 2992-3006.
89. Williams, N. C.; O'Neill, L. A. J., A Role for the Krebs Cycle Intermediate Citrate in Metabolic Reprogramming in Innate Immunity and Inflammation. *Front Immunol* **2018**, *9*, 141.
90. Nonnenmacher, Y.; Hiller, K., Biochemistry of proinflammatory macrophage activation. *Cell Mol Life Sci* **2018**, *75* (12), 2093-2109.
91. Yu, X. H.; Zhang, D. W.; Zheng, X. L.; Tang, C. K., Itaconate: an emerging determinant of inflammation in activated macrophages. *Immunol Cell Biol* **2019**, *97* (2), 134-141.
92. Patil, N. K.; Bohannon, J. K.; Hernandez, A.; Patil, T. K.; Sherwood, E. R., Regulation of leukocyte function by citric acid cycle intermediates. *J Leukoc Biol* **2019**, *106* (1), 105-117.
93. Ryan, D. G.; Murphy, M. P.; Frezza, C.; Prag, H. A.; Chouchani, E. T.; O'Neill, L. A.; Mills, E. L., Coupling Krebs cycle metabolites to signalling in immunity and cancer. *Nat Metab* **2019**, *1*, 16-33.
94. Hooftman, A.; O'Neill, L. A. J., The Immunomodulatory Potential of the Metabolite Itaconate. *Trends Immunol* **2019**, *40* (8), 687-698.
95. Viola, A.; Munari, F.; Sánchez-Rodríguez, R.; Scolaro, T.; Castegna, A., The Metabolic Signature of Macrophage Responses. *Front Immunol* **2019**, *10*, 1462.
96. Zasłona, Z.; O'Neill, L. A. J., Cytokine-like Roles for Metabolites in Immunity. *Mol Cell* **2020**, *78* (5), 814-823.
97. Wu, R.; Chen, F.; Wang, N.; Tang, D.; Kang, R., ACOD1 in immunometabolism and disease. *Cell Mol Immunol* **2020**, *17* (8), 822-833.

98. Li, R.; Zhang, P.; Wang, Y.; Tao, K., Itaconate: A Metabolite Regulates Inflammation Response and Oxidative Stress. *Oxid Med Cell Longev* **2020**, *2020*, 5404780.
99. Cordes, T.; Metallo, C. M., Exploring the evolutionary roots and physiological function of itaconate. *Curr Opin Biotechnol* **2020**, *68*, 144-150.
100. Lampropoulou, V.; Sergushichev, A.; Bambouskova, M.; Nair, S.; Vincent, E. E.; Loginicheva, E.; Cervantes-Barragan, L.; Ma, X.; Huang, S. C.; Griss, T.; Weinheimer, C. J.; Khader, S.; Randolph, G. J.; Pearce, E. J.; Jones, R. G.; Diwan, A.; Diamond, M. S.; Artyomov, M. N., Itaconate Links Inhibition of Succinate Dehydrogenase with Macrophage Metabolic Remodeling and Regulation of Inflammation. *Cell Metab* **2016**, *24* (1), 158-66.
101. Nair, S.; Huynh, J. P.; Lampropoulou, V.; Loginicheva, E.; Esaulova, E.; Gounder, A. P.; Boon, A. C. M.; Schwarzkopf, E. A.; Bradstreet, T. R.; Edelson, B. T.; Artyomov, M. N.; Stallings, C. L.; Diamond, M. S., Irg1 expression in myeloid cells prevents immunopathology during M. tuberculosis infection. *J Exp Med* **2018**, *215* (4), 1035-1045.
102. Brightman, V.; Martin, W. R., PATHWAY FOR THE DISSIMILATION OF ITACONIC AND MESACONIC ACIDS. *J Bacteriol* **1961**, *82* (3), 376-82.
103. Martin, W. R.; Frigan, F.; Bergman, E. H., Noninductive metabolism of itaconic acid by *Pseudomonas* and *Salmonella* species. *J Bacteriol* **1961**, *82* (6), 905-8.
104. Shimi, I. R.; Nour El Dein, M. S., Catabolism of itaconic acid by preformed mats of *Aspergillus terreus*. *Arch Mikrobiol* **1962**, *41*, 261-7.
105. Cooper, R. A.; Kornberg, H. L., The utilization of itaconate by *Pseudomonas* sp. *Biochem J* **1964**, *91* (1), 82-91.
106. Cooper, R. A.; Itiaba, K.; Kornberg, H. L., THE UTILIZATION OF ACONATE AND ITACONATE BY MICROCOCCUS SP. *Biochem J* **1965**, *94* (1), 25-31.
107. Chen, M.; Huang, X.; Zhong, C.; Li, J.; Lu, X., Identification of an itaconic acid degrading pathway in itaconic acid producing *Aspergillus terreus*. *Appl Microbiol Biotechnol* **2016**, *100* (17), 7541-8.
108. Wang, H.; Fedorov, A. A.; Fedorov, E. V.; Hunt, D. M.; Rodgers, A.; Douglas, H. L.; Garza-Garcia, A.; Bonanno, J. B.; Almo, S. C.; de Carvalho, L. P. S., An essential bifunctional enzyme in *Mycobacterium tuberculosis* for itaconate dissimilation and leucine catabolism. *Proc Natl Acad Sci U S A* **2019**, *116* (32), 15907-15913.
109. Adler, J.; Wang, S. F.; Lardy, H. A., The metabolism of itaconic acid by liver mitochondria. *J Biol Chem* **1957**, *229* (2), 865-79.
110. Wang, S. F.; Adler, J.; Lardy, H. A., The pathway of itaconate metabolism by liver mitochondria. *J Biol Chem* **1961**, *236*, 26-30.
111. Sasikaran, J.; Ziemski, M.; Zadora, P. K.; Fleig, A.; Berg, I. A., Bacterial itaconate degradation promotes pathogenicity. *Nat Chem Biol* **2014**, *10* (5), 371-7.
112. Torres, R.; Swift, R. V.; Chim, N.; Wheatley, N.; Lan, B.; Atwood, B. R.; Pujol, C.; Sankaran, B.; Bliska, J. B.; Amaro, R. E.; Goulding, C. W., Biochemical, structural and molecular dynamics analyses of the potential virulence factor RipA from *Yersinia pestis*. *PLoS One* **2011**, *6* (9), e25084.
113. Torres, R.; Lan, B.; Latif, Y.; Chim, N.; Goulding, C. W., Structural snapshots along the reaction pathway of *Yersinia pestis* RipA, a putative butyryl-CoA transferase. *Acta Crystallogr D Biol Crystallogr* **2014**, *70* (Pt 4), 1074-85.
114. Torres, R.; Chim, N.; Sankaran, B.; Pujol, C.; Bliska, J. B.; Goulding, C. W., Structural insights into RipC, a putative citrate lyase β subunit from a *Yersinia pestis* virulence operon. *Acta Crystallogr Sect F Struct Biol Cryst Commun* **2012**, *68* (Pt 1), 2-7.
115. Shen, H.; Campanello, G. C.; Flicker, D.; Grabarek, Z.; Hu, J.; Luo, C.; Banerjee, R.; Mootha, V. K., The Human Knockout Gene CLYBL Connects Itaconate to Vitamin B12. *Cell* **2017**, *171* (4), 771-782.e11.

116. Goulding, C. W.; Bowers, P. M.; Segelke, B.; Lekin, T.; Kim, C. Y.; Terwilliger, T. C.; Eisenberg, D., The structure and computational analysis of Mycobacterium tuberculosis protein CitE suggest a novel enzymatic function. *J Mol Biol* **2007**, *365* (2), 275-83.
117. Pham, T. V.; Murkin, A. S.; Moynihan, M. M.; Harris, L.; Tyler, P. C.; Shetty, N.; Sacchettini, J. C.; Huang, H. L.; Meek, T. D., Mechanism-based inactivator of isocitrate lyases 1 and 2 from Mycobacterium tuberculosis. *Proc Natl Acad Sci U S A* **2017**, *114* (29), 7617-7622.
118. Williams, J. O.; Roche, T. E.; McFadden, B. A., Mechanism of action of isocitrate lyase from Pseudomonas indigofera. *Biochemistry* **1971**, *10* (8), 1384-90.
119. Nolte, J. C.; Schurmann, M.; Schepers, C. L.; Vogel, E.; Wubbeler, J. H.; Steinbuchel, A., Novel characteristics of succinate coenzyme A (Succinate-CoA) ligases: conversion of malate to malyl-CoA and CoA-thioester formation of succinate analogues in vitro. *Appl Environ Microbiol* **2014**, *80* (1), 166-76.
120. Rosenberg, G.; Yehezkel, D.; Hoffman, D.; Mattioli, C. C.; Fremder, M.; Ben-Arosh, H.; Vainman, L.; Nissani, N.; Hen-Avivi, S.; Brenner, S.; Itkin, M.; Malitsky, S.; Ohana, E.; Ben-Moshe, N. B.; Avraham, R., Host succinate is an activation signal for Salmonella virulence during intracellular infection. *Science* **2021**, *371* (6527), 400-405.
121. Duncan, D.; Auclair, K., The coenzyme A biosynthetic pathway: A new tool for prodrug bioactivation. *Arch Biochem Biophys* **2019**, *672*, 108069.
122. Duncan, D.; Chang, J. H.; Artyomov, M. N.; Auclair, K., Small molecule resensitizes Salmonella to itaconate and decreases bacterial proliferation in macrophages. *bioRxiv* **2020**, 2020.07.15.204305.
123. Barnard, L.; Mostert, K. J.; van Otterlo, W. A. L.; Strauss, E., Developing Pantetheinase-Resistant Pantothenamide Antibacterials: Structural Modification Impacts on PankK Interaction and Mode of Action. *ACS Infect Dis* **2018**, *4* (5), 736-743.
124. Howieson, V. M.; Tran, E.; Hoegl, A.; Fam, H. L.; Fu, J.; Sivonen, K.; Li, X. X.; Auclair, K.; Saliba, K. J., Triazole Substitution of a Labile Amide Bond Stabilizes Pantothenamides and Improves Their Antiplasmodial Potency. *Antimicrob Agents Chemother* **2016**, *60* (12), 7146-7152.
125. Guan, J.; Tjhin, E. T.; Howieson, V. M.; Kittikool, T.; Spry, C.; Saliba, K. J.; Auclair, K., Structure-Activity Relationships of Antiplasmodial Pantothenamide Analogues Reveal a New Way by Which Triazoles Mimic Amide Bonds. *ChemMedChem* **2018**, *13* (24), 2677-2683.
126. Spry, C.; Barnard, L.; Kok, M.; Powell, A. K.; Mahesh, D.; Tjhin, E. T.; Saliba, K. J.; Strauss, E.; de Villiers, M., Toward a Stable and Potent Coenzyme A-Targeting Antiplasmodial Agent: Structure-Activity Relationship Studies of N-Phenethyl- α -methyl-pantothenamide. *ACS Infect Dis* **2020**, *6* (7), 1844-1854.
127. Riquelme, S. A.; Prince, A., Airway immunometabolites fuel Pseudomonas aeruginosa infection. *Respir Res* **2020**, *21* (1), 326.
128. Juarez, P.; Jeannot, K.; Plésiat, P.; Llanes, C., Toxic Electrophiles Induce Expression of the Multidrug Efflux Pump MexEF-OprN in Pseudomonas aeruginosa through a Novel Transcriptional Regulator, CmrA. *Antimicrob Agents Chemother* **2017**, *61* (8).
129. Wongsaroj, L.; Saninjak, K.; Romsang, A.; Duang-Nkern, J.; Trinachartvanit, W.; Vattanaviboon, P.; Mongkolsuk, S., Pseudomonas aeruginosa glutathione biosynthesis genes play multiple roles in stress protection, bacterial virulence and biofilm formation. *PLoS One* **2018**, *13* (10), e0205815.
130. Riquelme, S. A.; Liimatta, K.; Wong Fok Lung, T.; Fields, B.; Ahn, D.; Chen, D.; Lozano, C.; Sáenz, Y.; Uhlemann, A. C.; Kahl, B. C.; Britto, C. J.; DiMango, E.; Prince, A., Pseudomonas aeruginosa Utilizes Host-Derived Itaconate to Redirect Its Metabolism to Promote Biofilm Formation. *Cell Metab* **2020**, *31* (6), 1091-1106.e6.
131. Bambouskova, M.; Gorvel, L.; Lampropoulou, V.; Sergushichev, A.; Loginicheva, E.; Johnson, K.; Korenfeld, D.; Mathyer, M. E.; Kim, H.; Huang, L.-H.; Duncan, D.; Bregman, H.; Keskin, A.; Santeford, A.; Apte, R. S.; Sehgal, R.; Johnson, B.; Amarasinghe, G. K.; Soares, M. P.; Satoh, T.; Akira, S.; Hai, T.; de Guzman Strong, C.; Auclair, K.; Roddy, T. P.; Biller, S. A.; Jovanovic, M.; Klechevsky, E.; Stewart, K. M.;

- Randolph, G. J.; Artyomov, M. N., Electrophilic properties of itaconate and derivatives regulate the I κ B ζ -ATF3 inflammatory axis. *Nature* **2018**, 556 (7702), 501-504.
132. Fisher, R. A.; Gollan, B.; Helaine, S., Persistent bacterial infections and persister cells. *Nat Rev Microbiol* **2017**, 15 (8), 453-464.
133. Van Acker, H.; Sass, A.; Bazzini, S.; De Roy, K.; Udine, C.; Messiaen, T.; Riccardi, G.; Boon, N.; Nelis, H. J.; Mahenthiralingam, E.; Coenye, T., Biofilm-grown *Burkholderia cepacia* complex cells survive antibiotic treatment by avoiding production of reactive oxygen species. *PLoS One* **2013**, 8 (3), e58943.
134. Sendra, M.; Saco, A.; Rey-Campos, M.; Novoa, B.; Figueras, A., Immune-responsive gene 1 (IRG1) and dimethyl itaconate are involved in the mussel immune response. *Fish Shellfish Immunol* **2020**, 106, 645-655.
135. Alfaro, A. C.; Nguyen, T. V.; Bayot, B.; Rodriguez Leon, J. A.; Domínguez-Borbor, C.; Sonnenholzner, S., Metabolic responses of whiteleg shrimp to white spot syndrome virus (WSSV). *J Invertebr Pathol* **2021**, 180, 107545.
136. Chun, H. L.; Lee, S. Y.; Lee, S. H.; Lee, C. S.; Park, H. H., Enzymatic reaction mechanism of cis-aconitate decarboxylase based on the crystal structure of IRG1 from *Bacillus subtilis*. *Sci Rep* **2020**, 10 (1), 11305.
137. Qin, W.; Qin, K.; Zhang, Y.; Jia, W.; Chen, Y.; Cheng, B.; Peng, L.; Chen, N.; Liu, Y.; Zhou, W.; Wang, Y. L.; Chen, X.; Wang, C., S-glycosylation-based cysteine profiling reveals regulation of glycolysis by itaconate. *Nat Chem Biol* **2019**, 15 (10), 983-991.
138. Ko, J. H.; Olona, A.; Papathanassiou, A. E.; Buang, N.; Park, K. S.; Costa, A. S. H.; Mauro, C.; Frezza, C.; Behmoaras, J., BCAT1 affects mitochondrial metabolism independently of leucine transamination in activated human macrophages. *J Cell Sci* **2020**, 133 (22).
139. Ogger, P. P.; Albers, G. J.; Hewitt, R. J.; O'Sullivan, B. J.; Powell, J. E.; Calamita, E.; Ghai, P.; Walker, S. A.; McErlean, P.; Saunders, P.; Kingston, S.; Molyneaux, P. L.; Halket, J. M.; Gray, R.; Chambers, D. C.; Maher, T. M.; Lloyd, C. M.; Byrne, A. J., Itaconate controls the severity of pulmonary fibrosis. *Sci Immunol* **2020**, 5 (52).