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Basic Mechanisms of Immunometabolites in Shaping the Immune Response

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Abstract
Background: Innate immune cells play a crucial role in responding to microbial infections, but their improper activation can also drive inflammatory disease. For this reason, their activation state is governed by a multitude of factors, including the metabolic state of the cell and, more specifically, the individual metabolites which accumulate intracellularly and extracellularly. This relationship is bidirectional, as innate immune cell activation by pathogen-associated molecular patterns causes critical changes in cellular metabolism. ***Summary:*** In this review, we describe the emergence of various "immunometabolites." We outline the general characteristics of these immunometabolites, the conditions under which they accumulate, and their subsequent impact on immune cells. We delve into well-studied metabolites of recent years, such as succinate and itaconate, as well as newly emerging immunometabolites, such as methylglyoxal. ***Key Messages:*** We hope that this review may be used as a framework for further studies dissecting the mechanisms by which immunometabolites regulate the immune system and provide an outlook to harnessing these mechanisms in the treatment of inflammatory diseases.

Introduction

Metabolites have traditionally been perceived as building blocks and intermediates in energy production and the synthesis of macromolecules, accumulating passively according to the metabolic demands of the cell. However, this viewpoint is now considered somewhat misguided. The complexity of metabolism in multicellular organisms necessitates signalling mechanisms for intercellular communication and the coordination of metabolic fluxes in response to environmental fluctuations. It is increasingly recognized that metabolites themselves, whether acting at the local or systemic level, exhibit signalling activities that fine-tune homoeostatic responses in both physiological and pathological conditions. This concept of metabolites as signalling molecules has been elegantly addressed in a recent review article, which we would direct the reader's attention to for a more general overview on this topic [1]. Herein, we will specifically explore the role of several well-defined intermediary metabolite signalling modalities in immunity while focussing on the innate immune system. We will introduce this by first defining the characteristics of "immunometabolites." This concept is akin to the emergence of "oncometabolites," which promote or contribute to tumour biology [2].

While the impact of metabolism on innate immune cell function has been a subject of long-standing consideration, early studies often oversimplified these metabolic changes as merely arising from the increased metabolic demands

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associated with innate immune cell activation [3, 4]. The discovery of mTOR as a kinase which synchronizes nutrient sensing with immune cell effector function [5] stimulated interest from immunologists in the field of metabolism, and the inhibitor of mTOR, rapamycin, provided early evidence of the potential therapeutic utility in targeting metabolic pathways [6, 7]. Over the years, the description of the metabolic changes occurring upon innate immune cell activation has become slightly more nuanced and stimulus-dependent. Generally speaking, classical activation of macrophages with lipopolysaccharide (LPS) drives an increase in glycolysis, while IL-4-stimulated alternatively activated macrophages are predominantly dependent on oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) [8]. The emergence of this field of "immunometabolism" has been greatly aided by the development of technologies to profile cells metabolically, including metabolomics, metabolic flux analyses, flow cytometry, and respirometry assays, which can subsequently be applied to a variety of models of infection and inflammation. The discoveries of various immunometabolites which will be described in this review have been greatly aided by the use of these methods. More recently, the emergence of mass spectrometry-based assays to study metabolite-protein interactions has placed further emphasis on the role that individual metabolites play as signalling molecules. These techniques include MIDAS [9], TPP [10], and LiP-SMap [11], among others, and will be invaluable tools in the further delineation of the mechanisms of metabolite-driven processes.

In this review, we will begin by broadly defining the properties of an immunometabolite before discussing the immunometabolites succinate, lactate, itaconate, fumarate, and α-ketoglutarate within this framework. We will also provide an overview of some newly emerging and historical immunometabolites, such as methylglyoxal and kynurenine, which may gain traction over the coming years.

Properties of Signalling Immunometabolites

Here, we define an "immunometabolite" as any intermediary metabolite whose change in abundance affects a response within immune cells or is secreted by non-immune cells to influence immune cell behaviour during the course of an infection or immune response. Of particular interest are metabolites whose primary mechanism of signal transduction is via non-covalent binding of protein effectors that govern innate immune cell behaviours, although in some cases, covalent interactions may also play a role. It is important to note that many of these metabolites serve signalling functions beyond the immune system, and our classification does not diminish their importance or imply exclusive relevance to immune responses compared to other biological processes. The immunometabolites under discussion fall under the umbrella of metabolic signalling, which encompasses metabolite signals associated with, arising from, or controlling intermediary metabolism [1]. Consequently, we will not delve into hormone signalling or second messengers used by classical G protein-coupled receptors (GPCRs), such as cyclic AMP (cAMP), or the emerging field of signalling cyclic dinucleotides (CDNs) produced downstream of cytosolic innate immune receptors, notably cGAS and cGAS-like effector proteins, whose primary function is as messengers and have been reviewed elsewhere [12, 13].

The metabolites we will primarily focus on are predominantly intermediates or products of the tricarboxylic acid (TCA) cycle, illuminating the important connection between mitochondrial metabolic signalling and immune cell function. Additionally, we will discuss the role of lactate and other emerging intermediary metabolite signals in this context. Often, the discussed immunometabolites engage in what can be described as classical intracellular metabolite signalling mechanisms, such as product inhibition or inhibition via a safety valve. However, in some instances, they can be secreted to engage specific receptors or are taken up by immune cells in the local microenvironment for incorporation as metabolic substrates. Changes in the abundance of these immunometabolites can occur through various mechanisms, including transcriptional up- or downregulation of enzymes involved in their production, alterations in metabolic fluxes, or through promiscuous and neomorphic enzyme activity. We have summarized these properties in Table 1. Please note that this list does not aim to be exhaustive, nor does it provide an in-depth analysis of each individual metabolite. Instead, our goal is to underscore the significance of metabolic signalling in immune cell function through clear examples.

Lactate

Lactate was first described in mammalian muscle tissue in the 19th century [14]. When immune cells upregulate glycolysis to facilitate effector function, lactate is produced as a by-product from lactate dehydrogenase (LDH)-mediated dehydrogenation of pyruvate to L-lactate, also producing NADH from NAD^+ [15]. Additionally, several tissue environments such as the tumour microenvironment are very rich in lactate due to the "Warburg effect" [16, 17]. As the accumulation of lactate at millimolar concentrations [18–20] has been found to have numerous effects on immune cell activation and function both intracellularly and extracellularly, lactate is

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Table 1. Signalling immunometabolites and their properties

Metabolite	Main pathway(s)	Immune cell behaviour impacted	Abundance regulation (key enzymes)	Abundance regulation (nutrient flux)	Intracellular signalling mechanism	Intracellular targets	Extracellular signalling mechanism
Succinate	TCA cycle	Macrophages, T cells	SDH/CII activity	Glutamine anaplerosis	Product inhibition	PHDs, α-KGDDs	Receptor agonist (SUCNR1/GPR91)
Fumarate	TCA cycle	Macrophages, DCs, T cells	FH suppression, ASS1 increase	Glutamine anaplerosis	Product inhibition, PTM (succination)	PHDs, α-KGDDs + many	N/A
Itaconate	TCA cycle-derived	Macrophages, neutrophils, MDSCs, T cells	IRG1/ACOD1 increase	Glucose, glutamine anaplerosis	Product inhibition, PTM (2,3-dicarboxypropylation), Allostery*	SDH/CII, PHDs, α-KGDDs + many	Receptor agonist (OXGR1)
α-KG	TCA cycle	Macrophages	OGDH, IDH, GOT1/2 activity	Glutamine anaplerosis	Substrate provision	PHDs, α-KGDDs	N/A
L-2-HG	TCA cycle-derived, glycolysis	Macrophages, T cells	IDH, MDH, LDH (promiscuous activity), L2HGDH	Glutamine anaplerosis	Product inhibition, competitive inhibition	PHDs, α-KGDDs	N/A
D-2-HG	TCA cycle-derived	Macrophages, T cells	IDH, (neomorphic activity), HOT, D2HGDH	Glutamine anaplerosis	Product inhibition	PHDs, α-KGDDs, LDH	N/A
Lactate	Glycolysis	Macrophages, T cells	LDH, activity	Glucose, pyruvate, lactate uptake	Allostery, PTM (lactoylation), acidification	Histones, NDRG3, MAVS, SENP1	Receptor agonist (GPR81)
MGO	Glycolysis	Macrophages	GLO1, GLO2	Glucose, aminoacetone	PTM (glycation), Metabolite modification	Many, arginine depletion	N/A
LGSH	Glycolysis	Macrophages	GLO1, GLO2	Glucose	PTM (lactoylation)	Histones	N/A
KYN	Tryptophan catabolism, NAD^+ synthesis	Monocytes, macrophages	IDO1	Tryptophan uptake	Ligand	AhR	N/A
KYNA	Tryptophan catabolism, NAD^+ synthesis	Monocytes, macrophages	IDO1, KAT	Tryptophan uptake	Ligand	AhR	Receptor agonist (GPR35), receptor antagonist (NMDA etc)

PTM, post-translational modification.

now considered to be a signalling metabolite, given its widespread regulation of gene transcription, rather than a mere waste product [18–20]. Upon lactate production, it is transported across the cell membrane via proton-linked monocarboxylate symporters (MCT1, MCT2, MCT3, and MCT4) as well as sodium-coupled lactate co-transporters (SLC5A12 and SLC5A8) [21, 22]. From the extracellular environment, numerous cell types can use lactate as a fuel, sustaining pyruvate production for the TCA cycle [23, 24].

As lactate began to emerge as an immunometabolite over a decade ago, early reports found that it had the ability to regulate immune cell polarization states [25–28]. Mechanistically, lactate has several modes of action which are context and cell-type specific. It is able to acidify the extracellular environment [25], interact with proteins, act as an extracellular signal via GPR81, as well as modify lysine residues through a post-translational modification known as "lactylation" [29, 30]. Histone lysine lactylation regulates gene transcription in murine and human cells, specifically M2-like genes such as *Arg1* [29]. Recently, this mechanism was found to be important in the regulation of macrophage polarization, as mitochondrial fragmentation increased lactate levels to drive *Arg1* via lactylation [31]. Additionally, lactylation has now been linked to autoimmunity as lactylation of IKZF1 was found to promote Th17 polarization which exacerbated experimental autoimmune uveitis [32]. Early work identified lactate as a key signal in the tumour microenvironment due to its ability to promote an M2 phenotype in infiltrating macrophages through hypoxia-inducible factor-1α (HIF-1α) stabilization. Further studies on lactate demonstrate that the upregulation of lactate in hypoxic cells leads to an interaction with NDRG3, preventing its PHD2/VHL-dependent degradation and resulting in the activation of the raf-ERK pathway to promote angiogenesis and cell growth [33]. The activation of ERK signalling in this manner may be responsible for some of the immunoregulatory roles of lactate as ERK activation has been found to support macrophage development and function [34, 35].

A notable description of lactate as a signalling immunometabolite was the discovery that lactate suppresses mitochondrial antiviral signalling protein (MAVS) activation [36]. Lactate was found to associate with and inhibit MAVS aggregation, thereby promoting its interaction with hexokinase 2 (HK2) to support glycolytic activity. Double-stranded RNA (dsRNA) sensing by RIG-I-like receptors (RLRs), resulting in MAVS oligomerisation, disrupts the association of MAVS and HK2, thereby impairing glycolytic activity and further decreasing lactate production in a positive feedback manner. A contradictory study found that lactate is able to suppress glycolytic enzymes in diverse tissues [37], indicating that the effect of lactate on glycolysis may be cell-type specific. Lactate-mediated suppression of glycolysis as well as its ability to acidify media has also been found to decrease TNF and IL-6 production, suggesting a role for lactate in preventing M1 polarization [38]. Consistent with this, lactate has also been found to decrease NF-κB activation in response to LPS, independent of its acidic properties, leading to a reduced release of TNF, IL-23, and other pro-inflammatory cytokines and chemokines [39]. A similar phenotype was reported in a separate study and found to be GPR81-dependent [40].

Recently, lactate has also been reported to promote cell cycle progression through an interaction with SENP1 to activate the anaphase-promoting complex, driving cell cycle progression [41]. This may have implications in activated immune cells which are rich in lactate and often require rapid proliferation during infection and inflammatory responses.

Succinate

Succinate is a TCA cycle intermediate which is metabolized by succinate dehydrogenase (SDH) to fumarate and is an early textbook example of a signalling immunometabolite. While succinate was a well-characterized oncometabolite in tumours [42], the first report of succinate as a pro-inflammatory intracellular signal was made only around a decade ago [43].

There are numerous diverse mechanisms of action for this metabolite that contribute to its overall role as an immunometabolite. As α-ketoglutarate-dependent dioxygenases (α-KGDDs) convert α-KG to succinate, succinate impairs the activity of α-KGDDs through product inhibition. There are several α-KGDDs involved in regulating immune responses such as prolyl hydroxylases (PHDs), ten-eleven translocases (TETs), histone demethylases (KDMs), and fat mass and obesity-associated protein (FTO) [44]. Inhibition of these enzymes includes the prevention of HIF-1α degradation leading to a hypoxic response, the prevention of DNA and histone demethylation, and the prevention of RNA demethylation. Extracellular succinate is also able to act as an extracellular signal by activating the GPCR GPR91 which was an orphan receptor until 2004 [45].

In macrophages, succinate is currently regarded as a pro-inflammatory signalling immunometabolite. When macrophages sense bacterial pathogen-associated molecular patterns, succinate is a metabolite which accumulates highly [43]. Recent studies have found that the

accumulation of succinate in macrophages is dependent on aconitate decarboxylase 1 (ACOD1)-mediated production of itaconate, a known competitive inhibitor of SDH [46–48]. Upon accumulation, succinate exits the cytosol via dicarboxylate transporters [43]. It then impairs PHDs and stabilizes HIF-1α, resulting in the transcriptional upregulation of IL-1β and other targets including glycolytic enzymes [49].

Importantly, the high accumulation of succinate through enhanced TCA cycle activity during early LPS stimulation and glutaminolysis leads to high levels of succinate oxidation by SDH [50]. Increased SDH activity results in levels of hydroquinone ($CoQH_2$) which are unable to be efficiently consumed by complex III. These are then oxidized to quinone (CoQ) by complex I in a compensatory mechanism known as reverse electron transport (RET) which generates high levels of mitochondrial reactive oxygen species (mtROS) in contexts such as ischaemia-reperfusion [51] and immunity [50, 52]. Within macrophages, mtROS production has numerous immunoregulatory functions [53, 54]. Notably, mtROS is also able to impair PHDs, resulting in HIF-1α stabilization and transcriptional upregulation of IL-1β, providing another mechanism for the pro-inflammatory role of succinate. Dimethyl malonate (DMM) is a well-described inhibitor of SDH and was shown to inhibit the generation of ROS production and IL-1β in LPS-activated macrophages [50]. The endogenously produced metabolite, itaconate, was also shown to inhibit IL-1β and ROS production [47]. However, as there are other mechanisms by which itaconate may reduce IL-1β expression [55], it is unclear how much of a role SDH inhibition may contribute to its overall immunoregulatory activities, as discussed in more detail below.

Several recent studies have reported that succinate is selectively able to be protonated in an acidic environment, resulting in a monocarboxylic acid, whereas most other similar metabolites are unable to be efficiently protonated in mildly acidic environments [56, 57]. This results in its ability to be actively transported through monocarboxylate transporters such as MCT1 to cross the cell membrane. Although neither study which made this finding focused on immune cells, it is certainly possible that immune cells, which often operate in acidic environments such as the tumour microenvironment, could secrete large amounts of protonated succinate. This is important as, within the extracellular space, succinate regulates immunity through activation of GPR91 [58].

Early studies on succinate implicated important roles for GPR91 signalling in regulating myeloid cell function [58]. Dendritic cells (DCs) were found to have a high expression of GPR91, and succinate signalling via its receptor was found to promote chemotaxis and cytokine production [59]. Therefore, mice lacking GPR91 exhibited dampened T-cell activation and weaker allograft rejection responses relative to their wild-type counterparts. Further studies have also found a role for GPR91 in regulating DC migration in a murine model of rheumatoid arthritis [60]. This study found that succinate signalling promoted DC trafficking to lymph nodes, which allowed for T helper 17 (Th17) cell activation. Additionally, a previous study had shown that succinate production from macrophages signals through GPR91 in an autocrine and paracrine manner to promote IL-1β release and tissue inflammation in rheumatoid arthritis [61]. Finally, it has recently also been shown that SDH-deficient tumours which accumulate and release succinate drive the polarization of tumour-associated macrophages. Tumour cell-secreted succinate binds macrophage GPR91, leading to PI3K/AKT/HIF-1α activation and polarization into a TAM state [62]. While most studies have identified pro-inflammatory roles for succinate and GPR91 signalling, a recent study has identified potentially anti-inflammatory roles for this signalling axis as GPR91-deficient macrophages exhibited increased pro-inflammatory cytokine production [63]. This indicates that there may be context-dependent effects of succinate and GPR91 signalling that contribute to their immunoregulatory roles.

Itaconate

Although the emergence of itaconate as an immunometabolite has been rapid and recent, its initial discovery and synthesis date back as far as 1836 [64], and since the 1960s, its synthesis by fermentation in various fungal species has been exploited for industrial purposes. Itaconate is an unsaturated dicarboxylic acid, a property which is responsible for its nature as an electrophile which can covalently modify thiol-reactive cysteine residues in a post-translational modification termed 2,3-dicarboxypropylation. Indeed, this characteristic of itaconate is responsible for its antimicrobial effects [65, 66], which were described long before its immunomodulatory role became apparent. Itaconate covalently modifies isocitrate lyase [67], an enzyme possessed by various bacteria including *Salmonella enterica* and *Mycobacterium tuberculosis* (Mtb) which is involved in the glyoxylate shunt, inhibition of which restricts bacterial growth. As well as being directly antimicrobial, itaconate can also modify transcription factor EB (TFEB), thereby driving lysosomal biogenesis and facilitating bacterial clearance [68].

Although itaconate is not a TCA cycle metabolite, its accumulation is dependent on Krebs cycle function as its synthesis from the decarboxylation of an intermediate, *cis*-aconitate, is catalysed by the enzyme immune-responsive gene 1 (IRG1), also known as aconitate decarboxylase 1 (ACOD1) [69]. A critical observation which actually predated the discovery of IRG1 as the itaconate-producing enzyme was that *Irg1* expression in a murine macrophage cell line could be upregulated by stimulation with the TLR4 ligand LPS [70]. The upregulation of *Irg1* was subsequently shown to be at least partly dependent on downstream type I interferon (IFN) signalling [55, 71], meaning that itaconate accumulation can occur during stimulation with a variety of pattern recognition receptor agonists. Acute iron deprivation, which occurs during pathogen infection, has also been shown to result in itaconate accumulation [72], strengthening the notion that itaconate is an inflammation-induced metabolite which may have an important role in host defence. The accumulation of itaconate appears to be restricted to myeloid cells, although itaconate secreted from myeloid cells may exert effects on neighbouring adaptive immune cells. For example, myeloid-derived suppressor cell (MDSC)-derived itaconate suppresses $CD8^+$ T-cell proliferation and function [73]. Itaconate can also influence T-cell differentiation by suppressing Th17 cell development while promoting regulatory T (Treg) cell expansion [74]. As Treg cell differentiation is dependent on FAO, these effects may be related to the stabilization of the mitochondrial fatty acid uptake enzyme carnitine palmitoyltransferase 1a (CPT1a) by itaconate [75].

The importance of extracellular secreted itaconate raises the prospect that some of these effects may be receptor-mediated. To date, the only receptor identified for itaconate is the GPCR OXGR1, ligation of which drives mucociliary clearance in respiratory epithelial cells [76]. This was not the first report of itaconate playing a role in the lung, as it was also shown to have an anti-fibrotic effect in models of pulmonary fibrosis [77] and has more recently been shown to stimulate ketogenesis in lung fibroblasts, facilitating disease tolerance during *Pseudomonas aeruginosa* infection [78].

The use of itaconate derivatives, such as dimethyl itaconate (DMI) and 4-octyl itaconate (4-OI), has been widespread in the study of the immunomodulatory properties of itaconate, and care should be taken when interpreting these studies given the potential off-target effects of these compounds. Where possible, we will clarify where certain mechanisms have been attributed to derivatives rather than unmodified itaconate, and we have displayed the various effects described using unmodified itaconic acid, *Irg1* overexpression/knockout models, and itaconate derivatives in Figure 1. Inhibition of SDH, activation of nuclear factor 2 erythroid 2-related factor 2 (NRF2), inhibition of glycolysis, and inhibition of the NLRP3 inflammasome are the best-described mechanisms by which itaconate modulates the innate immune response, and we will outline these in the following sections.

Inhibition of SDH by itaconate was first described over 70 years ago [79, 80], and subsequent studies have been able to reframe these findings in the context of innate immunity. Oxidation of succinate to fumarate by SDH promotes macrophage activation upon LPS sensing through the generation of mtROS and stabilization of hypoxia-inducible factor (HIF) [81]. Itaconate, much like the SDH inhibitor malonate [82], competitively inhibits SDH, thereby reducing the LPS-induced upregulation of specific cytokines including IL-1β, IL-6, and IL-12 [46, 47]. These studies were predominantly conducted using DMI but subsequently verified using unmodified itaconate and macrophages lacking IRG1 [48]. Inhibition of SDH by itaconate has been shown to contribute to the onset of innate immune tolerance, a state in which innate immune cells are less responsive upon restimulation with activating stimuli such as LPS. This process can be counteracted by treatment with the fungal cell wall component β-glucan, which reduces *IRG1* expression and itaconate accumulation, in a process called trained immunity [83]. Conversely, DMI, in the absence of other stimuli, can actually increase the responsiveness of human monocytes to secondary stimulation with LPS by increasing ROS production, and it also protected mice from secondary infection with *Staphylococcus aureus* [84].

As mentioned previously, many of the immunomodulatory functions of itaconate can be linked to its nature as a cysteine-modifying compound. Kelch-like ECH-associated protein (KEAP1), which usually sequesters and ubiquitinates the master antioxidant transcription factor NRF2 [85], is a sensor of oxidative stress and as such possesses several reactive cysteines which may be targeted by electrophiles such as itaconate [86]. 4-OI was found to modify several cysteines on KEAP1, of which C151 was identified as being critical for the 4-OI-driven induction of NRF2 [55]. However, given its reduced electrophilicity relative to derivatized itaconate, the ability of unmodified itaconate to exert the same effect has been the subject of much discussion [48]. Reduced activation of NRF2 has been observed in *Irg1*$^{-/-}$ macrophages [87], and modification of various cysteines on

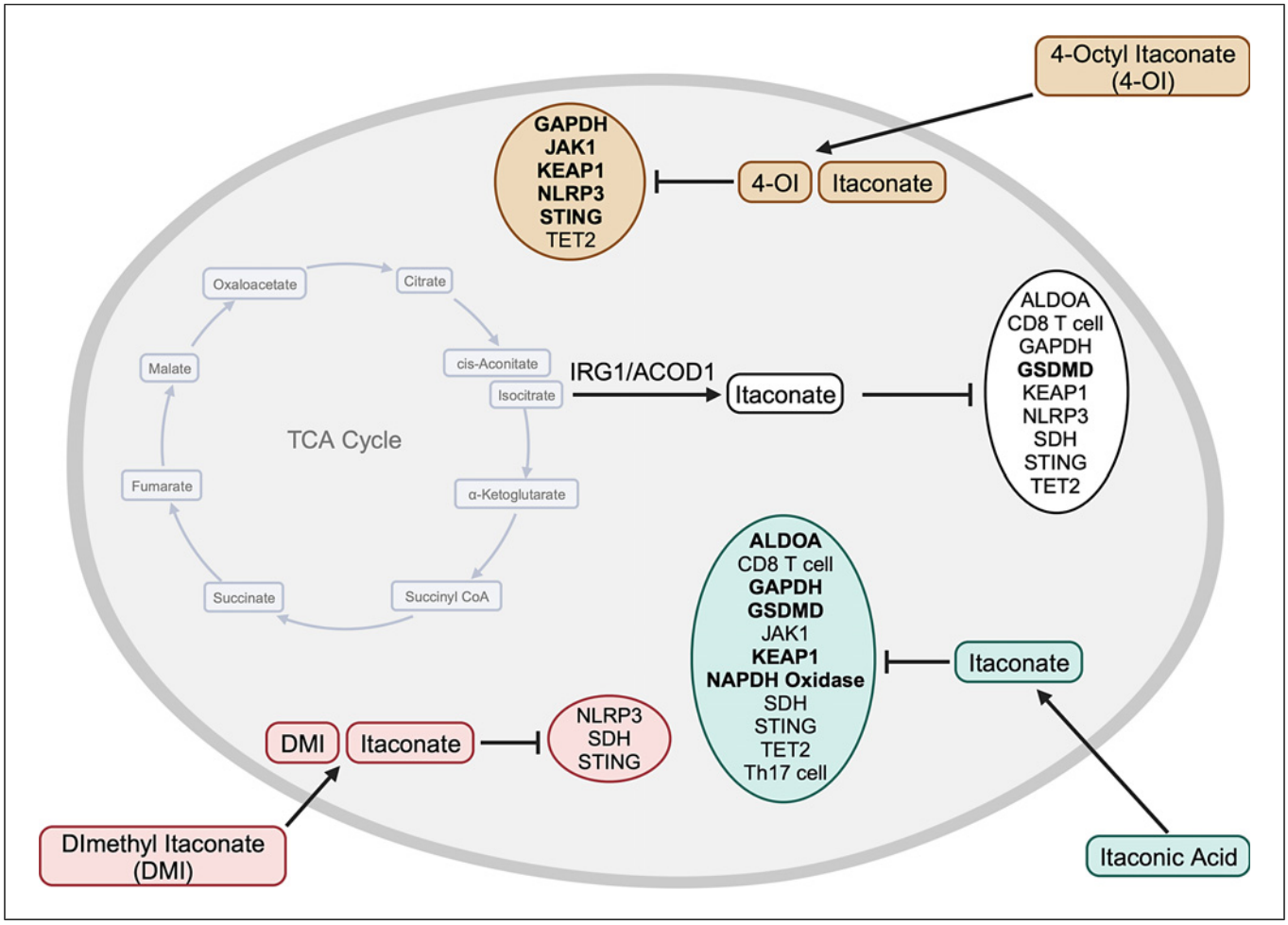

Fig. 1. Immunomodulatory effects described with different models of itaconate manipulation. Protein targets listed in bold denote covalent modifications (created with BioRender.com).

KEAP1 has been demonstrated with itaconate, although not on C151 [88]. Regardless of any disagreement in this area, the activation of NRF2 is clearly critical to the anti-inflammatory properties of 4-OI, including the suppression of IL-1β [55], stimulator of interferon genes (STING) [89], and type I IFN release, respectively [90]. Direct cysteine modification of STING by 4-OI, preventing its phosphorylation, has also been reported [91, 92]. NRF2 activation by unmodified itaconate has been demonstrated to occur in the livers of exercised mice, thereby protecting from hepatic ischemia/reperfusion injury through the promotion of an anti-inflammatory transcriptional program in liver-resident Kupffer cells [93]. More recently, the itaconate/KEAP1/NRF2 axis has been targeted in the context of anti-tumour immunity, through the generation of pro-inflammatory anti-tumour chimeric antigen receptor-expressing macrophages (CAR-iMACs) [94]. IRG1-depleted CAR-iMACs exhibited reduced NRF2 activation due to reduced itaconate levels, resulting in an increase in ROS production, phagocytosis, and ultimately cancer cell killing [94].

Various itaconated cysteines have been uncovered using chemoproteomic profiling. These include aldolase A (ALDOA) [95] and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [96], both of which function as enzymes in glycolysis and were found to be targeted by unmodified itaconate. The glycolytic shift observed in classically activated macrophages is required to support cytokine release, and the inhibition of glycolysis by itaconate was found to be essential to its inhibition of IL-1β [95, 96]. Transcriptional upregulation of IL-1β is followed by its processing into a smaller active subunit and release from the cell, processes which are regulated by a multi-protein complex called the NLRP3 inflammasome [97]. The NLRP3 inflammasome is

also subject to regulation by itaconate, which inhibits cytokine release and pyroptosis resulting from inflammasome activation [48, 98, 99]. Although modification of C548 of NLRP3 and C77 of the pyroptosis executioner protein gasdermin D (GSDMD) were identified following 4-OI [99] and itaconate [98] treatment, respectively, it is unclear whether these modifications occur endogenously during NLRP3 activation. Nevertheless, these reports are particularly exciting given that the NLRP3 inflammasome has been implicated in numerous inflammatory diseases, and various inhibitors of NLRP3 are currently in clinical development [100].

The effects of itaconate and its derivatives are not restricted to classical macrophage activation. 4-OI and unmodified itaconate were also shown to block IL-4-induced alternative macrophage activation, potentially through modification of janus kinase 1 (JAK1) [101], which functions together with signal transducer and activator of transcription 6 (STAT6) to drive alternative macrophage activation. Again, modification of JAK1 was only detected with itaconate derivatives. Interestingly, the cysteine targeted by 4-OI has also been shown to be susceptible to allosteric regulation by covalent modification [102]. Thus, while their cysteine-modifying capabilities render itaconate derivatives promiscuous in the proteins that they target, they may be unrepresentative of unmodified itaconate. The clearest example of this is the activation of activating transcription factor 3 (ATF3) and concomitant inhibition of NF-κB inhibitor zeta (IκBζ), a transcription factor which controls secondary transcriptional responses to LPS [87]. The ability to drive this axis is directly associated with the electrophilic strength of the compound in question [48], thus activation of this axis is observed with itaconate derivatives but not unmodified itaconate. One mechanism of immunomodulation which does not involve cysteine modification is the inhibition of the DNA dioxygenase ten-eleven translocation family member 2 (TET2) [103]. TET2 is an epigenetic modifier which catalyses the conversion of methylcytosine to 5-hydroxymethylcytosine, a modification which promotes the expression of LPS-induced genes. TET2 function requires α-ketoglutarate as a cofactor which, given its structural similarity, can be outcompeted by itaconate on the same binding site. Inhibition of various LPS-induced inflammatory genes by itaconate was found to be TET2-dependent [103].

As well as impacting macrophage and T-cell function, itaconate has been demonstrated to have profound effects on neutrophil function. A key weapon in the armoury of neutrophils in combating *Staphylococcus aureus* infection is the oxidative burst, which generates ROS through the activity of NADPH oxidase [104]. *S. aureus* infection of neutrophils drives an accumulation of itaconate, which suppresses the oxidative burst through covalent modification of NADPH oxidase, thereby restraining bacterial killing [105]. These observations were consistent with a prior report, which described the effect of 4-OI in blocking neutrophil ROS production and the formation of neutrophil extracellular traps (NETs) [106], which also contribute to the innate immune response by trapping and immobilizing pathogens. There is a growing appreciation of the role that neutrophils play in the tumour microenvironment, as tumour-infiltrating neutrophils (TINs) correlate strongly with poor outcome in multiple cancers. The upregulation of ACOD1 and the resulting accumulation of itaconate protect TINs from ferroptosis in an NRF2-dependent manner, promoting TIN persistence and cancer metastasis [107].

One outstanding question in the itaconate field is whether itaconate is actually the terminal effector metabolite in the pathway, or does itaconate catabolism also play a role? The CoA derivative of itaconate, itaconyl CoA, is an inhibitor of the human and Mtb enzyme methylmalonyl CoA mutase (MUT) [108, 109]. The consequences of this inhibition include the inactivation of host vitamin B_{12} [108] and inhibition of Mtb growth in macrophages [109]. Two isomers of itaconate, mesaconate, and citraconate, which differ in the position of their carbon-carbon double bond, accumulate in activated macrophages and have also been identified as potential immunomodulators [110]. Mesaconate accumulation is dependent on the presence of IRG1 [111], while citraconate accumulation appears to be independent of the itaconate pathway [112]. Despite their structural similarity and the fact that they affect LPS-induced cytokine responses in a similar manner to itaconate, few of the previously described mechanisms are shared between the 3 isomers. Relative to itaconate, mesaconate and citraconate are weak inhibitors of SDH activity, while citraconate is the strongest activator of NRF2, likely due to its increased electrophilicity [112, 113]. Some of citraconate's immunomodulatory effects may be mediated by inhibition of ACOD1 enzyme activity or alternatively may represent a feedback mechanism to regulate itaconate levels. Intriguingly, this represents the first description of an endogenous ACOD1 inhibitor, which clearly warrants further investigation.

Fumarate

The dicarboxylic acid fumarate is an intermediate of the TCA cycle, generated from the oxidation of succinate by SDH and further metabolized through its hydration to

malate by the enzyme fumarate hydratase (FH), encoded by the gene *Fh1*. Fumarate is frequently described as an "oncometabolite." Hereditary inactivating mutations in FH and resultant accumulation of fumarate are implicated in the onset of certain smooth muscle tumours and a highly aggressive form of kidney cancer, collectively termed hereditary leiomyomatosis and renal cell cancer (HLRCC) [114]. Its nature as an oncometabolite has been attributed to its ability to competitively inhibit the α-ketoglutarate (α-KG)-dependent dioxygenases (αKGDDs), causing stabilization of HIF as well as driving various epigenetic alterations which can promote tumorigenesis [115, 116]. Its tumour-promoting effects may also be partly attributable to its ability to suppress $CD8^+$ T-cell function and subsequent anti-tumour immune responses via inhibition of zeta-chain associated protein kinase 70 (ZAP70) [117], a kinase which plays a critical role in T-cell receptor (TCR) signalling. While the oncogenic properties of fumarate have been the subject of numerous fascinating studies, these have been capably reviewed elsewhere [118, 119], and herein we will focus on the growing body of evidence supporting an immunomodulatory role for fumarate.

At earlier time points of macrophage activation (e.g., 4 h LPS), the accumulation of fumarate in activated macrophages is fuelled by increased glutamine anaplerosis and an aspartate-argininosuccinate shunt, which is partly driven by increased expression of argininosuccinate synthase 1 (ASS1) [120, 121]. This is then supplemented by transcriptional suppression of *Fh1* at later time points (e.g., 24 h LPS) [121]. Although the accumulation of fumarate upon inflammation was only elucidated recently, the story of fumarate as an immunomodulator can be traced back to the late 1950s, when topical and oral administration of fumarate esters were used in the treatment of psoriasis [122]. This was followed by the development of more standardized oral formulations of these esters, including dimethyl fumarate (DMF) under the trade name Fumaderm®, which is licensed for the treatment of psoriasis [123]. Further indications for the use of DMF were explored, resulting in its licensing by the US Food and Drug Administration (FDA) for the treatment of relapsing-remitting multiple sclerosis (MS) [124], under the brand name Tecfidera®. Given its overwhelming success as an anti-inflammatory therapeutic with Tecfidera® reaching annual revenue of over $5 billion, what are the mechanisms supporting these effects?

Firstly, it is important to again clarify that differences exist between the effects of unmodified fumarate and fumarate derivatives such as DMF. Much like itaconate, fumarate is an unsaturated dicarboxylic acid which is capable of reacting with nucleophilic cysteine residues in a modification termed succination. Therefore, derivatization of the metabolite can make it more promiscuous in the cysteines that it reacts with. Given its numerous reactive cysteines, KEAP1 is also a target of fumarate, leading to the formation of 2-succinyl cysteine (2SC) adducts on multiple cysteines in KEAP1 [125]. The resulting activation of NRF2 has been deemed important for its neuroprotective effects in treating MS [126]. These neuroprotective effects may also be dependent on direct modulation of dendritic cell maturation and function, as fumarate and derivatives have been shown to reduce the activation of IL-12- and IL-23-producing dendritic cells while promoting the maturation of tolerogenic IL-10-producing dendritic cells [127, 128]. IL-12 and IL-23 production by dendritic cells is critical to the development of pathogenic T helper cells, an axis which is associated with disease progression in psoriasis and MS [129, 130]. Inhibition of this axis may be through the covalent modification of the NF-κB member p65 [131], although this effect appears to be DMF specific [132].

KEAP1 is not the only shared covalent target between itaconate and fumarate. Fumarate was found to modify GAPDH in vitro and in vivo, as evidenced by the presence of 2SC adducts on C149 and C244 of rat GAPDH [133]. These results were built on a seminal paper describing how modification of GAPDH blocked the glycolytic shift in murine macrophages activated with LPS [134]. Both DMF, and its active derivative monomethyl fumarate (MMF) which is the predominant form detected in serum, modified the active site cysteine C152, and this was also detected in peripheral blood mononuclear cells (PBMCs) obtained from MS patients on DMF therapy. Inhibition of LPS-induced IL-1β expression in murine macrophages was dependent on the modification of GAPDH, indicating that this is an important mechanistic insight into the anti-inflammatory nature of fumarate derivatives [134]. GSDMD is a pore-forming protein which is cleaved into its active form upon inflammasome activation, subsequently inserting into the cell membrane and thereby driving pyroptosis, an inflammatory form of cell death, and permitting the release of the inflammasome-driven cytokines IL-1β and IL-18 [135, 136]. DMF was found to block pyroptosis and IL-1β release through modification of C192 on GSDMD [137], which is again similar to what has been described for itaconate [98]. Although the authors detected modification of multiple cysteines with DMF treatment, it was modification of C192 specifically which prevented oligomerization of the GSDMD N-terminal fragment, indispensable for its pore-forming function [137]. Use of a

specific, competitive inhibitor of FH [138], which augments endogenous fumarate levels, also blocked pyroptosis but did not cause modification of C192 [137].

However, fumarate should not be pigeonholed as an anti-inflammatory metabolite. The process of trained immunity is underpinned by changes in innate immune cell metabolism, typically an increase in glycolysis, which subsequently cause epigenetic modifications that alter the responsiveness to activating stimuli [139]. Increased glutaminolysis during trained immunity also leads to an accumulation of fumarate which boosts TNF and IL-6 release upon LPS restimulation [140]. Using MMF, the authors showed that fumarate could promote the activating H3K4me3 mark at the promoters of these cytokines. This was proposed to occur through reduced activity of lysine-specific demethylase 5 (KDM5) [140]. DMF, MMF, and FH inhibition also increased TNF release from LPS-stimulated macrophages in results from a more recent publication, yet this was attributed to the fumarate-mediated dysregulation of an AP-1/IL-10/TNF axis [121]. This publication also placed FH at the centre of a mitochondrial retrograde signalling cascade, whereby inhibition or knockout of FH in macrophages led to mitochondrial dysfunction and the release of double-stranded mitochondrial RNA (mtdsRNA), thereby amplifying LPS-induced type I IFN release. The aberrant release of type I IFN is pathogenic in certain inflammatory disorders such as systemic lupus erythematosus (SLE). Indeed, *FH* expression was found to be suppressed in whole blood samples isolated from SLE patients, indicating that FH suppression and associated mtdsRNA signalling may contribute to disease pathogenesis in SLE [121]. These results were broadly consistent with a separate publication by Zecchini et al., which described the fumarate- and sorting nexin 9 (SNX9)-dependent release of mitochondrial-derived vesicles (MDVs) containing mtDNA, which subsequently drove a cGAS-STING-dependent type I IFN response in kidney epithelial cells. The authors placed these results in the context of *FH*-deficient cancers, describing a type I IFN transcriptional signature in tumour tissue harvested from HLRCC patients [141]. These papers, in combination, highlight the importance of fumarate and FH in regulating inflammation and raise new awareness of the context-dependent roles that fumarate may play. The endogenous accumulation of fumarate, as described in these papers, may be a means of communicating mitochondrial dysfunction which occurs during infection or cancer. As such, fumarate may be seen as a danger signal which couples mitochondrial health to the mounting of an innate immune response. This is juxtaposed to the use of more electrophilic fumarate derivatives which could be viewed as being anti-inflammatory, given their ability to inactivate pro-inflammatory proteins such as GSDMD and GAPDH.

α-Ketoglutarate (α-KG)

α-KG is a dicarboxylic acid generated from isocitrate in the third step of the TCA cycle through isocitrate dehydrogenase (IDH), a reaction referred to as oxidative decarboxylation [142]. The process of α-KG formation is crucial as it yields the first molecule of NADH and is a rate-limiting step due to IDH's allosteric regulation by citrate and ADP (activation) or ATP (inhibition). Additionally, α-KG can be synthesized sequentially through glutamine anaplerosis. In this pathway, glutamine is converted to glutamate by the enzyme glutaminase (GLS), and then glutamate is further transformed into α-KG through glutamate dehydrogenase (GDH)-mediated oxidative deamination. α-KG also serves as a vital substrate/product of the cytosolic and mitochondrial aspartate aminotransferases, namely glutamic-oxaloacetic transaminase 1/2 (GOT1/2). As such, the abundance of α-KG can be modulated across subcellular compartments, placing it at the intersection of central carbon and amino acid metabolism.

In addition to its role in the TCA cycle, α-KG is an important substrate for the αKGDDs. Notable examples of aKGDD members include the prolyl hydroxylases (PHDs), the Jumonji-C-domain-containing histone demethylases (JMJDs, also KDM2-7), and the TET1-3 family of 5 mC hydroxylases. These enzymes are involved in various processes, including HIF-1α stability, histone demethylation, and DNA demethylation, respectively. Glutamine incorporation into the TCA cycle, known as glutamine anaplerosis, represents an essential metabolic module of alternatively activated macrophages [120]. α-KG has been found to suppress the pro-inflammatory phenotype of classically activated macrophages and support endotoxin tolerance [143]. Mechanistically, maintaining a low α-KG/succinate ratio is required to prevent PHD-mediated hydroxylation of IKK-β, which in turn suppresses NF-κB nuclear translocation in pro-inflammatory macrophages. In contrast, an increase in the α-KG/succinate ratio following IL-4 stimulation supports anti-inflammatory gene expression via JMJD3-mediated histone demethylation.

Furthermore, adipocyte-derived exosomal α-KG also alleviates obesity-associated adipose tissue inflammation in response to melatonin. This effect is mediated by TET-dependent DNA demethylation in adipose tissue macrophages. [144]. More recently, endothelial-derived Rspondin3 has been found to promote anti-inflammatory interstitial macrophage activation to dampen lung inflammation and

injury [145]. The authors subsequently demonstrated that this anti-inflammatory macrophage state is driven by β-catenin-mediated glutamine anaplerosis, α-KG production, and TET2-mediated DNA demethylation. In summary, α-KG serves as an excellent example of how substrate provision via alterations in metabolic fluxes acts as a signal to shape innate immune responses.

2-Hydroxyglutarate (2-HG)

2-HG is a dicarboxylic acid derived from α-KG. While it is considered a metabolic intermediate associated with the TCA cycle, it does not actively participate in this central metabolic pathway or in energy transformation. 2-HG exists in two enantiomeric forms, L-2-HG (also known as S-2-HG) and D-2-HG (also known as R-2-HG). The conversion of these enantiomers back to α-KG is regulated by two mitochondrially localized enzymes, L-2-HG dehydrogenase (L2HGDH) and D-2-HG dehydrogenase (D2HGDH) [142]. These enantiomers are typically limited in abundance in normal tissues, but they can accumulate under specific environmental conditions.

The synthesis of L-2-HG and D-2-HG is intricate and involves both specific enzymes and promiscuous or neomorphic enzyme activities. L-2-HG can be synthesized from α-KG by promiscuous enzyme activity of malate dehydrogenase 1 and 2 (MDH1/2) or lactate dehydrogenase A and C (LDHA/C) [146–149]. This promiscuity is reportedly promoted by hypoxic and acidic pH microenvironments [147–149]. D-2-HG, on the other hand, is a product of mitochondrial hydroxyacid-oxacid transhydrogenase (HOT), which uses α-KG as an electron acceptor to metabolize β-hydroxybutyrate to succinic semialdehyde [150]. D-2-HG can also be synthesized by cytosolic IDH1 or mitochondrial IDH2 with single-point mutations in R132 or R172, respectively [151, 152]. These gain-of-function mutations result in neomorphic IDH activity due to increased affinity for NADPH, which promotes α-KG reduction at the expense of isocitrate. IDH1 and IDH2 are among the most frequently mutated metabolic enzymes in human cancers, and the accumulation of D-2-HG in certain tumours prompted the adoption of the term "oncometabolite." While the role of D-2-HG as an oncometabolite has been an intense area of focus for the field of cancer metabolism, an unanticipated role for L- and D-2-HG enantiomers as regulators of innate and adaptive immunity has now begun to emerge.

Recent reports on inflammatory macrophages have identified an increase in both L-2-HG and D-2-HG enantiomers following TLR4 activation [150, 153]. D-2-HG levels were found to be the most abundant in both studies, likely owing to the induction of HOT and reduced D2HGDH expression. Similarly, L-2-HG accumulation following LPS stimulation was linked to a decrease in L2HGDH levels. Despite the fact that both enantiomers are reported to inhibit α-KGDDs, though to varying extents [154, 155], they elicit distinct responses in inflammatory macrophages. L-2-HG was found to support glycolytic reprogramming and the expression of the pro-inflammatory cytokine IL1β, likely via PHD inhibition and HIF-1α stabilization [153]. In contrast, D-2-HG was found to act as a negative regulator of inflammatory signalling in late-stage LPS responses, independently of α-KGDDs [150]. While these studies shed light on the accumulation of 2-HG enantiomers during macrophage activation and highlight the importance of metabolite signalling in the regulation of innate immunity, several questions remain unanswered. The specific enzyme(s) responsible for L-2-HG production in macrophages are yet to be identified. The mechanism by which D-2-HG reduces the production of certain pro-inflammatory cytokines, such as TNF-α and IL-6, or how HOT induction impacts inflammatory signalling, is also an area for future investigation.

Prior to the discovery of 2-HG enantiomers in macrophages, the divergent impact of L-2-HG and D-2-HG was observed in adaptive immunity. T cell subtypes undergo dramatic metabolic reprogramming that instructs their proliferation, differentiation, and effector functions [156]. Th17 cells are a subset of $CD4^+$ T cells with important roles in the protection against infection and in promoting autoimmunity. Importantly, Th17 differentiation from naïve $CD4^+$ T cells requires a HIF-1α and RORγt-mediated switch from oxidative phosphorylation to glycolysis. In contrast, induced Treg cells (iTregs), which are an essential immunosuppressive $Foxp3^+$ T cell subset, differentiate in a HIF-1α-independent manner. D-2-HG levels are reported to increase during Th17 differentiation relative to iTregs owing to elevated GOT1-mediated α-KG production [157]. This subsequently leads to increased DNA methylation and the suppression of the *Foxp3* locus. D-2-HG treatment or TET1/2 silencing also initiated Th17 cell differentiation from $CD4^+$ T cells, which suggests a causal link between D-2-HG accumulation and an inhibition of TET-mediated DNA methylation. Furthermore, inhibition of GOT1 with aminooxyacetic acid (AOAA) decreased D-2-HG levels, promoted differentiation of Th17 cells to iTregs, and ameliorated disease progression in experimental autoimmune encephalomyelitis (EAE), a model of MS. This study identified a key role for 2-HG signalling in instructing cell fate and/or function while also suggesting that therapeutic strategies to lower its levels may be applicable to Th17-mediated diseases.

However, these findings have recently come under scrutiny as GOT1-deficient $CD4^+$ T cells produce more IL-17A and exhibit reduced *Foxp3* expression [158].

Furthermore, the impact of AOAA was confirmed in GOT1-deficient $CD4^+$ T cells, raising concerns of off-target effects. This discrepancy was countered by the finding that chronically deleting GOT1 leads to major metabolic compensation, including an increase in another transaminase, glutamic-pyruvic transaminase 2 (GPT2). In contrast, acute deletion of GOT1 using a tamoxifen-inducible $Got1^{fl/fl}$Cd4-creERT2 model recapitulated the study's previous findings [159]. As such, current evidence suggests this GOT1-D-2-HG-epigenetic mechanism is indeed operative in Th17 cells.

Similarly, the loss of complex III activity in haematopoietic stem cells (HSCs) or iTregs results in the accumulation of 2-HG [160, 161]. While L-2-HG was reported to increase in HSCs, the contribution of each enantiomer to 2-HG in iTregs was not defined. In the case of HSCs, increased L-2-HG impaired their differentiation, whereas iTregs lost their suppressive function without impairments in *Foxp3* expression, proliferation, or survival. In both cases, elevated 2-HG coincides with histone and DNA hypermethylation, which was likely to mediate the observed impact of complex III deficiency on cellular function.

An intriguing discovery is the secretion of D-2-HG into the tumour microenvironment (TME) by IDH1-mutant tumours and its uptake by T cells, which impairs anti-tumour immunity. This effect was first described in the context of IDH1-mutant glioma, where D-2-HG was found to impair T cell activation by inhibiting ATP-dependent TCR signalling, polyamine biosynthesis, and calcium-dependent nuclear factor of activated T-cell (NFAT) activity [162]. Similarly, a later study confirmed the inhibitory effect of D-2-HG on cytotoxic $CD8^+$ T cells. In this case, D-2-HG, but not L-2-HG, altered $CD8^+$ T cell metabolism and impaired anti-tumour functions via inhibition of LDH [163]. These studies therefore attributed a novel, non-tumour cell-autonomous role to an oncometabolite in shaping the TME to promote tumorigenesis. In contrast to the inhibitory role of D-2HG on anti-tumour T cell immunity, L-2-HG is reported to increase following TCR stimulation in $CD8^+$ T cells. $CD8^+$ T cells are highly dependent on the activation of HIF-1α and glycolysis, and increased L-2-HG occurred in a HIF-1α and LDHA-dependent manner. L-2-HG enhanced the in vivo proliferation, persistence, and anti-tumour capacity of adoptively transferred $CD8^+$ T cells through modulation of their histone and DNA methylation landscape. More recently, inhibition of IDH2-dependent reductive carboxylation in $CD8^+$ T cells has been shown to increase 2-HG, fumarate, and succinate levels and induce features of memory T cells during ex vivo manufacturing of chimeric antigen receptor (CAR) T cells [164]. Importantly, this improves CAR T cell anti-tumour activity via an epigenetic-dependent mechanism. In summary, these studies highlight a crucial role for 2-HG enantiomer signalling in governing cell fate decisions and behaviour under different (patho)physiological contexts and illustrate the importance of metabolite signalling in the regulation of adaptive immunity, which may be co-opted for therapeutic gain.

Emerging and Historical Metabolites in Immune Signalling

Methylglyoxal and S-D-Lactoylglutathione

Methylglyoxal (MGO) is a highly reactive dicarbonyl compound formed as a by-product of glycolysis. Recent research suggests that MGO has the potential to act as an immunomodulatory metabolic intermediate [165]. Approximately 90% of MGO is believed to originate from the non-enzymatic degradation of glyceraldehyde 3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP). It is also reported to increase from acetol by acetone/actol monooxygenase (AMO) or aminoacetone by semicarbazide-sensitive amine oxidase (SSAO) [165, 166]. MGO reacts with proteins to form advanced glycation end products (AGEs). These AGEs accumulate in immune cells in inflamed tissues, such as during infections and sepsis. To counter the toxicity of MGO, the cytosolic glyoxalase system metabolizes MGO to D-lactate. The glyoxalase system is composed of two enzymes, glyoxalase I (GLO1) and glyoxalase II (GLO2), along with glutathione (GSH). GSH reacts with MGO to form hemithioacetal, which GLO1 converts to S-D-lactoylglutathione (LGSH). GLO2 then converts this metabolite into D-lactate. GLO1 is the rate-limiting enzyme in the detoxification of MGO and is reportedly downregulated by inflammatory stimuli such as TNF-α, which may contribute to its accumulation in immune cells [167].

A prime example whereby endogenous MGO may exert immunomodulatory signalling is in the context of innate-adaptive immune crosstalk in the TME [166]. MGO is produced by metabolically quiescent human myeloid-derived suppressor cells (MDSCs) in a SSAO-dependent mechanism. This leads to a reduction in the metabolic activity of MDSCs and the transfer of MGO to $CD8^+$ T cells. MGO transfer happens through direct contact, resulting in reduced proliferation and cytokine production by $CD8^+$ T cells and suppressing their anti-tumour functions. Mechanistically, MGO rapidly reacts with L-arginine, leading to its depletion and inhibits glucose uptake in $CD8^+$ T cells. Thus, MGO signalling is crucial for the immunosuppressive activity of MDSCs, which has implications in various contexts, from inflamed to cancerous tissues. For a more in-depth exploration of MGO and its role in immune cell regulation, refer to the source [165].

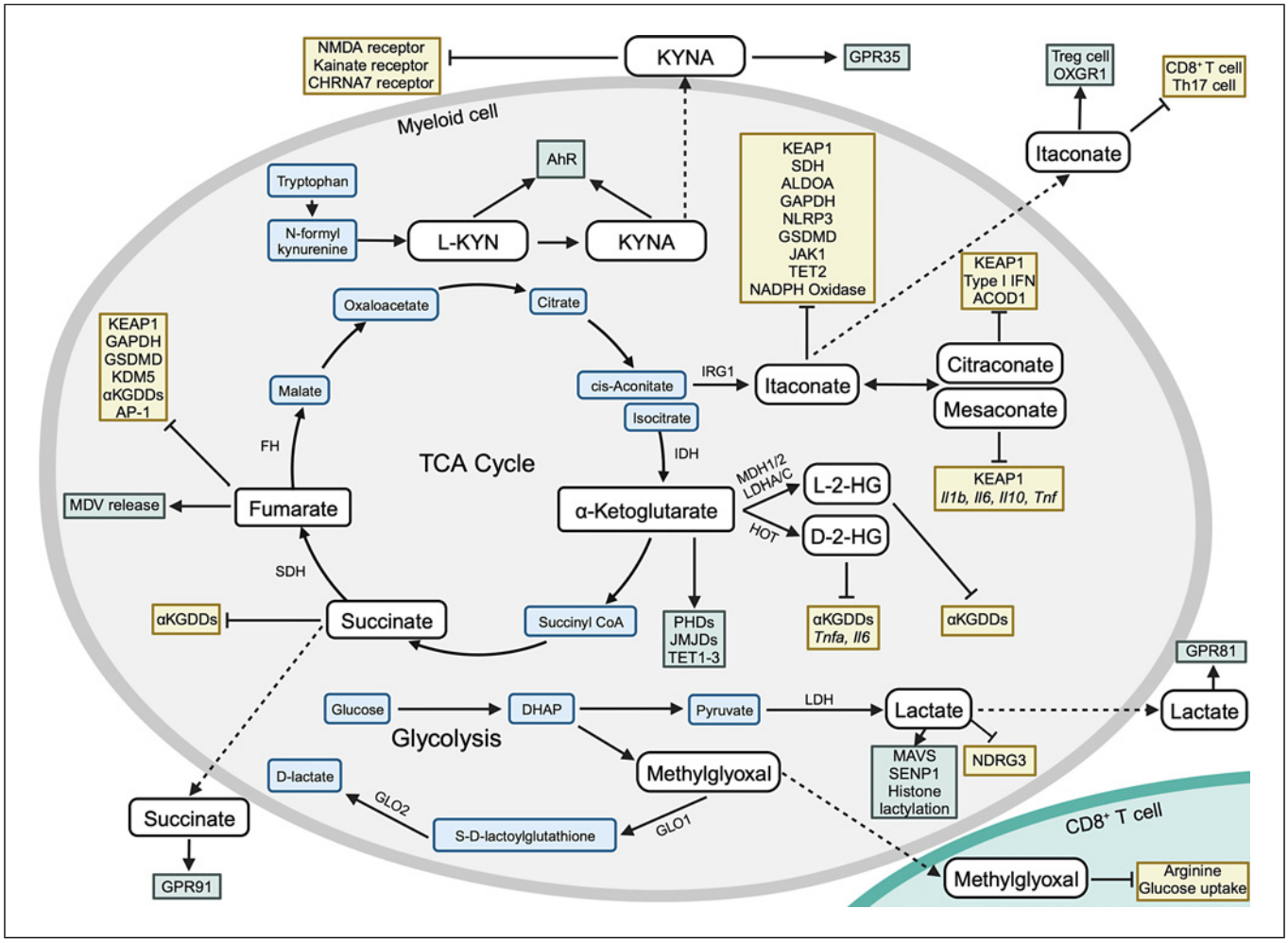

Fig. 2. Signalling immunometabolites in myeloid cells (created with BioRender.com).

In addition to MGO, LGSH has recently been shown to modulate the inflammatory response of macrophages exposed to LPS mimetic, kdo2-Lipid A. Here, the authors suggest that LGSH, as opposed to lactate, is the source of lactoyl-CoA and histone-mediated lactoylation in macrophages [168]. This role in inflammatory gene regulation is supported by deletion of GLO2, which increased LGSH and lactoyl-CoA levels, potentiated inflammatory cytokine production, including TNF-α and IL-6 expression, and altered site-specific histone lactoylation [168].

Tryptophan-Derived Metabolites

Kynurenine (L-KYN) and kynurenic acid (KYNA) are two metabolites generated by tryptophan uptake and catabolism, a key pathway required for de novo NAD^+ synthesis. Tryptophan is first metabolized to N-formyl kynurenine by indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO) before conversion to kynurenine by arylformamidase (AFMID). IDO1 is a key rate-limiting enzyme that is transcriptionally upregulated in macrophages by pro-inflammatory cytokines, such as IFN-γ, and exhibits immunosuppressive activity against T-cell responses [169]. L-KYN can be further metabolized to nicotinamide via the kynurenine pathway to feed NAD^+ synthesis [170] or directed towards KYNA synthesis by kynurenine aminotransferase (KYAT) [171].

Both L-KYN and KYNA are now recognized agonists of the aryl hydrocarbon receptor (AhR), a stress-responsive transcription factor with role in immunomodulation [172, 173]. In addition, KYNA is a reported agonist of GPR35 and antagonist of the NMDA, kainate, and CHRNA7 receptors [171]. L-KYN- and KYNA-mediated activation of AhR is now reported to modulate the behaviour of many innate and adaptive immune cell populations. For

instance, the production of L-KYN in DCs following inflammatory stimuli, such as LPS, is required for inducing the differentiation of Tregs and preventing Th17 cell differentiation from naïve $CD4^+$ T cells, and this occurs in an AhR-dependent manner [174, 175]. Tumour-derived L-KYN has also been found to contribute to an immunosuppressive TME through modulation of Tregs and tumour-associated macrophages (TAMs) in an AhR-dependent manner [176]. Mechanistically, kynurenine uptake and AhR activation are restricted to T cell-expressing system L transporter, SLC7A5, which is increased in cells activated by T cell antigen receptor or pro-inflammatory cytokines [177]. KYNA is also reported to synergistically induce IL-6 production in tumour cells via AhR activation [173] and to act in an anti-inflammatory manner via GPR35, which is highly expressed in immune cell populations. Specifically, a KYNA/GPR35 signalling axis induced by social stress in mice has been shown to restrict NLRP3 inflammasome activation and worsen experimental colitis [178]. In summary, MGO, S-D-lactoylglutathione, L-KYN, and KYNA are examples of emerging and historical metabolic signals that act as critical regulators of cell fate and behaviour under various physiological contexts, affecting both innate and adaptive immunity.

Conclusion

Overall, we hope to have given immunologists a concise overview of the metabolites which have emerged as signalling molecules in immunity over the last several decades and expanded the view of metabolites from biosynthetic substrates and molecules required for energy production, to signals which orchestrate widespread changes in immune cell function at the level of transcription, epigenetics, and protein function. This overview is depicted diagrammatically in Figure 2. Given the major phenotypic effects in a variety of animal models caused by artificially regulating the accumulation of these metabolites or by using derivatized forms of these metabolites, they may be exploited in the therapeutic treatment of a multitude of diseases caused by immunopathology. The success of DMF as a highly effective treatment for MS demonstrates that future targeting of metabolism or use of metabolite derivatives is a safe and effective approach in finding novel therapeutics. Future studies further delineating the metabolic changes that occur and metabolites which are dynamically regulated during immune responses will give more insight into the importance of metabolism in controlling immune cell effector functions and cytokine production. as well as providing understanding into the mechanisms that immune cells have evolved to prevent aberrant inflammatory responses, such as the production of itaconate during M1 polarization.

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Figures 1 and 2 were created using BioRender.com.

Conflict of Interest Statement

D.G.R., C.P., and A.H. are listed as inventors on a pending patent for the treatment of interferon-driven inflammation.

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Author Contributions

D.G.R., C.P., and A.H. contributed equally to the writing and editing of this review.

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