The role of the electron transport chain in immunity

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Abstract
The electron transport chain (ETC) couples oxidative phosphorylation (OXPHOS) with ATP synthase to drive the generation of ATP. In immune cells, research surrounding the ETC has drifted away from bioenergetics since the discovery of cytochrome c (Cyt c) release as a signal for programmed cell death. Complex I has been shown to generate reactive oxygen species (ROS), with key roles identified in inflammatory macrophages and T helper 17 cells (T_{H17}) cells. Complex II is the site of reverse electron transport (RET) in inflammatory macrophages and is also responsible for regulating fumarate levels linking to epigenetic changes. Complex III also produces ROS which activate hypoxia-inducible factor 1-alpha (HIF-1α) and can participate in regulatory T cell (T_{reg}) function. Complex IV is required for T cell activation and differentiation and the proper development of T_{reg} subsets. Complex V is required for T_{H17} differentiation and can be expressed on the surface of tumor cells where it is recognized by anti-tumor T and NK cells. In this review, we summarize these findings and speculate on the therapeutic potential of targeting the ETC as an anti-inflammatory strategy.

KEYWORDS
electron transport chain (ETC), immunometabolism, macrophage, mitochondria, oxidative phosphorylation, T-lymphocytes

Abbreviations: 2HG, 2-hydroxyglutarate; 3NP, 3-nitropropionic acid; AMPK, AMP-activated protein kinase; BATF, basic leucine zipper ATF-like transcription factor; CoQ, coenzyme Q; COX, cytochrome c oxidase; CXCL10, C-X-C motif chemokine ligand 10; Cyt c, cytochrome c; DC, dendritic cell; DHODH, dihydroorotate dehydrogenase; DMM, dimethyl malonate; ERK, extracellular signal-regulated kinases; ETFQO, electron transferring-flavoprotein dehydrogenase; FAD, flavin adenine dinucleotide; FoxP3, forkhead box protein P3; GDF15, growth/differentiation factor 15; GPDH, glycerol-3-phosphate dehydrogenase; HIF-1α, hypoxia-inducible factor 1-alpha; HSC, hematopoietic stem cell; IFN, interferon; IL, interleukin; iNKT, invariant natural killer T cells; IR, ischemia-reperfusion; IRG-1, immuno-responsive gene 1; LPS, lipopolysaccharides; MCJ, methylation-controlled J protein; M-CSF, macrophage colony forming factor; mtDNA, mitochondrial DNA; mTOR, mechanistic target of rapamycin; NAD, nicotinamide adenine dinucleotide; ND6, NADH:ubiquinone oxidoreductase core subunit 6; NDUFAF1, NADH:ubiquinone oxidoreductase complex assembly factor 1; NDUFS4, NADH:ubiquinone oxidoreductase core subunit 4; NFAT, nuclear factor of activated T-cells; NLRP3, NLR family pyrin domain containing 3; OXPHOS, oxidative phosphorylation; PD-L2, programmed cell death 1 ligand 2; PHD, prolyl hydroxylase; RANKL, receptor activator of nuclear factor kappa-B ligand; RELMa, resistin-like molecule-alpha; RET, reverse electron transport; RISP, Rieske iron-sulfur protein; RORγt, RAR-related orphan receptor gamma 2; ROS, reactive oxygen species; SC, supercomplex; SDH, succinate dehydrogenase; STAT, Signal Transducer and activator of transcription; T2DM, type II diabetes mellitus; TCR, T cell receptor signaling; T_{reg}, T effector; TET, ten-eleven translocation; T_{H17}, T helper; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-alpha; T_{reg}, regulatory T cell; TTFA, 2-thienoyltrifluoroacetone; UQCRFS, ubiquinol-cytochrome C reductase, Rieske iron-sulfur polypeptide.

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1 | INTRODUCTION

Immunometabolism has become a major focus for immunologists interested in how metabolic pathways govern the immune response. Extensive studies have been conducted to study the role of metabolites in glycolysis, the Krebs cycle, the pentose phosphate pathway, fatty acid oxidation, fatty acid synthesis, and amino acid metabolism in immune cell functions. The role of mitochondria in immunity has been an area of interest since the discovery of the electron transport protein cytochrome c (Cyt c) as a driver of apoptosis. This unexpected finding brought the electron transport chain (ETC) into a new realm outside its role in bioenergetics.

The ETC consists of two electron carriers (coenzyme Q [CoQ] and Cyt c) and a series of complexes (I, II, III, IV, and V) situated in the inner mitochondrial membrane (Figure 1). The breakdown of nutrients through glycolysis, the Krebs cycle, amino acid, and fatty acid oxidation generates high energy reducing equivalents, NADH and FADH₂. NADH and FADH₂ are oxidized to NAD⁺ and FAD⁺ respectively stepwise by redox centers located in each of the ETC complexes, a process known as oxidative phosphorylation (OXPHOS). Electrons derived from NADH and FADH₂ are transferred from complex I and complex II, respectively, to complex IV, where they are used to reduce molecular oxygen into water. This process of electron transfer drives proton pumping from the mitochondrial matrix into the intermembrane space, creating a proton motive force that ultimately leads to ATP production at the ATP synthase, or complex V.

The elucidation of OXPHOS occurred in the ETC was a major triumph for biochemistry and OXPHOS forms the basis for energetics in most living systems. However, the protein complexes in the ETC participate in cellular processes beyond respiration. New roles for the ETC components outside OXPHOS have recently emerged besides Cyt c, with much influence on the immune system. In this review we summarize recent findings linking changes in the ETC to immune cell activation, focusing on macrophages (Figure 2 and Table 1) and T cells (Table 2). We further discuss therapeutic possibilities for targeting the ETC components for the treatment of immune-mediated diseases.

2 | COMPLEX I

NADH: ubiquinone oxidoreductase, Type I NADH dehydrogenase, or complex I, is the first and the largest protein complex in the mitochondrial ETC. It catalyzes the transfer of two electrons from NADH to CoQ and concomitantly pumps four protons into the intermembrane space (Figure 1).

Mitochondrial reactive oxygen species (ROS) produced by the ETC during OXPHOS are the primary source of free radicals. ROS are known to cause oxidative damage and are responsible for damage upon ischemia-reperfusion (IR), but increasing evidence has shown that ROS also function as signaling molecules. Complex
I is a major producer of ROS within the mitochondria, mainly via RET.\textsuperscript{13,14}\textsuperscript{13,14} Although superoxide can be generated from forward electron transfer, the amount is negligible (<0.1% of total electron flow via OXPHOS).\textsuperscript{14,15}\textsuperscript{14,15} RET occurs when there is a high mitochondrial membrane potential and when the pool of CoQ becomes highly reduced by electrons from complex II,\textsuperscript{16,17}\textsuperscript{16,17} possibly also from other electron donors like glycerol-3-phosphate dehydrogenase (GPDH), electron-transferring flavoprotein dehydrogenase (ETFQO), or dihydroorotate dehydrogenase (DHODH).\textsuperscript{11}\textsuperscript{11}

The ROS produced by complex I significantly impact the immune system. In 2016, Mills et al. demonstrated that in response to lipopolysaccharides (LPS), macrophages shifted from OXPHOS to glycolysis while also increasing succinate levels. The increased succinate oxidation by complex II and an elevation of the mitochondrial membrane potential combined to boost ROS production via RET at complex I. Importantly, ROS generated via this pathway activated hypoxia-inducible factor 1-alpha (HIF-1α), a key transcription factor in the expression of proinflammatory genes, a notable example being an increased production of the proinflammatory cytokine, interleukin 1 beta (IL-1β).\textsuperscript{17}\textsuperscript{17} Accordingly, reducing ROS production using the complex I inhibitors metformin or rotenone, or MitoQ, an antioxidant targeting mitochondria, decreased pro-IL-1β but increased anti-inflammatory IL-10 production.\textsuperscript{18}\textsuperscript{18} The effect of metformin on pro-IL-1β might be partly mediated by IL-10 as metformin no longer suppressed pro-IL-1β mRNA and protein production in IL-10 knockout macrophages.\textsuperscript{18}\textsuperscript{18} A more recent study by Xian et al. showed that metformin inhibited complex I and reduced the severity of LPS-induced acute respiratory disease in non-diabetic mice by limiting the production of IL-1β and IL-6.\textsuperscript{19}\textsuperscript{19} Although the inhibitory effect on IL-6 was independent of complex I, the decrease in IL-1β was due to blunted
NLRP3 inflammasome activity as a downstream effect of targeting of complex I, resulting in reduced synthesis and oxidation of mitochondrial DNA (mtDNA), an NLRP3 ligand. Combined, HIF-1α likely activates the production of pro-IL-1β, an inactive precursor which requires NLRP3 to be processed into the mature effector form of IL-1β. Further, complex I-derived ROS were also necessary to differentiate peripheral blood monocytes into dendritic cells (DCs) in vitro, as reflected by a substantial reduction in CD1a surface expression when cells were treated with rotenone or an antioxidant catalase to reduce ROS content.20 Interestingly, although blocking complex I-induced ROS is anti-inflammatory in LPS-activated macrophages, the complete ablation of complex I (Ndufs4*/*) appeared to trigger substantial ROS production and in turn caused systemic inflammation and an intrinsic lineage shift from osteoclasts to macrophages.21 Therefore, complex I-derived ROS act as an important rheostat of innate immunity and bone homeostasis.

Complex I is also frequently implicated in lymphocyte activation, proliferation, and function. Its activity is required in proliferating cells as it maintains an appropriate cellular NAD+/NADH ratio to support aspartate biosynthesis, and the nucleotides essential for cell proliferation.22 Indeed, blocking complex I with rotenone inhibited proliferation in all T effector (Teff) cell subsets; and aspartate supplementation partially restored the proliferative capacity of rotenone treated Th1 cells.23 Although the effect of aspartate supplementation on rotenone treated Th2 and Th17 cells was not measured, it was expected to result in similar effects.23 In an earlier report, decreasing complex I-derived ROS also consistently reduced CD8+ T lymphocytes’ proliferation by hindering intracellular calcium flux and extracellular signal-regulated kinases (ERK) 1/2 phosphorylation.24 The role of complex I in T lymphocytes is not limited to proliferation. Notch1 is a transmembrane receptor involved in Th0 cell differentiation in response to polarizing cytokines.25,26 In Th17-polarising conditions, rotenone abrogated Notch1 and RAR-related orphan receptor gamma 2 (RORγt) colocalization in the nucleus and reduced Th17 differentiation.27 In terms of function, again, complex I was required for IL-17 production in Th17 cells, but dispensable for IFN-γ production in Th1 or IL-4 in Th12 cells.23 However, conflicting results were shown by Ozay and colleagues, where rotenone treatment attenuated IFN-γ and IL-2 production.27 In effector and memory CD8+ T cells, rotenone ablated T cell cytotoxicity as measured by reduced IFN-γ and TNF-α production and degranulation.24 Together, it is suggested that complex I plays an integral role in lymphocyte differentiation, although its exact role remains to be elucidated.

### Table 1: Macrophage function regulated by the ETC

| ETC complex | Specifics | Experimental validation |
|-------------|----------|-------------------------|
| I           | Produces ROS to initiate the expression of proinflammatory cytokine including IL-1β through HIF-1α | Rotenone and alternative oxidase |
|             | Reduced ROS at complex I increase IL-10 production | Rotenone and Metformin |
|             | Reduced ROS at complex I alleviate LPS-induced acute respiratory disease in non-diabetic mice by limiting the production of IL-1β. The effect was likely due to a combined effect of reduced HIF-1α and NLRP3 inflammasome activity | Metformin |
|             | Complete ablation of complex I triggers substantial ROS production, followed by systemic inflammation and a lineage shift of osteoclasts to macrophages | Ndufs4*/* |
| II          | Source of electrons that drives RET at complex I; inhibition of complex II activity with DMM decreases IL-1β but increases IL-10 production | DMM |
|             | Elevated complex II expression in β-glucan-trained macrophages restores proinflammatory responses in immune paralysis | Methylfumarate as a fumarate supplement |
| III         | Produces ROS which destabilizes PHDs and activates HIF-1α | shRNA-Uqcrfs |
| IV          | Complex IV knockdown causes an increase in IL-1β, IL-6, and TNF-α, and the anti-inflammatory cytokine IL-10, as well as inducing macrophage differentiation to osteoclasts via RANK-L | shRNA-IvI1 and shRNA-Vβ |
| V           | Inhibition of complex V enhances the mRNA expression of IL-6, IL-1β, TNF, and CXCL10 in M-CSF-conditioned media | Oligomycin |
|             | Inhibition of complex V reduces LPS-induced IL-1β | Oligomycin |
|             | Required for arginase activity, PD-L2, RELMα, and STAT6 phosphorylation in M2 macrophages | Oligomycin |
| ETC complex | T cell type | Process | Specifics | Experimental validation |
|-------------|-------------|---------|----------|--------------------------|
| I | CD8<sup>+</sup> | Proliferation<sup>34</sup> | Induces intracellular calcium flux and ERK1/2 phosphorylation to support CD8<sup>+</sup> T cell proliferation | Rothenone |
| I | Function<sup>34,36</sup> | Required for IFN-γ, TNF-α production, and degranulation by CD8<sup>+</sup> T cells<sup>34</sup>; complex I-associated supercomplex aids in IFN-γ and IL-2 production by CD8<sup>+</sup> T cells and provides superior protection against influenza virus infection<sup>36</sup> | Rothenone<sup>34</sup>, Mej<sup>−/−</sup><sup>36</sup> |
| I | TH1 (potentially TH2 and TH17) | Proliferation<sup>23</sup> | Maintains the NAD<sup>+</sup>/NADH balance for aspartate biosynthesis that is used in nucleotide synthesis during TH1 proliferation | Rothenone |
| I | TH17 | Differentiation<sup>27</sup> | Maintains Notch1 and ROARyt colocalization that are important for TH17 differentiation | Rothenone |
| I | Treg | Differentiation<sup>27</sup> | Induces Foxp3 expression and cytokine production during iTreg differentiation but is dispensable in fully differentiated iTreg | Rothenone |
| I | Function<sup>23</sup> | Important for Treg to suppress effector T cell proliferation | mt-ND6<sup>P51L</sup> and rotenone<sup>31</sup>, rotenone<sup>32</sup> |
| II | TH1 | Proliferation<sup>23</sup> | Knockdown of complex II increases TH1 proliferation | Sdhc<sup>fl/fl</sup>TetO-cre<sup>−/−</sup>Rosa26<sup>TTA/+</sup> |
| II | Function<sup>23</sup> | Supports IFN-γ production by TH1 cells | sgRNA-Sdhc; Sdhc<sup>fl/fl</sup>TetO-cre<sup>−/−</sup>Rosa26<sup>TTA/+</sup>; DMM, 3NP, TFFA, and atpenin A5 |
| III | CD4<sup>+</sup> | Activation and proliferation<sup>43</sup> | Produces ROS to activate IL-2 production through NFAT and antigen-specific proliferation in CD4<sup>+</sup> T cells | Uqcrfs<sup>fl/fl</sup>Cd4-Cre |
| III | CD8<sup>+</sup> | Function<sup>44</sup> | Persistently elevated ROS produced from complex III hyper-stimulates NFAT by suppressing tyrosine phosphatases. CD8<sup>+</sup> T cells with hyper-stimulated NFAT displays exhausted-like dysfunction with high co-inhibitory molecule expression and decreased IFN-γ and TNF production | Antimycin A |
| III | iNKT | Proliferation<sup>45</sup> | Required to sustain the number of iNKTs in the thymus and periphery | Uqcrfs<sup>fl/fl</sup>Cd4-Cre |
| III | Activation<sup>45</sup> | Maintains the expression of iNKT activation markers, functional TCR signaling, and appropriate response to IL-5 stimulation | Uqcrfs<sup>fl/fl</sup>Cd4-Cre |
| III | Function<sup>45</sup> | Required for the activity of NFATc2, that leads to IFN-γ and IL-4 production | Uqcrfs<sup>fl/fl</sup>Cd4-Cre |
| III | Treg | Function<sup>46</sup> | Signals through the TET family of DNA demethylases to maintain expression of genes associated with Treg suppressive function | Uqcrfs<sup>fl/fl</sup>Foxp3<sup>YFP-cre</sup> |

(Continues)
In addition to its role in activation, complex I is also important in dampening the immune system. Through the use of either a siRNA-mediated knockdown of a complex I chaperone NDUFAF1 or metformin inhibition of complex I, it was shown that complex I was involved in inducing the expression of CD95L during activation-induced T-cell death,\(^{28}\) a process important for immune homeostasis.\(^{29}\) Rotenone treatment also selectively reduced Foxp3 expression and cytokine production during induced-regulatory T-cell (iTreg) differentiation but did not affect Foxp3 expression in fully differentiated iTreg cells, suggesting a critical role of complex I in iTreg differentiation.\(^{27,30}\) Rotenone treatment or complex I mutation (ND6P25L) also altered Treg suppressive functions, as illustrated by a lower capacity to limit T\(_{\text{eff}}\)-cell proliferation.\(^{31,32}\)

The five complexes in the respiratory chain, except complex II, can dynamically assemble as larger molecular supercomplexes (SCs) in the mitochondrial inner membrane,\(^{33}\) with complex I forming SCs with either complex III and complex IV, or with complex III alone.\(^{34}\) The function of SCs remains controversial but is postulated to contribute to efficient respiration and limit the amount of ROS produced during OXPHOS.\(^{35}\) Methylation-controlled J protein (MCJ) is an endogenous inhibitor of complex I that acts as a negative regulator of the respiratory chain by interfering with the formation of complex I into SCs.\(^{35}\) MCJ deficiency in CD8\(^{+}\) T cells resulted in an increase in OXPHOS and subcellular ATP accumulation, which elevated the secretion of IFN-γ and IL-2 but not proliferation or gene expression of activation markers.\(^{36}\) Memory CD8\(^{+}\) T cells lacking MCJ also showed superior protection against influenza virus infection.\(^{36}\)

### TABLE 2 (Continued)

| ETC complex | T cell type | T cell type |
|-------------|-------------|-------------|
| IV          | CD4\(^{+}\) and CD8\(^{+}\) | T\(_{\text{reg}}\) |
|            | T\(_{\text{h}1}\) | T\(_{\text{h}1}\) and T\(_{\text{h}2}\) |
|            | T\(_{\text{h}17}\) | T\(_{\text{reg}}\) |

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### 3 | COMPLEX II

Succinate dehydrogenase (SDH) or succinate-CoQ reductase or respiratory complex II is the only member of the ETC that participates in both the Krebs cycle and the ETC.\(^{51}\) SDH catalyzes the oxidation of succinate to fumarate in the Krebs cycle with the reduction of CoQ to ensure electron flow in the respiratory chain.

As stated previously, complex II has been implicated in M1 macrophage activation via succinate oxidation, which drives RET at complex I, leading to ROS production.\(^{17}\) LPS-activated macrophages break at isocitrate dehydrogenase in the Krebs cycle, an enzyme that catalyzes the conversion of cis-aconitate to isocitrate.\(^{52}\) Immune-responsive gene 1, a highly expressed protein in M1 macrophages, combines with a build-up of its substrate cis-aconitate to produce itaconate via decarboxylation.\(^{1}\) Itaconate exerts anti-inflammatory properties through several pathways,\(^{53–56}\) one of which is a competitive inhibitor of
complex II, reducing RET-induced ROS and the downstream production of IL-1β. Accumulated succinate can also be exported from mitochondria into the cytosol where it directly suppresses the activity of PHDs to stabilize HIF-1α. The inflammatory role of complex II was reinforced by using dimethyl malonate (DMM), a potent competitive inhibitor of succinate oxidation by complex II, which abrogated LPS-induced IL-1β but increased IL-10 in macrophages.

Itaconate is critical for generating tolerized macrophages which are hyporesponsive to restimulation with LPS, a process that may lead to immune paralysis. However, this may be rescued by trained immunity through the alleviation of complex II suppression. Trained immunity is the persistent hyperresponsiveness that innate immune cells develop after brief stimulation and is achieved by modifying epigenetic markers of specific genes. Training macrophages with β-glucan increased H3K27Ac markers at the Sdh gene and elevated the expression of SDH subunits, restoring fumarate production by complex II and the downstream inflammatory response. Interestingly, fumarate can also indirectly increase histone modifications (H3K4me3 and H3K27Ac) at the promoters of proinflammatory cytokines through suppression of the histone demethylase KDM5.

Impairing complex II activity with inhibitors (i.e., DMM, chenoyl trifluoroacetone, 3-nitropropionic acid, or atpenin AS) or through genetic ablation of complex II subunits (i.e., sgRNA targeted Sdhα knockdown or Sdhc knockout) significantly reduced IFN-γ produced by TH1 cells. However, a dichotomy was observed as knockouts of Sdhc in TH1 led to increased proliferation compared to WT. Complex II potentially has a key role in uncoupling the processes of TH1 proliferation and effector function.

4 | COMPLEX III

The mitochondrial cytochrome bc1 complex (complex III; ubiquinol: Cyt c oxidoreductase) is the third complex that sits in the mitochondrial respiratory chain and transfers electrons from ubiquinol to Cyt c while pumping protons from the mitochondrial matrix to the intermembrane space.

Complex III is another major ROS producer besides complex I in the ETC. In complex IV, oxygen is reduced to water one electron at a time. Complex IV retains the partially reduced product until a complete reduction is achieved. However, while transferring electrons to Cyt c, complex III leaks electrons to oxygen, partially reducing it to a superoxide anion. Superoxide produced from complex III is of particular interest because it releases ROS to the mitochondrial matrix and the intermembrane space. From the intermembrane space, superoxide travels through voltage-dependent anion channels in the outer mitochondrial membrane to the cytosol, where it can be converted to H2O2 by superoxide dismutase 1. Therefore, ROS produced by complex III provides an easier route to the cytosol, allowing its participation in cellular signaling events.

In addition to the ROS production by complex I, ROS produced from the Qo site of complex III are recognized as another major activation signal for HIF-1α suppressing the upstream PHDs. HIF-1α is a crucial transcription regulator in cellular metabolism and immune cell effector functions. It is widely expressed and detected in virtually all innate and adaptive immune cells including macrophages, neutrophils, DCs, and lymphocytes. HIF-1α is a critical transcription factor for the maturation of DCs and the activation of T cells. HIF-1α also acts through ROR-γt to drive Th17 differentiation. Besides HIF-1α, a recent study by Ahmed et al. illustrated that in macrophages the sensing of double-stranded RNA by TLR3 was associated with a more pronounced complex III expression, which increased mitochondrial and cytosolic ROS from the Qo site of complex III to provide enhanced inflammatory and antiviral cytokines.

Studies that examined the specific roles of complex III in the immune system have mainly been focused on the adaptive arm. The loss of an essential complex III subunit Rieske iron-sulfur protein (RISP; Uqcrfs<sup>−/−</sup>) led to impaired differentiation of hematopoietic stem cells (HSCs). Without a functional complex III, the cells displayed histone hypoacetylation due to the reduced citrate produced from the mitochondria and increased methylation of DNA and histones which resulted from an accumulation of 2-hydroxyglutarate (2-HG). Additionally, ROS produced specifically at complex III are required for antigen-specific CD4<sup>+</sup> T cell activation. By utilizing a T cell-specific mutation in complex III (T-Uqcrfs<sup>−/−</sup>), Sena et al. demonstrated that complex III-ROS were required for IL-2 production through the activation of nuclear factor of activated T-cells (NFAT), as well as antigen-specific proliferation. Although NFAT signaling is required for T cell activation, persistent stimulation of NFAT promotes T cell exhaustion, a state of T cell dysfunction that arises from chronic infection and tumors. Indeed, persistently elevated ROS resulting from antimycin A treatment (a complex III inhibitor) led to continuous NFAT stimulation through suppression of tyrosine phosphatases.

Antimycin A-treated T cells also displayed exhausted-like dysfunction with high co-inhibitory molecule expression and decreased polyfunctionality (IFN-γ and TNF production). T cell-specific deletion of Uqcrfs significantly reduced the number of invariant natural killer T cells (iNKTs) in the thymus and periphery. Uqcrfs<sup>−/−</sup> iNKTs
also displayed reduced activation marker expression, reduced T cell receptor signaling (TCR), and an altered response to TCR and IL-5 stimulation. Consistently, in mature iNKT cells, depletion of Uqcrfs ablated IFN-γ and IL-4 production likely resulting from a decrease in NFATc2 activity. Similar to the observation in HSCs, Treg-specific knockout of Uqcrfs increased DNA methylation, 2-HG, and succinate accumulation that inhibited the ten-eleven translocation family of DNA demethylases. This change was associated with a loss of T cell-suppression capacity as illustrated by a decrease in gene expression associated with Treg function, but without altering Treg proliferation and survival. Complex III is therefore essential to the general development of T cells. However, more studies are needed to understand how complex III is regulated, and how it supports both TH17 and Treg functions.

5 | COMPLEX IV

The enzyme cytochrome c oxidase (COX) or complex IV is the last enzyme in the respiratory ETC that catalyzes the transfer of electrons from reduced Cyt c to the final acceptor of electrons, O₂, in a process that is coupled to proton translocation for ATP synthesis. Unlike complex I and complex III which produce ROS as a secondary messenger for cellular activation and function, complex IV does not produce ROS but is vital for apoptosis (due to Cyt c release) and ultimately cell fate. Complex IV assembly requires more than 30 structural components, and the delivery of redox-active metal centers and prosthetic groups. The non-structural component COX10 functions in the maturation of one of the heme prosthetic groups and is a common target used to study the association of complex IV and the immune system.

Loss of complex IV renders macrophages more inflammatory. Knockdown of Cox in macrophages increased IL-1β, IL-6, and TNF-α, favored M1 polarization and enhanced phagocytosis. Nonetheless, an increase in the anti-inflammatory cytokine IL-10 was also seen with the decrease in Cox. Further studies are needed to explain these contrasting observations. Diminished levels of COX induced the differentiation of macrophages to osteoclasts via RANK-L, in contrast to what was observed with complex I inhibition. In NK cells, complex IV is required for antigen-specific amplification. NK-specific deletion of Cox10 impaired the expansion of murine cytomegalovirus-specific NK cells and NK cell memory formation, which was associated with upregulation of glycolysis, activation of AMP-activated protein kinase (AMPK), and the mechanistic target of rapamycin (mTOR).

Despite adopting a glycolytic metabotype, OXPHOS also runs at a lower rate during primary T cell activation. Since complex IV plays a central regulatory role in OXPHOS, T cell-specific knockdown of Cox10 impaired T cell activation. Indeed, COX10-mediated OXPHOS is important for T cell exit from quiescence and acts as a metabolic checkpoint for cell fate decisions following activation. Cox10-deficient T cells could differentiate into Treg but were defective in developing into the more metabolically demanding T effector17 subsets. In terms of functions, COX10 ablation led to increased apoptosis and immunodeficiency in vivo. Treg-specific knockout of COX10 also impaired Treg maturation and suppressive functions.

6 | COMPLEX V

Mitochondrial F₁F₀-ATP synthase or complex V couples ATP synthesis during OXPHOS to an electrochemical gradient generated by proton pumping across the inner mitochondrial membrane.

In the absence of activating signals (e.g., LPS, IFN-γ or IL-4) but the presence of macrophage colony-stimulating factor, inhibiting mitochondrial respiration by oligomycin in naïve macrophages enhanced the expression of a range of proinflammatory genes Il6, Tnf, Il1b, and Cxcl10. In polarized macrophages, inhibition of complex V continues to suppress both M1 and M2 activities. Oligomycin decreased LPS-induced IL-1β in M1 macrophages and suppressed M2 arginase activity, differentiation markers PD-L2 and RELMα, and STAT6 phosphorylation. Hence, it is likely both naïve and polarized macrophages require some level of ATP synthesis within the mitochondrial matrix.

In TH1-polarising conditions, oligomycin caused a modest but significant decrease in cell proliferation. Still, it did not alter Ifng expression, whereas in TH12 and TH17-polarising conditions, blocking complex V with oligomycin did not affect cellular proliferation or IL-4 and IL-17 production, respectively. Conversely, in a different study, complex V promoted TH17 lineage commitment and oligomycin reduced IL-17a production. RORγT and BATF are two essential transcription factors for TH17 fate decision. RORγT is the master regulator for TH17 differentiation, whereas BATF supports the expression of TH17-related genes by binding to the IL17a/IL17f gene locus, increasing chromatin accessibility for STAT3. Treatment with oligomycin in differentiating TH17 cells blocked RORγT and BATF expression and in turn, reduced IL-17a. While inhibiting TH17 lineage specification, blocking complex V with oligomycin interestingly favored FoxP3 induction, shifting TH17 to a functionally suppressive Treg population. Consistent with this finding, oligomycin reduced IL-17 production in in vivo-derived TH17 cells and
alleviated a T_{H}17-driven murine model of colitis by reducing the number of IL17+ IFN-γ+ T_{H}17 cells at the site of colonic inflammation.\textsuperscript{50} BATF expression was also down-regulated by oligomycin in T_{H}1 and T_{H}2 cells,\textsuperscript{49} although it is unclear whether this alters the effector function of these subsets.

Another intriguing role of complex V concerns its expression on the cell surface.\textsuperscript{97} The ectopic expression of ATP synthase has been shown to occur on tumor cells targeted by natural killer cells, lymphokine-activated killer cells, and cytotoxic T cells to promote cell death.\textsuperscript{97–102} Further analysis of a cell surface role of complex V is warranted.

### 7 | THE THERAPEUTIC POTENTIAL OF TARGETING THE ETC COMPLEXES

Given these findings, might it be possible to target the ETC in immune cells for therapeutic gain in inflammatory diseases? In this regard, metformin is of interest. This is a first-line treatment for type II diabetes mellitus (T2DM). It has a validated low toxicity profile and is widely used clinically.\textsuperscript{103} Metformin has multiple mechanisms of action, including decreasing hepatic gluconeogenesis,\textsuperscript{104} insulin resistance,\textsuperscript{105} and increasing GDF15 secretion which suppresses appetite.\textsuperscript{106} The pathogenesis of T2DM involves a significant proinflammatory component, with IL-1β inducing apoptosis in pancreatic beta cells\textsuperscript{107} as well as being produced from amyloid deposits via NLRP3 in the T2DM pancreas.\textsuperscript{108} Metformin not only participates in correcting glucose homeostasis in T2DM but also dampens down the associated inflammatory responses. As discussed earlier, metformin reduced IL-1β in LPS-activated macrophages by inhibiting RET-induced ROS.\textsuperscript{18,109} In type I diabetes mellitus, metformin activates AMPK signaling through its inhibition of complex I.\textsuperscript{110} AMPK restores splenic T cell homeostasis by decreasing the number of proinflammatory IFN-γ+ and IL-17+ CD4+ T cells and increasing the percentage of regulatory IL-10+ and Foxp3+ CD4+ T cells. The beneficial effect of metformin extends to many other inflammatory diseases, including models of systemic lupus erythematosus,\textsuperscript{111} colitis,\textsuperscript{112} experimental autoimmune arthritis,\textsuperscript{113} psoriasis,\textsuperscript{114} and obesity.\textsuperscript{115} However, whether these effects are mediated through complex I inhibition or other molecular targets is unclear. Nonetheless, these studies suggest that inhibition of complex I may have therapeutic potential for treating inflammatory diseases.

DMM is a cell-permeable, potent competitive inhibitor of complex II.\textsuperscript{58} In LPS-activated macrophages, DMM directly inhibits succinate oxidation by complex II, abrogating IL-1β and increasing IL-10 production.\textsuperscript{16,17} In a rat model of cardiac arrest, DMM reduced ROS generated from succinate oxidation, mitigating brain damage.\textsuperscript{116} When administered intraperitoneally, DMM alleviated LPS/d-galactosamine-induced acute hepatic damage in mice by suppressing the proinflammatory cytokines TNF-α and IL-6 in plasma and inhibiting hepatocyte apoptosis.\textsuperscript{117} DMM also protected against a murine model of cardiac IR injury.\textsuperscript{16} The clinical relevance of DMM has been further demonstrated recently in both animal and human ex vivo models of renal IR injury.\textsuperscript{118} In that administration of DMM at reperfusion inhibited the succinate-driven ROS production that underlies IR injury.\textsuperscript{118} The safety of the use of DMM has yet to be determined in human clinical studies, but molecules based on it may be a new avenue for the treatment of inflammatory diseases.

### 8 | CONCLUDING REMARKS

Immunometabolism is an area that has opened up in recent years for immunology. While extensive research has dissected the role of metabolites in the complex regulation of immune cells, we are just beginning to explore the therapeutic potential of the ETC in inflammatory diseases. The ETC sits at the center of cellular energy metabolism, regulating the levels of NADH, FADH, and ATP in conjunction with the Krebs cycle, fatty acid oxidation, amino acid oxidation, and pyrimidine biosynthesis pathway. This review discussed the recent advances in the ETC functions beyond respiration and highlighted the ETC’s role in immune cell activation, proliferation, and differentiation. Future work will continue to unravel the functions of the ETC in inflammation and explore exciting therapeutic possibilities for treating inflammatory diseases.

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### DISCLOSURES

The authors declare no competing interests.

### AUTHOR CONTRIBUTIONS

Maureen Yin and Luke A. J. O’Neill cowrote the manuscript.

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