Trends in
Immunology

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Feature Review

Unlocking potential: the role of the electron transport chain in immunometabolism

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The electron transport chain (ETC) couples electron transfer with proton pumping to generate ATP and it also regulates particular innate and adaptive immune cell function. While NLRP3 inflammasome activation was initially linked to reactive oxygen species (ROS) produced from Complexes I and III, recent research suggests that an intact ETC fueling ATP is needed. Complex II may be responsible for Th1 cell proliferation and in some cases, effector cytokine production. Complex III is required for regulatory T (Treg) cell function, while oxidative phosphorylation (OXPHOS) and Complexes I, IV, and V sustain proliferation and antibody production in B lymphocytes, with OXPHOS also being required for B regulatory (Breg) cell function. Despite challenges, the ETC shows therapeutic targeting potential for immune-related diseases and in immuno-oncology.

Highlights

Reliance on the electron transport chain (ETC) in mammalian innate immunity is demonstrated by its role in NLRP3 inflammasome activation. Recent studies challenge the conventional link between reactive oxygen species (ROS) and NLRP3, emphasizing that forward electron transfer and mitochondria-generated ATP are required for NLRP3 activation via the phosphocreatine shuttle.

Activity of the ETC can modulate the phenotype of mouse T and B cell populations, sustaining their proliferation and effector functions. This might be exploited to promote immune tolerance and inhibit autoimmunity.

Immune cells infiltrating the tumor microenvironment undergo metabolic rewiring depending on the nutrients they require. This metabolic switch may allow tumor-driven immune evasion, potentially dampening novel candidate therapies targeting mitochondria. Anticancer strategies might involve respiration inhibitors to target both immune and cancer cells.

Significance

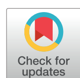
Recent findings show that remodeling of the mitochondrial ETC can regulate several mammalian immune processes. The ETC can support NLRP3 inflammasome activation and proinflammatory cytokine production. It can also promote effector and regulatory functions in T and B lymphocytes. This is relevant, highlighting the potential role of future therapies targeting the ETC to boost immune-mediated defenses against infection and cancer. However, since the ETC supports oxidative phosphorylation, its targeting might be deleterious, meriting attention.

The ETC and its regulation

The mitochondrial ETC is a crucial cellular process that occurs in the mammalian inner mitochondrial membrane. It plays a pivotal role in energy production through OXPHOS (Figure 1) [1]. The ETC comprises Complexes I–IV and facilitates proton pumping from the mitochondrial matrix into the intermembrane space (IMS) to synthesize ATP. Complex I (NADH coenzyme Q reductase; labeled I) and Complex II (succinate dehydrogenase; SDH; labeled II) transfer electrons from NADH and succinate respectively to ubiquinone (CoQ) and reduce it to ubiquinol ($CoQH_2$) [1]. $CoQH_2$ freely diffuses within the membrane and passes electrons to Complex III (cytochrome bc1 complex; labeled III), which passes them to Complex IV (cytochrome *c* oxidase; labeled IV) through cytochrome *c* (Cyt *c*). Through the transferred electrons, the energy is released to pump protons from the mitochondrial matrix into the IMS at Complexes I, III, and IV, generating an electrochemical gradient. This gradient is used by the F_oF_1 ATP synthase (sometimes also known as Complex V) to synthesize ATP via OXPHOS [2].

Of particular interest to immunologists was the discovery in 1996 linking the ETC and cell death, showing that Cyt *c* release from the ETC acts as a key driver of apoptosis [3]. Cyt *c* functions as an electron shuttle in the respiratory redox chain and interacts with cardiolipin [4]. Under proapoptotic conditions, Cyt *c* is released into the cytosol, where it mediates the allosteric activation and oligomerization of the adaptor molecule apoptosis-protease activating factor 1 (APAF-1), forming the apoptosome complex [5–9]. Apoptosome proteins then lead to the recruitment and activation of caspases and ultimately cause apoptotic cell death [10,11].

Besides Cyt *c*, the ETC also produces ROS and ATP to signal to the rest of the cell [12–15]. The production of ROS is important because it is an indicator of oxidative damage in many pathologies and contributes to redox signaling from the mitochondria to the cytosol and the nucleus [16]. Electrons leaking from the ETC can prematurely react with oxygen, resulting in the generation of superoxide ($O_2^{\bullet-}$) and H_2O_2 [17]. While there are several potential mitochondrial sources of



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$O_2^{\bullet -}$, Complex I is considered to be the major contributor. It was first demonstrated in submitochondrial particles from beef hearts where the reduction of the CoQ pool and generation of a high proton motive force led to H_2O_2 production [18]. **Reverse electron transport (RET)** (see Glossary) has been observed in ischemic stroke, activated macrophages in response to bacterial infection, cancer settings, as well as aged *Drosophila*; here, electron transfer can reverse direction from $CoQH_2$ to NAD^+ at Complex I, producing a significant amount of ROS [19–22]. Complex III is

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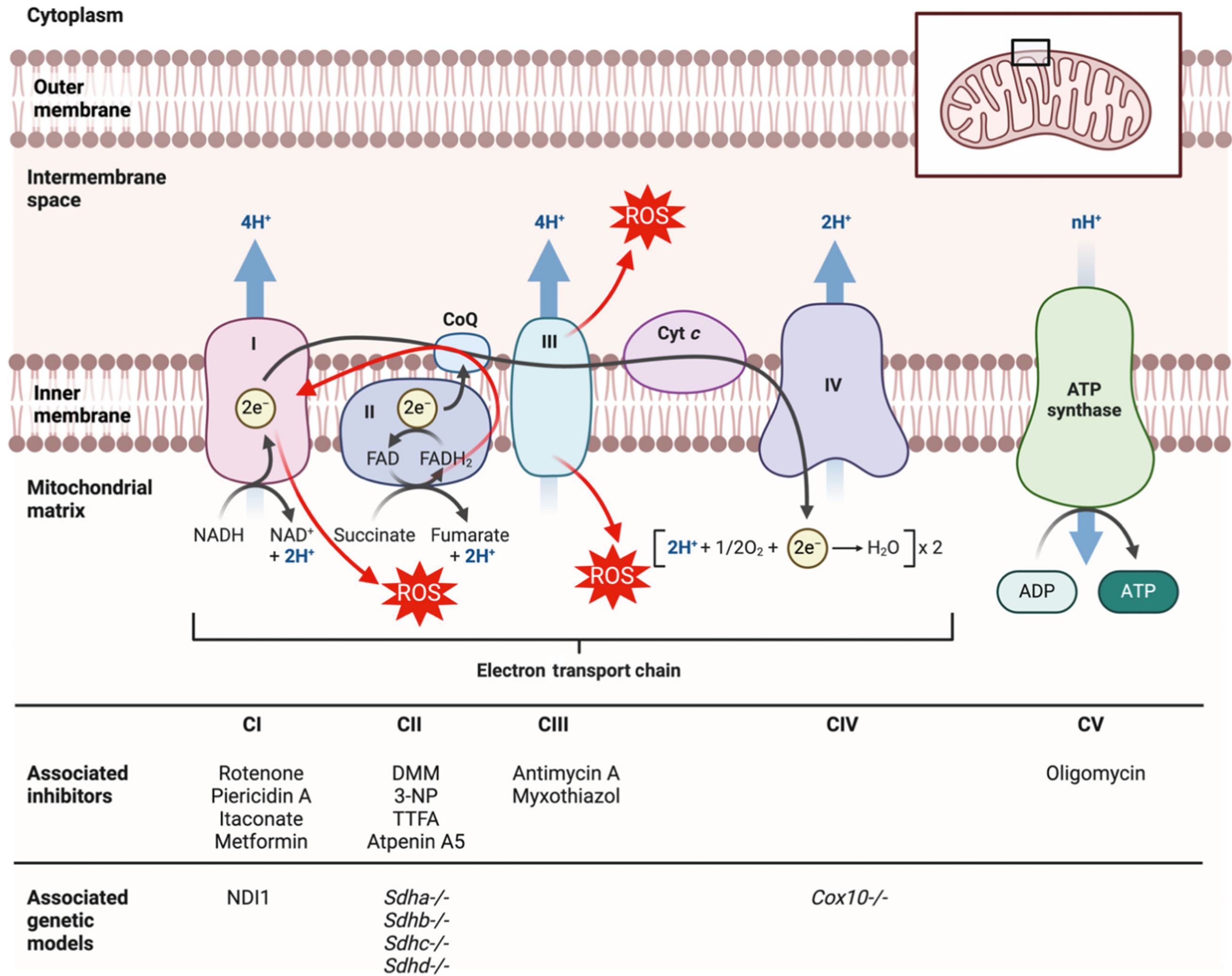

	CI	CII	CIII	CIV	CV
Associated inhibitors	Rotenone Piericidin A Itaconate Metformin	DMM 3-NP TTFA Atpenin A5	Antimycin A Myxothiazol		Oligomycin
Associated genetic models	NDI1	*Sdha-/-* *Sdhb-/-* *Sdhc-/-* *Sdhd-/-*		*Cox10-/-*	

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Figure 1. The mammalian ETC, chemical inhibitors, and genetic models utilized in recent immunological studies. It is well established that the mammalian ETC consists of four complexes (I–IV) and two electron carriers (CoQ and Cyt *c*) situated in the inner mitochondrial membrane [1]. The breakdown of nutrients through glycolysis, the Krebs cycle, amino acid, and fatty acid oxidation generates high energy-reducing equivalents NADH and $FADH_2$. NADH and $FADH_2$ are oxidized into NAD^+ and FAD^+ respectively and the electrons are then transferred to Complex III by CoQ, ultimately through Cyt *c* to Complex IV, where it is used to reduce oxygen into water. The transfer of electrons coupled with the translocation of protons across the inner mitochondrial membrane creates an electrochemical proton gradient that drives the synthesis of ATP at Complex V or ATP synthase [2]. Complexes I and III are the two major sites for ROS production in the ETC, with ROS production at Complex I often triggered by succinate-driven reverse electron transfer, leading to a highly reduced CoQ pool [19–22], whereas ROS generated at Complex III can enter both the mitochondrial matrix and intermembrane space [16,23]. Chemical inhibitors and genetic models utilized to study the role of individual complexes in the immune system are illustrated below. Note that many of the genetic models are immune-cell-type-specific and/or conditional knockouts. Abbreviations: 3-NP, 3-nitropropionic acid; CoQ, coenzyme Q; Cyt *c*, cytochrome *c*; DMM, dimethyl malonate; ETC, electron transport chain; NDI1, NADH dehydrogenase; ROS, reactive oxygen species; TTFA, thenoyltrifluoroacetone. Figure created with BioRender.com.

another major site of ROS, although the amount is overlooked compared to the ROS produced at Complex I [16]. Complex III ROS can disperse into both the mitochondrial matrix and IMS in which the $O_2^{\bullet-}$ can be converted to the more stable H_2O_2 and freely disperses through the outer membrane of the mitochondria [23].

Many studies have delved into the significance of respiration and individual ETC complexes within various immune cells by utilizing chemical inhibitors or genetic models (Figure 1) [15]. Indeed, the concept of the ETC specifically influencing immune processes is still relatively novel. Cyt *c*, ROS, or ATP might serve as potential links between the mitochondria and the rest of the cell, but other ETC-derived factors might also play a role in this interplay.

This Review focuses on recent discoveries on the ETC in macrophages, T and B lymphocytes, as well as immune cells in the tumor microenvironment (TME), and explores the potential therapeutic implications of targeting the ETC in autoimmunity and immuno-oncology. Understanding these connections will shed light on promising avenues for intervention in diseases where immune dysregulation plays a critical role.

The ETC and NLRP3

NLRP3 is a crucial component of the innate immune system that detects a wide range of factors, and its activation leads to the release of inflammatory cytokines interleukin (IL)-1β and IL-18, as well as driving an inflammatory form of cell death termed pyroptosis [7]. The activation of NLRP3 typically requires two signals. Signal 1 triggers priming that is mainly provided by the detection of **pathogen-associated molecular patterns (PAMPs)** which leads to the transcriptional activation of NLRP3 components via NF-κB. Signal 2 – activation – is induced by PAMPs, **damage-associated molecular patterns (DAMPs)**, or phagocytosed material, and causes NLRP3 assembly [24]. NLRP3 triggers the activation of caspase-1, which then cleaves pro-IL-1β and pro-IL-18. NLRP3 also cleaves Gasdermin D (GSDMD), prompting its insertion into the cell membrane, inducing **pyroptosis** [7]. While various events have been proposed as potential triggers for NLRP3 activation, such as membrane permeation, lysosomal damage, and fluxes of ions such as K^+, Cl^-, and Ca^{2+}, the role of the ETC in this process remains a subject of interest and discussion.

ROS produced from Complexes I and III of the ETC were initially linked to the activation of the NLRP3 inflammasome [14]. In this study, the respective Complex I and III inhibitors rotenone and antimycin A both induced ROS production and NLRP3 activation in THP-1 cells and murine bone marrow-derived macrophages (BMDMs), but not thenoyltrifluoroacetone (TTFA), an inhibitor of Complex II. Inflammasome activation was abolished with the addition of a ROS scavenger, APDC. Of note, sustained incubation of rotenone and antimycin A alone was sufficient for NLRP3-induced IL-1β release without the need for lipopolysaccharide (LPS) priming in THP-1 cells. Similarly, inhibition of **mitophagy/autophagy** caused the accumulation of ROS-producing damaged mitochondria which also led to the activation of NLRP3, further strengthening the link between ROS and NLRP3 activation. However, a later report indicated that K^+ efflux, but not ROS, was a common trigger of NLRP3 inflammasome activation [25]. In that study, rotenone, antimycin A, the autophagy inhibitor 3-methyladenine, or H_2O_2, failed to activate NLRP3 in LPS-primed BMDMs. Also, the ROS scavenger N-acetyl cysteine (NAC) could not impede NLRP3 activation triggered by the second signal, namely, nigericin or ATP. The ongoing debate persisted when ROS-dependent activation of NLRP3 by imiquimod and the related molecule CL097, were observed [12]. This activation was found to occur partly through inhibition on Complex I of the ETC because these two molecules selectively blocked Complex-I-mediated respiration without affecting respiration via Complex II or Cyt *c*–Complex IV, increased the Complex I substrate NADH, and decreased the NAD^+/NADH ratio in digitonin-permeabilized murine BMDMs [12].

Glossary

Alternative oxidase (AOX): single-subunit protein found in many eukaryotes, but not in arthropods or vertebrates; conveys electrons directly from the ubiquinol pool to oxygen and bypasses the cytochrome pathway; prevents the over-reduction of the ubiquinone pool, which is a major source of superoxide.
Autophagy: cellular process responsible for the degradation and recycling of various cellular components, such as damaged organelles, proteins, and pathogens; involves the formation of autophagosomes, which engulf the targeted cellular material and deliver it to lysosomes for degradation.
Chimeric antigen receptor (CAR) T cells: immunotherapy involving engineered T cells which possess a modified TCR that is redirected against specific antigens, thus able to specifically target a single type of cell. CAR T cells are mainly used as a treatment against certain blood cancers.
Class switch recombination (CSR): molecular process in the nucleus of B lymphocytes following their activation; involves the recombination of specific DNA fragments coding for the constant domain of an antibody's heavy chain (class); leads to switches in different immunoglobulin classes.
Damage-associated molecular patterns (DAMPs): group of endogenous danger molecules (e.g. HMGB1, ATP, uric acid, DNA), released from damaged or dying cells due to trauma or infection; recognized by PRRs. DAMPs include proteins such as heat-shock proteins and HMGB1 or non-proteins such as ATP, uric acid, and DNA.
Exhaustion: $CD8^+$ cytotoxic T cells lose their effector ability to kill pathogens and transformed cells; usually reached after a long time of T cell activation. In this state, T lymphocytes express more autoinhibitory receptors to avoid autoimmunity.
Immune checkpoint blockade: innovative technique of cell immunotherapy targeting checkpoint molecules expressed by immune cells; for example, PD-1/PD-L1 and CTLA-4 signaling pathways.
Mitophagy: selective degradation of mitochondria by autophagy; mainly in damaged mitochondria due to injury or stress.
NADH dehydrogenase (NDI1): rotenone-insensitive internal NADH-

IL-1β release induced by imiquimod and CL097 in LPS-primed BMDMs and bone marrow-derived dendritic cells (BMDCs) was dose-dependently reduced by the mitochondrial ROS scavengers Ebselen and PDTC, suggesting the link between ROS and NLRP3 activation [12].

In 2022, forward electron transfer and mitochondria-generated ATP were shown to be imperative for the activation of NLRP3 in murine BMDMs (Figure 2) [13]. Piericidin A- or myxothiazol-treated macrophages had impaired Complex I or III, respectively, and were defective in NLRP3 activation by ATP and/or nigericin. This suggested that a functional ETC might be required for NLRP3 activation. Indeed, BMDMs expressing *Saccharomyces cerevisiae* **NADH dehydrogenase (NDI1)** and/or *Ciona intestinalis* **alternative oxidase (AOX)** (allowing electron transport but not ROS generation at either Complex I or III), restored NLRP3 activation. IL-1β concentrations in serum showed no significant change following LPS administration in wild-type (WT) mice compared to mice expressing NDI1. Similarly, LPS induced comparable amounts of secreted IL-1β protein in serum from WT mice and from those lacking QPC (Complex III knockout) but expressing AOX (which sustains electron flow in Complex III without generating ROS). In addition, Mitotempo, a mitochondria-specific antioxidant, along with S1QEL and S3QEL (suppressing mitochondrial Complex-I- or III-generated superoxide production, respectively) [26,27] did not prevent CL097- or extracellular ATP-induced NLRP3 activation [13]. By using these genetic models combined with respiratory chain inhibitors, the research group showed that $O_2^{\bullet -}$ generated at Complex I via RET or Complex III was not required for NLRP3 activation in *vitro* or *in vivo* (in mice) in response to ATP [13]. The discrepancies concerning ROS involvement in NLRP3 activation between the different studies may be due to different stimuli being used or by considering temporal factors [12–14]. Perhaps NLRP3 activation by phagocytosed material such as cholesterol crystals might involve mitochondrial ROS, although this remains conjectural [28,29].

Importantly, the latter study proposed that it is the mitochondria-generated ATP that supports NLRP3 activation rather than ROS, acting via phosphocreatine (PCr) [13]. High-energy phosphate is transferred from OXPHOS-generated ATP to creatine by creatine kinase, mitochondrial 2 (CKMT2) in the mitochondria, forming PCr [30,31]. PCr then diffuses into the cytosol, where it can be reversed back to creatine by cytosolic creatine kinase B (CKB), and donate the phosphate to a molecule of ADP, generating cytosolic ATP. In this study, all the ETC inhibitors that suppressed NLRP3 activation also reduced PCr [13]. By using cyclocreatine – a creatine analog that inhibits the donation of phosphate to ADP in the cytosol, and RNAi against cytosolic CKB, IL-1β release was diminished in LPS-primed murine BMDMs. The data support the involvement of CKB and PCr in NLRP3 activation through the regulation of intracellular ATP concentrations. This mechanism is plausible, given previous findings of ATP directly binding to the NACHT domain to induce NLRP3 activation [32]. Complete depletion of ATP by 2-deoxyglucose (2-DG) and sodium azide under glucose starvation also led to an absence of IL-1β release from murine BMDMs [33].

These findings shed light on the intricate connection between mitochondrial metabolism and NLRP3, highlighting the potential significance of targeting mitochondrial ATP production pathways in modulating NLRP3 activation and thus, the inflammatory response. Future investigations should include comprehensive measurements of PCr, intracellular ATP, and ROS concentrations in response to various stimuli to better understand the mechanisms underlying NLRP3 activation. Additionally, utilizing genetic models such as NDI1 or AOX, may provide further insights into the regulation of NLRP3 activation pathways.

Complex II and IL-1β production

Complex II consists of four subunits comprising the enzyme succinate dehydrogenase, SDHA–SDHD, which functions in both the Krebs cycle and the ETC. SDH is a crucial branch point in

quinone is a component of *Saccharomyces cerevisiae*, as well as other yeast and certain bacteria. NDI1 functions by directly transferring electrons from NADH to ubiquinone, bypassing the need for direct interaction with oxygen molecules (does not lead to ROS).

Pathogen-associated molecular patterns (PAMPs): vast array of molecular motifs (e.g., LPS) conserved within a class of microbes recognized by TLRs and other pattern recognition receptors (PRRs).

Phosphocreatine (PCr) shuttle: PCr is a phosphorylated form of creatine; serves as a rapidly mobilizable reserve of high-energy phosphates. In the PCr shuttle, high-energy phosphate is transferred from ATP generated by OXPHOS in the mitochondria to creatine by CKMT2, thus generating PCr and ADP. PCr then diffuses into the cytosol, is converted back to ATP and creatine by cytosolic CKB. ATP is used by ATPases, while creatine returns to the mitochondria.

Pyroptosis: form of lytic programmed cell death triggered by proinflammatory signals; causes the release of inflammatory mediators such as IL-1β and IL-18; driven by inflammasomes activating caspase-1, cleaving Gasdermin D, leading to pore formation in the cell membrane, and cell lysis.

RAR-related orphan receptor (ROR)γt: member of the family of NR1 nuclear receptors; specific marker for Th17 lineage. When in the nucleus, acts as a transcription factor impacting genes encoding IL-17 and IL-23.

Regulatory B (Breg) cells: subset of B lymphocytes with immunosuppressive functions; mainly produce IL-10, TGF-β, and IL-35 as regulatory cytokines. Whether they originate from a unique progenitor or they have a specific lineage marker remains to be elucidated.

Regulatory T (Treg) cells: a unique subpopulation of helper T cells; suppress immune responses and maintain immune tolerance by inhibiting the activation and function of other immune cells. TGF-β is essential for *Foxp3 transcription and Treg development*.

Reverse electron transport (RET): phenomenon where electrons are transferred in the opposite direction compared to the usual flow in the ETC; occurs when there is a high mitochondrial membrane potential and when the pool of CoQ becomes highly reduced by electrons from Complex II.

the ETC because it reversibly converts succinate to fumarate while reducing FAD to $FADH_2$, transferring an electron into the ETC. Inflammatory, so-called M1-like macrophages produce increased succinate from a break at SDH [34,35]. Succinate accumulation stabilizes hypoxia-inducible factor (HIF)-1α, which activates *Il1b* (encoding IL-1β) transcription (Figure 2) [20,35]; an effect that is reversed by the SDH inhibitor, dimethyl malonate (DMM) [20]. When murine BMDMs were challenged with live *Escherichia coli* (*E. coli*), Complex I was destabilized in macrophages with increased Complex II activity, as evidenced by the reduced abundance of Complex I and Complex-I-containing supercomplexes, as well as Complex-I-mediated respiration [36]. The Complex II inhibitor 3-nitropropionic acid (3-NP) increased susceptibility in mice to infection with *Salmonella enterica* Typhimurium, as evidenced from the decreased mouse survival upon infection. Also, 3-NP-treated mice presented with greater splenic bacterial burden after intraperitoneal infection with live *E. coli* compared with their littermates treated with vehicle [36]. The greater bacterial burden in 3-NP-treated mice post *E. coli* infection correlated with lowered serum IL-1β concentrations and increased production of the anti-inflammatory cytokine IL-10 [36]. A recent study utilized CRISPR-Cas9-based deletion of *Sdha* and *Sdhb*, the two catalytic subunits of Complex II, and confirmed that these subunits were necessary for LPS-mediated IL-1β production and HIF-1α stabilization in murine BMDMs [37]. Of note, *Sdha* and *Sdhb* were essential for IL-10 production and signal transducer and activator of transcription (STAT)3 phosphorylation. This is interesting because it contradicts the previous report demonstrating an inhibitory role of Complex II on IL-10 production [36]. The differences in IL-10 production could be attributed to the specific experimental conditions, with compromised respiration being more pronounced in *Sdha/Sdhb* knockout cells compared to 3-NP treated cells. This distinction suggests that long-term loss of Complex II in knockouts may be associated with metabolic adaptations that lead to the reduction of IL-10.

As mentioned above, RET at Complex I, with electrons coming from the oxidation of succinate by SDH appears to be required for HIF-1α stabilization leading to IL-1β production [20,35]. At a later time-point, however, succinate itself can activate HIF-1α, most likely via inhibition of the prolyl hydroxylases (PHDs) and promote IL-1β production [38]. Thus, understanding the dual role of succinate and succinate oxidation in HIF-1α activation, both through RET at Complex I, and direct inhibition of PHDs can shed light on the temporal dynamics of HIF-1α activation and subsequent IL-1β production. This distinction is crucial in scenarios such as ischemic reperfusion injury, where succinate-oxidation-fueled RET is implicated, underscoring the importance of differentiating early and late HIF-1α activation for targeted therapeutic strategies [19]. Of note, the metabolite itaconate, derived from aconitate, might inhibit this process because it can inhibit SDH, thereby blocking IL-1β production as part of its anti-inflammatory effect [39–42].

The interplay between Complex II and succinate metabolism in macrophages is therefore complex and context dependent. While succinate accumulation from reduced SDH activity can lead to HIF-1α stabilization and IL-1β production, the role of Complex II in modulating IL-10 production is less clear, possibly being influenced by experimental conditions. Additionally, the direct activation of HIF-1α by succinate and its inhibition by itaconate highlight the intricate balance between pro- and anti-inflammatory metabolites, suggesting potential therapeutic approaches to modulate these metabolites in immune-related disorders.

Complexes I and II interplay in T cells

It is well known that activation of T cell effector function is initiated by engaging the T cell receptor (TCR) in conjunction with co-stimulatory signals. Complex I inhibition with rotenone has been demonstrated to result in cell-cycle arrest at the G_2 or M phase in murine $CD4^+$ T cells cocultured with **T helper (Th)1**, Th2, or **Th17 cells**, suggesting a conserved role for Complex I in regulating

Somatic hypermutation: molecular process involving the mutation of the immunoglobulin variable region gene in B lymphocytes; begins after the B cell encounters antigen; essential for antibody diversity and to recognizing an enormous array of foreign peptides.
Th1 cells: lead to an increased cell-mediated response; triggered by the polarizing cytokine IL-12, and through the transcription factor T-bet, producing effector cytokines IFN-γ and IL-2.
Th17 cells: specific helper subset mediating host defense; are also pathologic in autoimmune disorders such as multiple sclerosis. Characterized by IL-17 production and the presence of transcription factor RORγt; generated following exposure to IL-6, IL-23, and TGF-β.
T helper cells: $CD4^+$ helper T cells that can differentiate into different subsets upon various environmental stimuli; the major subsets include Th1, Th2, Th17, and Treg cells, each with distinct functions.

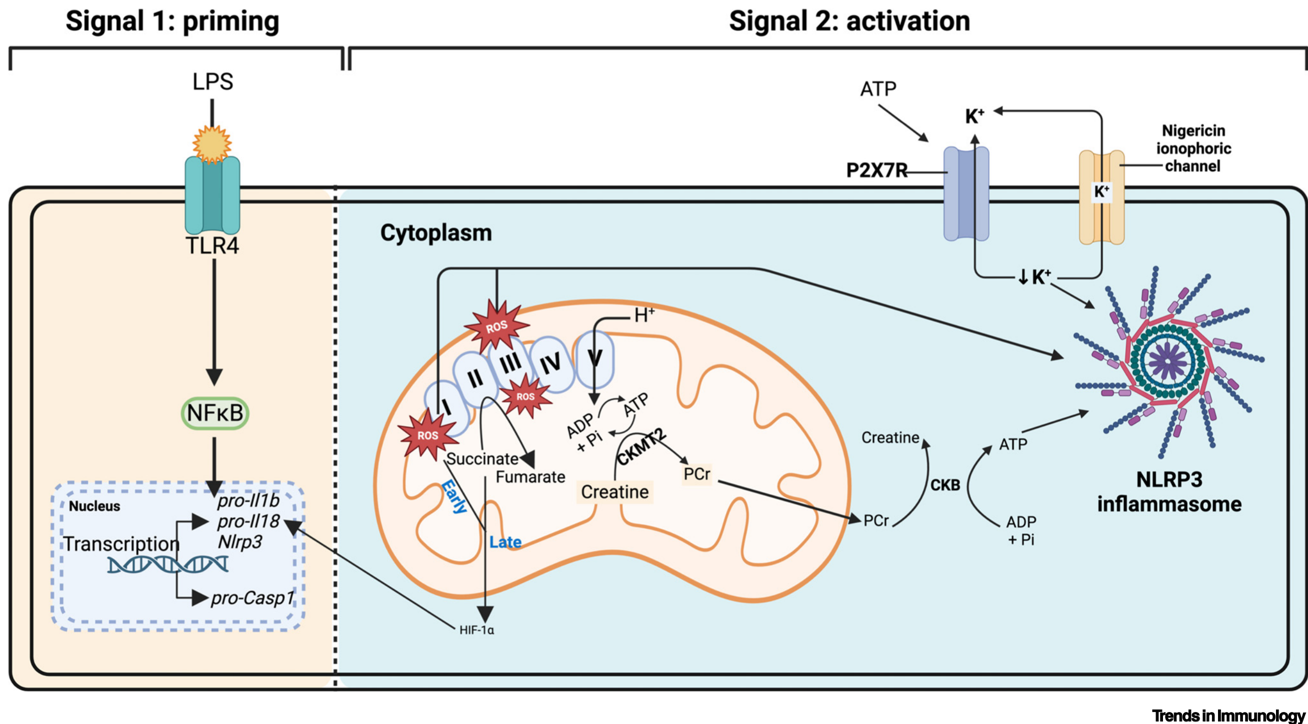

Figure 2. The ETC and NLRP3 activation in macrophages. Signal 1 or the 'priming' step in macrophages typically involves the sensing of LPS by TLR4. This then activates NF-κB, which leads to the transcription of pro-IL-1β, pro-IL-18, components of NLRP3, and pro-caspase-1. Under inflammatory conditions, electrons in the ETC can flow in RET resulting in ROS production at Complex I [20]. At an early time-point, ROS generated from RET at Complex I is known to stabilize HIF-1α, which drives the production of pro-IL-1β; while at a later time point, succinate alone can also aid in the transcription of pro-IL-1β by acting directly on HIF-1α [38]. The second step of NLRP3 activation requires signal 2, in which ROS generated at Complexes I and III are recognized as a common activator [12,14,24]. Extracellular ATP acting on the ATP receptor P2X7R causes K^+ efflux, and also activates NLRP3 [25]. Recently, iATP has emerged as another NLRP3 activator. In this case, creatine and the ETC-generated ATP are converted to PCr by CKMT2, and then released into the cytosol where it is converted back to creatine by cytosolic CKB, transferring the phosphate group to ADP, thus generating iATP [30,31]. This mitochondrial ETC-derived iATP is necessary for the activation of NLRP3 via binding to the NACHT domain on NLRP3 [32]. Abbreviations: Casp1, caspase-1; CKB, creatine kinase B; CKMT2, creatine kinase, mitochondrial 2; ETC, electron transport chain; HIF-1α, hypoxia-inducible factor 1α; LPS, lipopolysaccharide; P2X7R, P2X purinoceptor 7; PCr, phosphocreatine; RET, reverse electron transport; ROS, reactive oxygen species; TLR4, Toll-like receptor 4. Created with BioRender.com.

Th cell proliferation [43]. However, effector cytokine production in these cells varied: while Complex I inhibition had little impact on interferon (IFN)γ production in Th1 cells, it moderately boosted IL-4 in Th2 cells and reduced IL-17 in Th17 cells [43]. Another study showed that rotenone, combined with antimycin A treatment also reduced the frequency of IL-17A$^+$ and forkhead box (Fox)P3$^+$ murine CD4$^+$ T cells when cultured in Th17 conditions, supporting the role of Complex I in Th17 effector functions [44]. These results suggest that Complex I supports cell division regardless of the cytokine environment but has specific roles in regulating the effector functions of Th cells [43].

In the same report, *Sdhc*-deficient Th1 cells exhibited enhanced proliferation compared to WT controls, indicating that Complex II might be suppressing Th1 proliferation [43]. Inhibition of Complex II with DMM, 3NP, TTFA, or atpenin A5, or by genetically ablating *Sda or Sdhc,* both resulted in decreased IFN-γ production in Th1 cells, accompanied by elevated concentrations of α-ketoglutarate (α-KG), which is a key metabolite in the malate–aspartate shuttle [43]. The malate–aspartate shuttle and mitochondrial citrate export system supported rapid cell division and epigenetic remodeling during the early phase of cell differentiation in CD4$^+$ T cells [43]. Disruption of the malate–aspartate shuttle by targeting *Slc25a11* using sgRNA in murine CD4$^+$ T cells led to a decreased NADH/NAD$^+$ ratio, implicating these shuttling systems in

providing mitochondrial NADH for Complex I. In summary, these findings suggest that early activated Th cells can fuel Complex I through the malate–aspartate shuttle and mitochondrial citrate export. As differentiation progresses, Complex II can redirect carbon away from α-KG, thereby diminishing NADH availability for Complex I and facilitating the termination of the differentiation process, allowing Th1 cells to fully engage their terminal effector cell program (Figure 3).

However, in yet another conflicting report, *Sdhb* deletion in murine CD4+ T cells significantly delayed cell cycle progression from G_0–G_1 to the S phase [45]. Activated but not naïve *Sdhb*- and *Sdhd*-deficient murine CD3+ T cells exhibited an increased succinate/α-KG ratio (as opposed to the increased α-KG in the previous study [43]) and upregulated expression of genes encoding IFN-γ, IL-22, IL-17A, and IL-17F expression (via RNA-seq) [45] (Figure 4). These authors indicated that the formation of succinate due to Complex II inhibition, elicited the proinflammatory cytokine profile [45]. Reduced proliferation caused by deletion of *Sdhb* in T cells also protected mice from disease progression in an experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis. This was evidenced by the reduced EAE clinical score and leukocyte accumulation in the spinal cord of animals. Moreover, cultured CD4+ T cell supplementation of cell-permeable succinate, NV118, effectively restored the expression of RNA molecules encoding IL-17F, IFN-γ, and IL-22 in CD4+ T cells. Conversely, supplementation with α-KG resulted in reduced expression of these cytokines in the same cell population [45]. The increased succinate/α-KG ratio in *Sdhb*-depleted CD4+ T cells led to a concordant increase in the expression

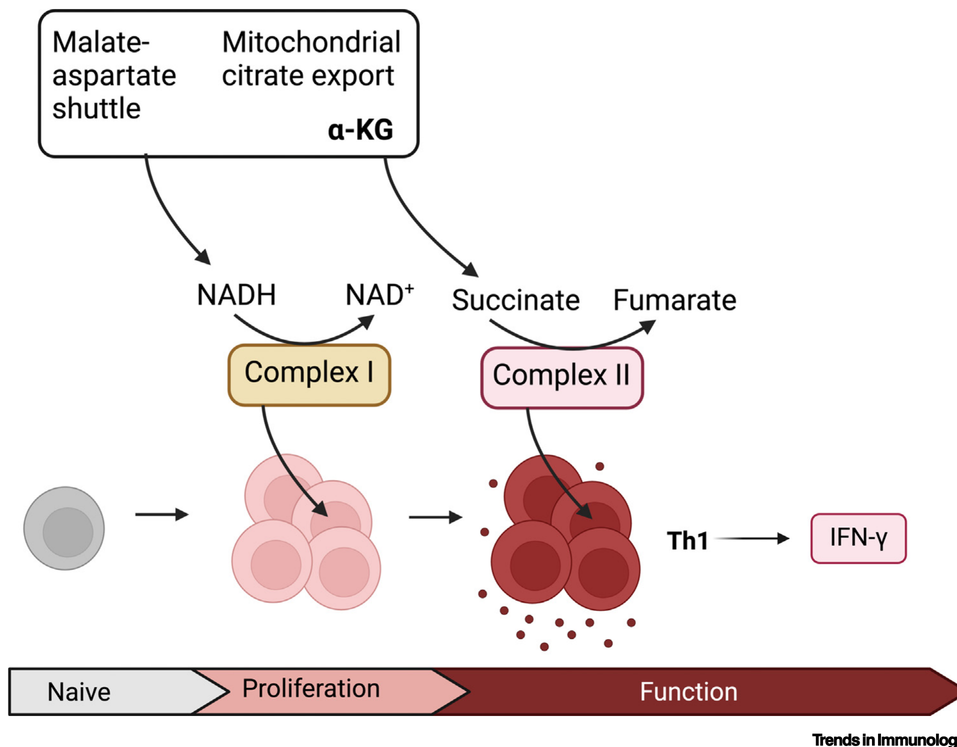

Figure 3. Complexes I and II can support mouse Th1 cell activation during various stages of differentiation. The malate-aspartate shuttle and mitochondrial citrate export results in the net movement of NAD+ to the cytosol and NADH into the mitochondria, through which the cycle can fuel the activity of Complex I during the early phase of T cell activation, leading to epigenetic remodeling and rapid cell division [43]. As differentiation continues, Complex II draws carbon away from α-KG, fueling the oxidation of succinate to fumarate, pulling out activated T cells out of the differentiation process and enabling them to fully engage in the effector cell program [43]. Abbreviations: α-KG, α-ketoglutarate; IFN-γ; interferon-γ; Th1, T helper 1. Created with BioRender.com.

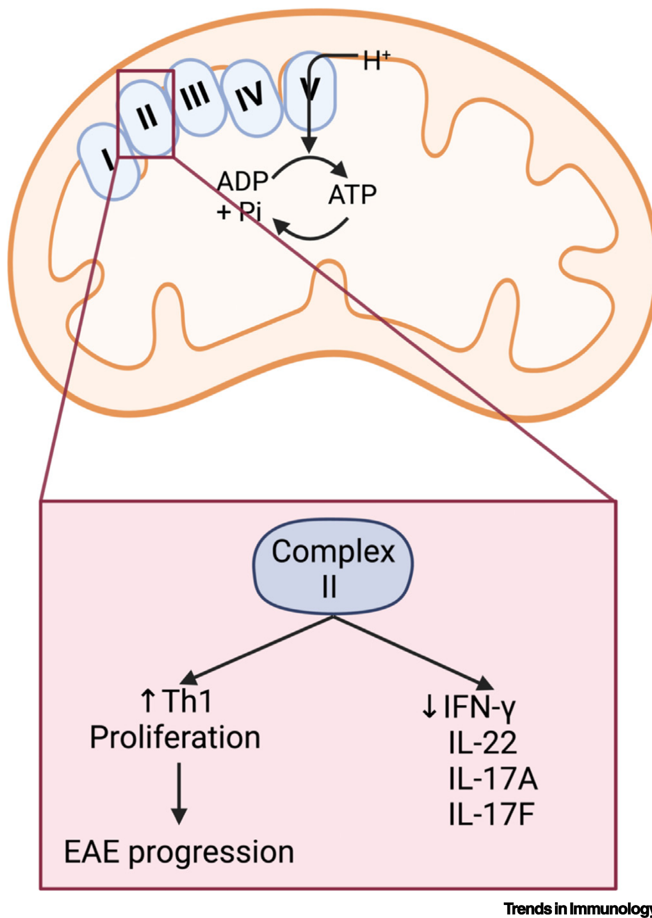

Figure 4. Complex II can modulate mouse T cell functions. Complex II can support Th1 cell proliferation; indeed, deletion of Complex II reduced proliferation, hence protected mice from EAE [45]. However, Complex II has also been shown to suppress effector cytokine production from activated $CD4^+$ T cells, including IFN-γ, IL-22, IL-17A, and IL-17F [45]. Abbreviations: EAE, experimental autoimmune encephalomyelitis; IFN-γ, interferon-γ; IL, interleukin; Th1, T helper 1. Created with Biorender.com.

and the motif accessibility of the transcription factor *Prdm1* (encoding Blimp-1), a known regulator of T cell differentiation and inflammation [45]. Of note, α-KG has also been shown to be a fundamental controller of Th1 against **Treg cell** differentiation [46]. Its supplementation in the media during Treg- and Th1-polarizing conditions boosted OXPHOS in both cases but restrained *Foxp3* expression and increased IFN-γ production in murine $CD4^+$ T cells [46]. By contrast, culturing Th1 cells with diethyl succinate (DES), a cell-permeable form of succinate, increased the percentage of $FoxP3^+$ cells from naïve $CD4^+$ T cells cultured in Treg-cell-polarizing conditions [46].

The discrepancies regarding the role of Complex II in T cell proliferation and function may stem from differences in experimental models (e.g., the use of inhibitors or genetic models) and naïve versus polarizing environments. Importantly, T cells must navigate between proliferation and differentiation pathways, a decision guided by complex transcriptional and epigenetic programs that differ between naïve, activated, and polarized states [47,48]. As such, future investigations should carefully consider these dynamic processes to unravel the precise role of SDH in T cell biology within distinct cytokine environments and during fate decision-making processes.

Itaconate as an anti-inflammatory metabolite in T cell immunity

Itaconate can block $CD4^+$ T cell differentiation *in vitro;* specifically*,* itaconate-treated lymphocytes isolated from murine spleens and cultured in Th17 and Treg cell conditions exhibited decreased

production of IL-17A and IL-17F and enhanced FoxP3 expression [49]. These changes were accompanied by decreased basal and maximal respiration rates and ATP production, suggesting that itaconate targeted Complex II [49]. Itaconate was therefore shown to have an immunomodulatory effect on T cells, as has been extensively indicated in macrophages [40]. Additionally, itaconate produced by myeloid-derived suppressor cells (MDSCs) lowered the activity of cytotoxic CD8$^+$ T cells and promoted MC38 tumor growth *in vivo* in mice, as evidenced from the analysis of MC38-OVA (ovalbumin) tumor cell viability following coculture with OT-1 splenic CD8$^+$ T cells. This supported the role of itaconate as being able to target T cells and in this case, promote tumor growth [49]. Metabolic profiling confirmed the accumulation of succinate and decreased fumarate amounts in CD8$^+$ T cells, suggesting SDH inhibition [50]. Another report demonstrated that itaconate could drive the exhaustion of cytotoxic CD8$^+$ T cells during hepatocellular carcinoma (HCC) progression through SDH inhibition [51]. Also, macrophage-derived itaconate was taken up by CD8$^+$ cytotoxic T cells, with increased mRNA expression of the transcription factor gene encoding EOMES (responsible for the induction of T cell-specific exhaustion genes such as those encoding PD-1 and TIM-3). Moreover, CD8$^+$ T cells cocultured with *Irg1*$^{-/-}$ BMDMs (unable to synthesize itaconate), showed decreased expression of exhaustion markers compared to controls [51].

Collectively, these studies suggest that itaconate, potentially acting via SDH inhibition, might promote a regulator phenotype in T cells, and also suppress the cytotoxic CD8$^+$ T cell phenotype via succinate-mediated epigenetic reprogramming-driven exhaustion; in the latter case, this can allow tumor expansion [50,51]. Nevertheless, further and rigorous studies are warranted to assess the effect of itaconate on T cells within different contexts.

Complexes III and V in the Treg /Th17 cell balance

Complex III is fundamental for Treg cell inhibitory functions. In one study, mice lacking subunit VII of Complex III (QPC) in the FoxP3$^+$ CD4$^+$ T cell lineage did not develop tumors in the mouse B16F10 melanoma model compared to WT controls; this suggested that the immunosuppressive function of Treg cells relied on Complex III [52]. Moreover, human Treg cells isolated from peripheral blood cells and activated with anti-CD3 and anti-CD28 antibodies and treated with the Complex III inhibitor antimycin A exhibited decreased FOXP3, IL-10, and cytotoxic T lymphocyte-associated protein (CTLA)-4 expression together with decreased STAT5 phosphorylation [53]. This indicated that Complex III was required for triggering the transcription of Treg-specific transcription factor and cytokine genes, and presumably, Treg cell function, at least in this model.

The relevance of OXPHOS and Complex V in the fate commitment of murine CD4$^+$ T cells has also been examined. The blockage of OXPHOS with Complex V inhibitor oligomycin during Th17-differentiating conditions elicited a subsequent decreased expression of HIF-1α- and respiration-related genes (*Sdhd*, *Idh1*, and *Mdh1)*, as well as those encoding IL-17A and **RAR-related orphan receptor (ROR)γt** [44]. Th17 cells treated with oligomycin were unable to sustain demyelination in the EAE model, as assessed by disease severity scores in *Rag1*$^{-/-}$*(B6.129S7-Rag1*^{tm1Mom}*/J)* mice, which lack mature T and B cells. This indicated that respiration in Th17 cells was essential for developing autoimmune disease *in vivo* in this model [44]. Oligomycin-treated Th17 lymphocytes showed an upregulation of suppressor of cytokine signaling (SOCS)3 and IL-10R, which impaired the progression of EAE disease, as evidenced from RNA-seq analysis of pathogenic and non-pathogenic gene signatures [44]. Inhibition of OXPHOS by oligomycin led to CD4$^+$ T cell FoxP3 expression during Th17 differentiating conditions, thus redirecting these cells towards the Treg phenotype, in turn blocking the proliferation of naïve CD4$^+$ T cells [43]. Th17 function therefore seems to involve Complex V while Treg cell function seems to require Complex III. This implies that the use of ETC-complex-specific modulators might redirect the immune response towards a beneficial outcome in this disease context, meriting further attention.

Regarding T cells and the ETC, a rather complex picture emerges. Complex I and II may control T cell phenotypes, but more work is needed to define their precise roles. Itaconate, most likely via inhibition of Complex II, may promote Treg cell functions while also contributing to $CD8^+$ T cell exhaustion. Complex III seems to be required for Treg cell function, while Complex V may be key in supporting Th17 cell activity.

ETC complexes in B cells

Compared to T cells, the studies of the ETC in B cells are more limited. The role of the ETC in B cell activation and immunity has been investigated with innovative approaches involving mitochondrial DNA (mtDNA) depletion instead of specific inhibitors [54]. Here, researchers used the dominant negative K320E mutation of the murine mitochondrial helicase Twinkle (DNT), which is essential for mtDNA replication [54]. Cre-recombinase-driven expression of DNT in $CD23^+$ B cells in mice ($DNTXCD23^{Cre}$) led to decreased amounts of Cyt *c* oxidase subunit I (COX1) protein, which impaired mitochondrial supercomplex formation and subsequent OXPHOS in LPS-activated B cells, as evidenced by electron microscopy and decreased oxygen consumption rate in DNT-B cells [54]. Mitochondrial respiration was required for LPS-driven IgG **class switch recombination (CSR)** from IgM, suggesting that the lack of OXPHOS and subsequent promotion of glycolysis and HIF-1α stabilization might impair B cell activation and CSR [54].

In another study, OXPHOS was reported to be necessary to establish the phenotype of a specific population of B cells – **regulatory B (Breg) cells**. Specifically, LPS-induced IL-10 production, characteristic of Breg cell effector functions, was restrained if respiration was inhibited via oligomycin [55]. This was paralleled by decreased expression of surface markers CD24, CD43, CD5, and CD23, unlike the survival and proliferation of B cells under normal respiration conditions [55]. *In vivo* activated B cells with impaired OXPHOS were incapable of ameliorating dextran sulfate sodium (DSS)-induced colitis and epithelial damage in mice, as evidenced from colon shortening and histopathology scores (immune cell infiltration into the sub-mucosal area); this suggested that OXPHOS in Breg cells might be fundamental for promoting the resolution of inflammation in the colon [55]. Of note, respiration has been implicated in B cell development and maturation in germinal centers. Specifically, B cells that undergo **somatic hypermutation** of the variable chains of the B cell receptor (BCR) after exposure to antigen exhibit higher OXPHOS than inactivated B cells, with higher expression of mitochondrial complex subunits such as ATP synthase subunits (ATP5A and ATP5E), Complex IV subunits (COX 8 and 10), and Complex I (NDUFAB4) [56]. Accordingly, B cell-specific ablation of COX10, an essential protein for the assembly of Complex IV, inhibited the proliferation and positive selection of B cells [56]. Therefore, mitochondrial respiration can sustain certain immune functions in B cells, at least in mice (Figure 5).

The ETC and antitumor immunity

One particular situation where the ETC in immune cells might be especially important is in antitumor immunity. As an example, persistent exposure of tumor antigens to T cells brings them to a state of **exhaustion**, which involves mitochondrial dysfunction [57,58] and oxidative stress, culminating in a decrease in ATP and an increase in the $NADH/NAD^+$ balance [59]. To assess the exhaustion state *in vitro*, T cells were isolated from murine spleens and subsequently activated and expanded in coculture with B16F10-OVA cells. OXPHOS restoration through overexpression of NADH oxidases from *Lactobacillus* in $CD8^+$ T cells improved mitochondrial fitness; furthermore, treatment with the antioxidant NAC, restored T cell effector function when these cells were chronically stimulated with antigen (OVA), suggesting a putative role for ROS in modulating exhaustion [60]. In another study, peritumoral supplementation of tumor-bearing mice with a recombinant fusion protein of IL-10 and IgG1 Fc (IL-10/Fc) restored the exhausted state of $CD8^+$ T cells, rescued

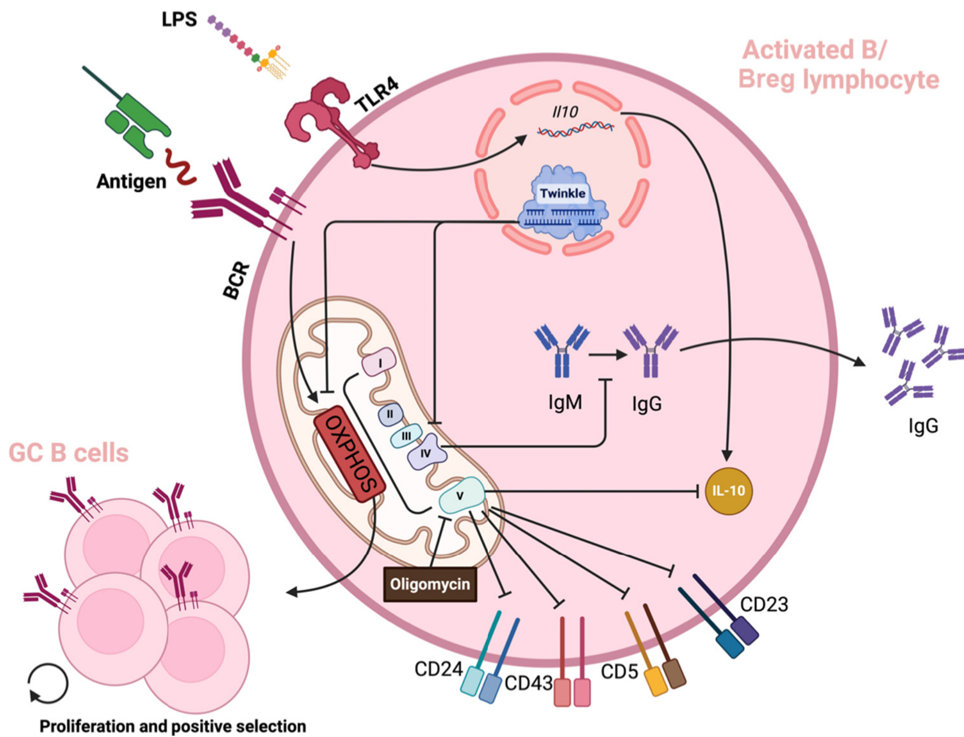

Figure 5. The ETC can sustain B cell function and proliferation in mice. When in contact with LPS through TLR4, Breg cells produce IL-10 [55]. LPS-induced production of IL-10 is impaired by oligomycin, a Complex V inhibitor [55]. Oligomycin also restrains the expression of CD24, CD23, CD5, and CD43 on the surface of Breg cells. Additionally, the binding of LPS to TLR4 induces a class switch from IgG to IgM production, which is blocked by Twinkle-mediated Complex IV deficiency [54]. In GCs, BCR engagement with antigen is essential to trigger OXPHOS, thus allowing B cell proliferation and positive selection [56]. Abbreviations: BCR, B cell receptor; Breg, regulatory B cell; ETC, electron transport chain; GC, germinal center; IL-10, interleukin 10; LPS, lipopolysaccharide; OXPHOS, oxidative phosphorylation; TLR4, Toll-like receptor 4. Created with BioRender.com.

OXPHOS, expanded $CD8^+$ tumor-infiltrating lymphocytes (TILs), and boosted the response to adoptive cell therapy and **immune checkpoint blockade** in B16F10-OVA and YUMM1.7-OVA tumor models [61] (Figure 6). These two studies highlighted how mitochondrial perturbation in cytotoxic $CD8^+$ T cells contributed to their exhaustion, and suggested that improvement of mitochondrial function was fundamental for restoring the killing activity of T cells and achieving tumor clearance, at least in these models.

The role of OXPHOS and the ETC has also been examined in the context of the therapeutic effects of **Chimeric antigen receptor (CAR) $CD8^+$ T cells** [62]. In one study, the CAR sequence included the mouse CD8 signal peptide, an antigen-specific scFv, a mouse CD8α hinge and transmembrane domain, a CD3ζ intracellular domain, and 4-1BB, or the CD28 co-stimulatory domain. These cells, which were transfected with retroviruses expressing IL-10 gene fragments into the CAR-containing viral vector, showed increased OXPHOS and mitochondrial fitness compared to CAR T cells not expressing IL-10. Mice could clear MC38 colorectal, PANC1 pancreatic adenocarcinoma, and B16F10 melanoma tumor burdens in xenograft models, exhibiting enhanced tumor cell killing activity and the establishment of antitumor immune memory; this was evidenced by the extent of tumor clearance upon rechallenge of surviving mice after 3 months [62]. This result is important because it contributes to our understanding of how CAR T cells may help overcome

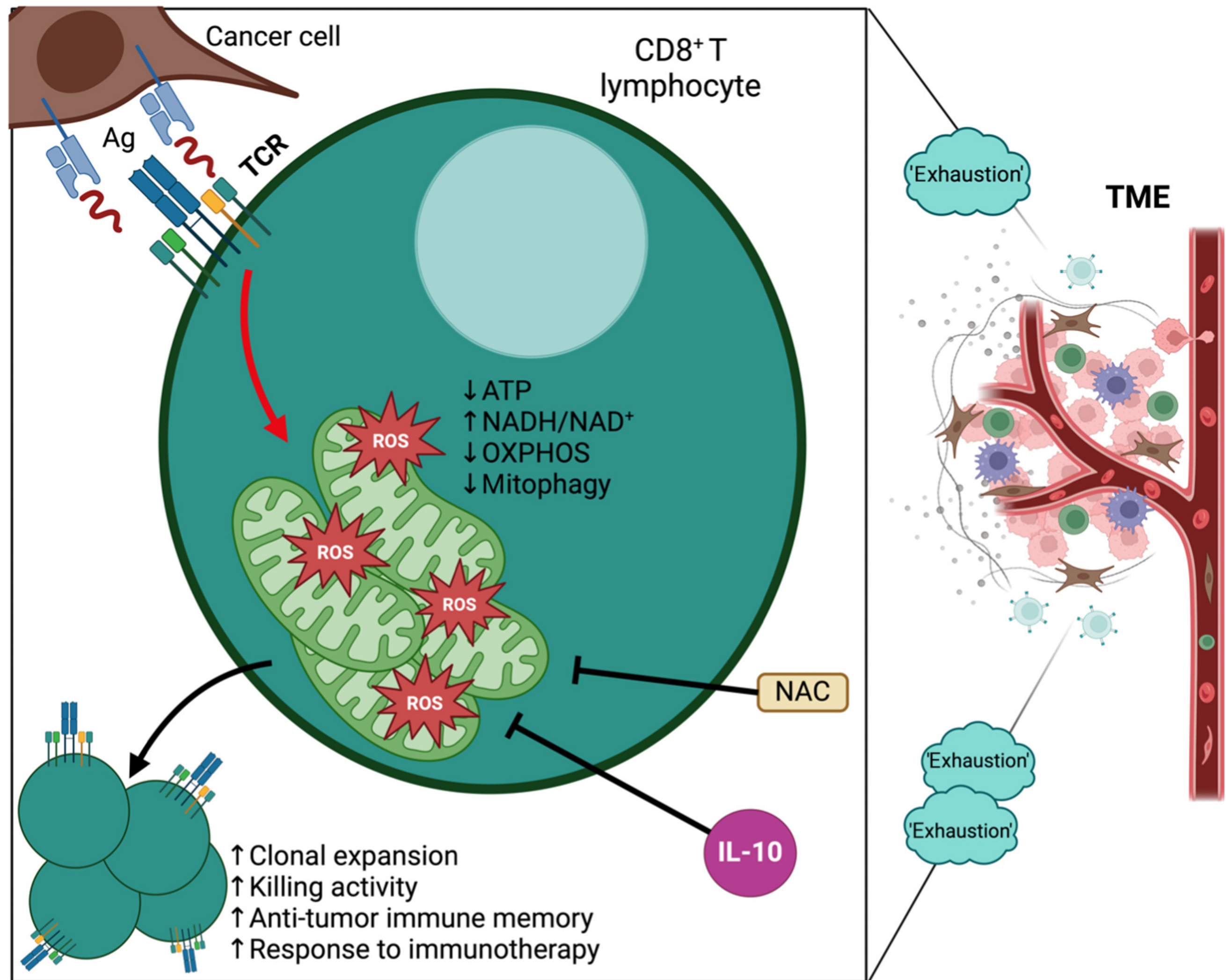

Figure 6. Cytotoxic CD8$^+$ T cells can undergo mitochondrial exhaustion in the TME. When in contact with cancer cells and chronically exposed to tumor antigens through the TCR, CD8$^+$ T lymphocytes undergo a state of 'exhaustion', characterized by dysfunctional mitochondria, which accumulate ROS [57,58]. Mitochondria fail to perform OXPHOS and mitophagy, producing ATP, and leading to an increase in the NADH/NAD$^+$ ratio compared to nonexhausted CD8$^+$ T cells [59]. Treatment with the antioxidant NAC or transfection of T lymphocytes with recombinant IL-10 rescued the damaged mitochondria (assessed via reduced amounts of mitochondrial DNA), promoting clonal expansion, cytotoxic activity, and an anti-tumor response in mouse models [60,61]. Abbreviations: Ag, antigen; IL-10, interleukin 10; NAC, N-acetyl cysteine; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species; TCR, T cell receptor; TME, tumor microenvironment.

T cell exhaustion. OXPHOS in CD8$^+$ T cells would therefore appear to be significant for their antitumor effect, although further experiments and models are warranted.

However, somewhat paradoxically, precise metabolic signatures showed enhanced OXPHOS in CD8$^+$ T cell subpopulations in a group of melanoma patients who were unresponsive to checkpoint blockade, suggesting that functioning mitochondria are not always linked to a good prognosis of patients [63,64]. Considering these conflicting findings which might stem from the use of different models and the availability of clinical samples, the precise role of OXPHOS in T cell antitumor immunity remains to be robustly explored, especially in a tumor- and species-dependent manner.

Overall, the ETC and OXPHOS play a role in the TME by shaping the ability of tumor-infiltrating lymphocytes to attack cancer cells. Promoting the ETC and OXPHOS in adaptive immune cells is therefore worthy of further exploration.

Concluding remarks

When it comes to examining the role of complexes in the ETC in immune cell function, there has perhaps been an over-reliance on the use of small molecule inhibitors; often used without appropriate assessment of their effects on the activity of various complexes being targeted and their consequences on other ETC components. A better understanding of the role of ATP generated by the ETC during OXPHOS, relative to the role of ROS generated by Complex I or III in immune cell activation and function is needed. In macrophages, Complex I and II are needed to induce IL-1β (via RET at Complex I), while an intact ETC generating ATP for the **PCr shuttle** is responsible for NLRP3 inflammasome activation in response to ATP. There may be a role for ROS from Complex I at later time points or in response to other NLRP3 activators. Perhaps the clearest studies to date on lymphocytes point to a key role for Complex II in Th1 cell proliferation (but not cytokine production), Complex III and OXPHOS in T_{reg} differentiation, and Complex V in B cell proliferation and CSR. Finally, for anti-tumor immunity, undamaged ETC and OXPHOS appear to be essential for cytotoxic T cell-mediated tumor targeting in the models studied.

Considering the knowledge acquired on ETC so far, respiratory complexes might represent a potential target for the development of candidate therapies in immuno-oncology (see Outstanding questions). In mice, Complex I inhibitors have shown promising results against melanoma progression through a dual effect on tumor and immune cells [65], while in humans, their use has been stopped due to their neurotoxicity and lactate acidosis [66]. Therefore, careful evaluation of ETC inhibitor efficacy, pharmacokinetics, and side effects is imperative before fully testing such compounds in clinical trials.

To improve the development of mitochondria-targeted inhibitors, the extracellular microenvironment must be considered, since it will rewire the metabolic necessities of immune cells through nutrient restriction, via compensatory mechanisms that are activated to generate ATP when OXPHOS is blocked. Some options to enhance immunotherapy might include the use of more specific inhibitors of respiration, which might discriminate between cancer and immune cells. More -omics and single-cell approaches for a personalized therapy may be needed, according to the type of tumor and immunophenotype of the patient. Moreover, future clinical studies should determine the beneficial effects of combinatorial treatments, modulating both specific immune cell components but also metabolic targets in the ETC in immune cells; this remains pertinent in immune-mediated diseases as well as oncology. Thus, much remains to be elucidated in this fascinating aspect of immunometabolism.

Outstanding questions

How does the knockout of ETC complexes in animal models impact immune processes, and how relevant are these findings for human immunity?

What is the precise role, origin, and temporal presence of ETC-generated ROS in modulating immune function or tumors?

How do different stimuli and cell models affect NLRP3 activation pathways, particularly in terms of ROS and ATP involvement, and how can genetic models aid in understanding these processes?

What are the clinical implications and relevance of ETC modulators, and how can the development of compounds that decrease OXPHOS activity address safety concerns while demonstrating efficacy?

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Declaration of interests

No interests are declared.

References

1. Zhao, R.Z. *et al.* (2019) Mitochondrial electron transport chain, ROS generation and uncoupling (Review). *Int. J. Mol. Med.* 44, 3–15
2. Nolfi-Donegan, D. *et al.* (2020) Mitochondrial electron transport chain: oxidative phosphorylation, oxidant production, and methods of measurement. *Redox Biol.* 37, 101674

3. Liu, X. *et al.* (1996) Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* 86, 147–157
4. Kagan, V.E. *et al.* (2004) Oxidative lipidomics of apoptosis: redox catalytic interactions of cytochrome c with cardiolipin and phosphatidylserine. *Free Radic. Biol. Med.* 37, 1963–1985
5. Srinivasula, S.M. *et al.* (1998) Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Mol. Cell* 1, 949–957
6. Zou, H. *et al.* (1997) Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* 90, 405–413
7. Zou, H. *et al.* (1999) An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. *J. Biol. Chem.* 274, 11549–11556
8. Li, P. *et al.* (1997) Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91, 479–489
9. Hu, Y. *et al.* (1999) Role of cytochrome c and dATP/ATP hydrolysis in Apaf-1-mediated caspase-9 activation and apoptosis. *EMBO J.* 18, 3586–3595
10. Acehan, D. *et al.* (2002) Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. *Mol. Cell* 9, 423–432
11. Rodriguez, J. and Lazebnik, Y. (1999) Caspase-9 and APAF-1 form an active holoenzyme. *Genes Dev.* 13, 3179–3184
12. Gross, C.J. *et al.* (2016) K(+) Efflux-independent NLRP3 inflammasome activation by small molecules targeting mitochondria. *Immunity* 45, 761–773
13. Billingham, L.K. *et al.* (2022) Mitochondrial electron transport chain is necessary for NLRP3 inflammasome activation. *Nat. Immunol.* 23, 692–704
14. Zhou, R. *et al.* (2011) A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469, 221–225
15. Yin, M. and O'Neill, L.A.J. (2021) The role of the electron transport chain in immunity. *FASEB J.* 35, e21974
16. Murphy, M.P. (2009) How mitochondria produce reactive oxygen species. *Biochem. J.* 417, 1–13
17. Chance, B. and Hollunger, G. (1961) The interaction of energy and electron transfer reactions in mitochondria. I. General properties and nature of the products of succinate-linked reduction of pyridine nucleotide. *J. Biol. Chem.* 236, 1534–1543
18. Hinkle, P.C. *et al.* (1967) Partial resolution of the enzymes catalyzing oxidative phosphorylation. XV. Reverse electron transfer in the flavin-cytochrome beta region of the respiratory chain of beef heart submitochondrial particles. *J. Biol. Chem.* 242, 5169–5173
19. Chouchani, E.T. *et al.* (2014) Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature* 515, 431–435
20. Mills, E.L. *et al.* (2016) Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell* 167, 457–470.e413
21. Ojha, R. *et al.* (2022) Regulation of reverse electron transfer at mitochondrial complex I by unconventional Notch action in cancer stem cells. *Dev. Cell* 57, 260–276.e269
22. Rimal, S. *et al.* (2023) Reverse electron transfer is activated during aging and contributes to aging and age-related disease. *EMBO Rep.* 24, e55548
23. Muller, F.L. *et al.* (2004) Complex III releases superoxide to both sides of the inner mitochondrial membrane. *J. Biol. Chem.* 279, 49064–49073
24. Swanson, K.V. *et al.* (2019) The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat. Rev. Immunol.* 19, 477–489
25. Munoz-Planillo, R. *et al.* (2013) K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity* 38, 1142–1153
26. Goncalves, R.L.S. *et al.* (2020) The use of site-specific suppressors to measure the relative contributions of different mitochondrial sites to skeletal muscle superoxide and hydrogen peroxide production. *Redox Biol.* 28, 101341
27. Orr, A.L. *et al.* (2015) Suppressors of superoxide production from mitochondrial complex III. *Nat. Chem. Biol.* 11, 834–836
28. Duewell, P. *et al.* (2010) NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 464, 1357–1361
29. Rajamaki, K. *et al.* (2010) Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. *PLoS One* 5, e11765
30. Kazak, L. and Cohen, P. (2020) Creatine metabolism: energy homeostasis, immunity and cancer biology. *Nat. Rev. Endocrinol.* 16, 421–436
31. Bessman, S.P. and Carpenter, C.L. (1985) The creatine-creatine phosphate energy shuttle. *Annu. Rev. Biochem.* 54, 831–862
32. Duncan, J.A. *et al.* (2007) Cryopyrin/NALP3 binds ATP/dATP, is an ATPase, and requires ATP binding to mediate inflammatory signaling. *Proc. Natl. Acad. Sci. USA* 104, 8041–8046
33. Nomura, J. *et al.* (2015) Intracellular ATP decrease mediates NLRP3 inflammasome activation upon nigericin and crystal stimulation. *J. Immunol.* 195, 5718–5724
34. Jha, A.K. *et al.* (2015) Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* 42, 419–430
35. Tannahill, G.M. *et al.* (2013) Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. *Nature* 496, 238–242
36. Garaude, J. *et al.* (2016) Mitochondrial respiratory-chain adaptations in macrophages contribute to antibacterial host defense. *Nat. Immunol.* 17, 1037–1045
37. Gobelli, D. *et al.* (2023) The mitochondrial succinate dehydrogenase complex controls the STAT3-IL-10 pathway in inflammatory macrophages. *iScience* 26, 107473
38. Selak, M.A. *et al.* (2005) Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell* 7, 77–85
39. Cordes, T. *et al.* (2016) Immunoresponsive gene 1 and itaconate inhibit succinate dehydrogenase to modulate intracellular succinate levels. *J. Biol. Chem.* 291, 14274–14284
40. Lampropoulou, V. *et al.* (2016) Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. *Cell Metab.* 24, 158–166
41. Mills, E.L. *et al.* (2018) Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. *Nature* 556, 113–117
42. Swain, A. *et al.* (2020) Comparative evaluation of itaconate and its derivatives reveals divergent inflammasome and type I interferon regulation in macrophages. *Nat. Metab.* 2, 594–602
43. Bailis, W. *et al.* (2019) Distinct modes of mitochondrial metabolism uncouple T cell differentiation and function. *Nature* 571, 403–407
44. Shin, B. *et al.* (2020) Mitochondrial oxidative phosphorylation regulates the fate decision between pathogenic Th17 and regulatory T cells. *Cell Rep.* 30, 1898–1909.e1894
45. Chen, X. *et al.* (2022) Succinate dehydrogenase/complex II is critical for metabolic and epigenetic regulation of T cell proliferation and inflammation. *Sci. Immunol.* 7, eabm8161
46. Matias, M.I. *et al.* (2021) Regulatory T cell differentiation is controlled by alphaKG-induced alterations in mitochondrial metabolism and lipid homeostasis. *Cell Rep.* 37, 109911
47. Pace, L. and Amigorena, S. (2020) Epigenetics of T cell fate decision. *Curr. Opin. Immunol.* 63, 43–50
48. Hosokawa, H. and Rothenberg, E.V. (2021) How transcription factors drive choice of the T cell fate. *Nat. Rev. Immunol.* 21, 162–176
49. Aso, K. *et al.* (2023) Itaconate ameliorates autoimmunity by modulating T cell imbalance via metabolic and epigenetic reprogramming. *Nat. Commun.* 14, 984
50. Zhao, H. *et al.* (2022) Myeloid-derived itaconate suppresses cytotoxic CD8(+) T cells and promotes tumour growth. *Nat. Metab.* 4, 1660–1673
51. Gu, X. *et al.* (2023) Itaconate promotes hepatocellular carcinoma progression by epigenetic induction of CD8(+) T-cell exhaustion. *Nat. Commun.* 14, 8154
52. Weinberg, S.E. *et al.* (2019) Mitochondrial complex III is essential for suppressive function of regulatory T cells. *Nature* 565, 495–499
53. Corte-Real, B.F. *et al.* (2023) Sodium perturbs mitochondrial respiration and induces dysfunctional Tregs. *Cell Metab.* 35, 299–315.e298
54. Urbanczyk, S. *et al.* (2022) Mitochondrial respiration in B lymphocytes is essential for humoral immunity by controlling the flux of the TCA cycle. *Cell Rep.* 39, 110912

55. Zhu, Y. *et al.* (2022) Oxidative phosphorylation regulates interleukin-10 production in regulatory B cells via the extracellular signal-related kinase pathway. *Immunology* 167, 576–589
56. Chen, D. *et al.* (2021) Coupled analysis of transcriptome and BCR mutations reveals role of OXPHOS in affinity maturation. *Nat. Immunol.* 22, 904–913
57. Wu, H. *et al.* (2023) Mitochondrial dysfunction promotes the transition of precursor to terminally exhausted T cells through HIF-1alpha-mediated glycolytic reprogramming. *Nat. Commun.* 14, 6858
58. Yu, Y.R. *et al.* (2020) Disturbed mitochondrial dynamics in CD8(+) TILs reinforce T cell exhaustion. *Nat. Immunol.* 21, 1540–1551
59. Scharping, N.E. *et al.* (2016) The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. *Immunity* 45, 374–388
60. Vardhana, S.A. *et al.* (2020) Impaired mitochondrial oxidative phosphorylation limits the self-renewal of T cells exposed to persistent antigen. *Nat. Immunol.* 21, 1022–1033
61. Guo, Y. *et al.* (2021) Metabolic reprogramming of terminally exhausted CD8(+) T cells by IL-10 enhances anti-tumor immunity. *Nat. Immunol.* 22, 746–756
62. Zhao, Y. *et al.* (2024) IL-10-expressing CAR T cells resist dysfunction and mediate durable clearance of solid tumors and metastases. *Nat. Biotechnol.*, Published online January 2, 2024. https://doi.org/10.1038/s41587-023-02060-8
63. Li, C. *et al.* (2022) A high OXPHOS CD8 T cell subset is predictive of immunotherapy resistance in melanoma patients. *J. Exp. Med.* 219, e20202084
64. Shorer, O. and Yizhak, K. (2023) Metabolic predictors of response to immune checkpoint blockade therapy. *iScience* 26, 108188
65. AbuEid, M. *et al.* (2021) Synchronous effects of targeted mitochondrial complex I inhibitors on tumor and immune cells abrogate melanoma progression. *iScience* 24, 102653
66. Yap, T.A. *et al.* (2023) Complex I inhibitor of oxidative phosphorylation in advanced solid tumors and acute myeloid leukemia: phase I trials. *Nat. Med.* 29, 115–126