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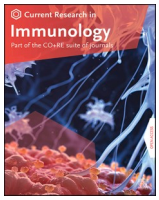

Mitochondrial electron transport chain in macrophage reprogramming: Potential role in antibacterial immune response

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ABSTRACT

Macrophages restrain microbial infection and reinstate tissue homeostasis. The mitochondria govern macrophage metabolism and serve as pivot in innate immunity, thus acting as immunometabolic regulon. Metabolic pathways produce electron flows that end up in mitochondrial electron transport chain (mtETC), made of super-complexes regulating multitude of molecular and biochemical processes. Cell-intrinsic and extrinsic factors influence mtETC structure and function, impacting several aspects of macrophage immunity. These factors provide the macrophages with alternate fuel sources and metabolites, critical to gain functional competence and overcoming pathogenic stress. Mitochondrial reactive oxygen species (mtROS) and oxidative phosphorylation (OXPHOS) generated through the mtETC are important innate immune attributes, which help macrophages in mounting antibacterial responses. Recent studies have demonstrated the role of mtETC in governing mitochondrial dynamics and macrophage polarization (M1/M2). M1 macrophages are important for containing bacterial pathogens and M2 macrophages promote tissue repair and wound healing. Thus, mitochondrial bioenergetics and metabolism are intimately coupled with innate immunity. In this review, we have addressed mtETC function as innate rheostats that regulate macrophage reprogramming and innate immune responses. Advancement in this field encourages further exploration and provides potential novel macrophage-based therapeutic targets to control unsolicited inflammation.

1. Introduction

Macrophages are the central mediators of innate immunity, which play an imperative role in restraining pathogens. The transition of macrophage functioning from microbicidal and inflammatory at initial phase of infection to immunomodulatory and tissue repairing roles also highlight its role in maintaining host homeostasis. The ability of macrophages to adapt to different fuel sources imparts extreme plasticity and allows them to alter their phenotypic and functional profile in response to diverse microenvironment (Kolliniati et al., 2022). Under normal circumstances, naïve macrophages are non-polarized immune cells that are referred to as "M0 macrophages". These cells are metabolically quiescent, characterized by reduced flow of glucose *via* glycolysis and TCA cycle, such that their ATP production occurs predominantly by oxidative phosphorylation (OXPHOS) through β-oxidation of fatty acids in the mitochondria (Kelly and O'Neill, 2015). M0 macrophages undergo change in their metabolic profile, referred as metabolic reprogramming, which drives specific effector functions in the macrophages. The common events that trigger metabolic reprogramming in macrophages include activation of pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), nutrient-based signals that involve kinases (AMP kinase or mTOR), and/or lipid nuclear receptors (ERRs, PPARs) and cytokines (Kolliniati et al., 2022). The two potential outcomes of the metabolic reprogramming are – differentiation of M0 macrophages into M1 macrophages (pro-inflammatory macrophages or classically activated macrophages) and M2 macrophages (anti-inflammatory macrophages or alternatively activated macrophages). The differentiation of M0 macrophages into two entirely different functional sets suggests that the role of metabolic reprogramming extends beyond ATP production (Galli and Saleh, 2021) (Fig. 1).

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It has been observed that metabolic reprograming in macrophages is essential for driving anti-bacterial responses as various intermediate metabolic products for example TCA derivatives, prostaglandins, itaconate, and tryptophan metabolism function as bactericidal agents or modulate innate immune signaling pathways resulting in the production of inflammatory cytokines, and anti-microbial peptides. Thus, depending upon the tissue microenvironment, macrophages can transit from aerobic glycolysis to anaerobic glycolysis, and *vice versa*.

The past decade has witnessed an impressive growth in the understanding of the intricate metabolic pathways that regulate macrophage activation and immunity leading to the coining of a new term, "immunometabolism". In simpler terms, metabolism can be visualized as the flow of electrons *via* numerous pathways where the metabolites act as potential carriers of electrons and the mitochondrial electron transport chain (mtETC) acts as the most decisive channels of electron flow. Various metabolic reactions supply electrons to the mtETC in the form of reducing equivalents such as flavin adenine dinucleotide ($FADH_2$) or nicotinamide adenine dinucleotide (NADH). The relative amount of these metabolites is contingent on the nature of the fuel used, and the respiratory chain adjusts to such fluctuations in the fuel sources (Enríquez, 2016). The capacity of the macrophages to utilize diverse cellular fuels (Galli and Saleh, 2021) is critical for these cells to adapt to the changing environmental cues and to compensate for the energy demands for carrying out various cellular functions during a pathogenic infection (Russell et al., 2019; S. Liu et al., 2021). In response to the changes in the cellular fuel, or signals received by upstream PRRs, mitochondria also regulate their biogenesis, location, shape, structure, and flux of metabolites inside the macrophages (Giallongo et al., 2020; Soto-Heredero et al., 2020; Kumar et al., 2022). The primary function of mtETC is to generate ATP, which eventually serves as an immediate donor of metabolic energy in the cells. The demand for ATP is quite high and since the total amount of ATP is limited each molecule is recycled between 1000–1500 times/day. In order to achieve this exorbitant degree of turnover, mtETC works in tandem to synthesize ATP from ADP and inorganic phosphate, for which energy is harnessed by OXPHOS.

In this review we provide a comprehensive treatise on mtETC with special focus on its role in metabolic regulation of macrophage activation, particularly in response to bacterial infection.

2. Structure and biology of mitochondrial electron transport chain (mtETC)

The mtETC consists of a series of transmembrane complexes: four respiratory chain Complexes (I, II, III, IV), a non-respiratory Complex (V), and two freely mobile electron carriers (coenzyme Q or ubiquinone and cytochrome *c*), which are present on the cristae at the IMM (Fig. 2).

Complex I (NADH-Q oxidoreductase), III (CoQ-cytochrome c oxidoreductase), and IV (Cytochrome *c* oxidase) act as electron-driven H^+ pumps. The breakdown of nutrients through TCA cycle, glycolysis, fatty acid and amino acid oxidation generates high-energy reducing equivalents, NADH and $FADH_2$, which are oxidized to NAD^+ and FAD^+ respectively, *via* the redox centers present in mtETC complex. The complex I delivers electrons from NADH to CoQ resulting in the generation of $CoQH_2$ (Fig. 3a). This results in the pumping of four H^+ from matrix into the IMS (Fig. 2) and mtROS generation through forward electron transfer (FET), although the amount is negligible.

Complex II catalyses oxidation of succinate to fumarate, and during this process FAD gets reduced to $FADH_2$, then electrons get transferred to the Fe–S clusters (Fig. 3a) reducing CoQ to $CoQH_2$ (Fig. 2). However, the transport of electrons through Complex II is not accompanied by H^+

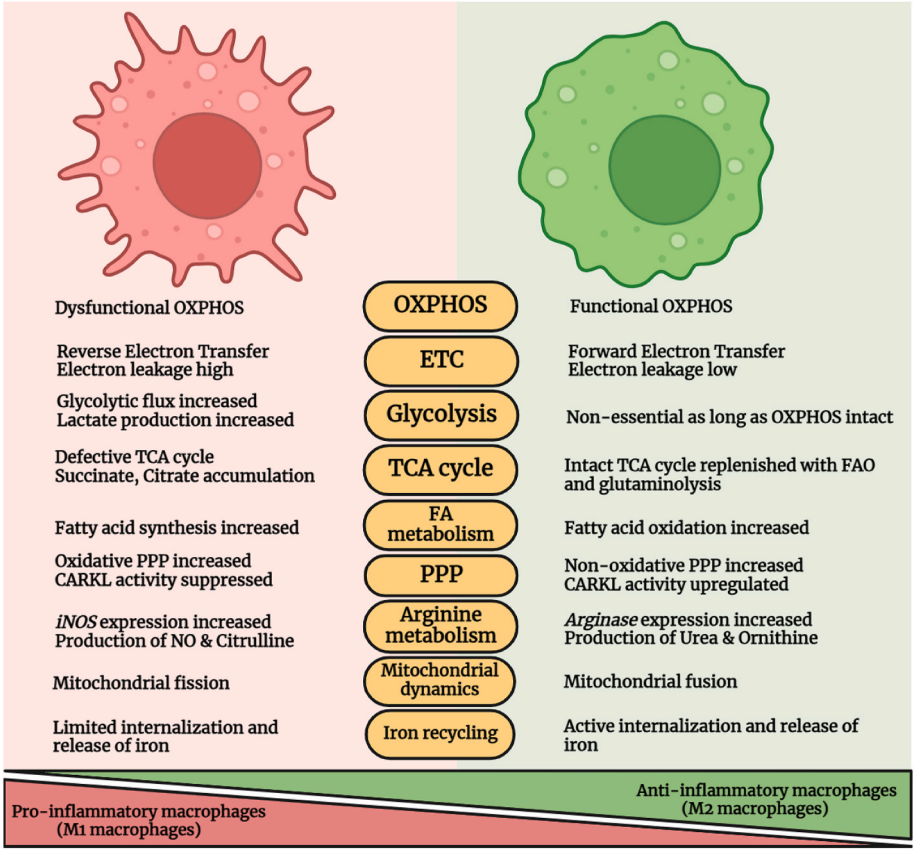

Fig. 1. Metabolic changes in M1 and M2 macrophages. This illustration depicts various changes in biochemical pathways that occur during metabolic reprogramming of the macrophages. Note that this illustration as well as all the others in this review were built on universal biochemical pathways, to which the various antibacterial pathways of the macrophage have been added. All drawings were created with the help of BioRender.com.

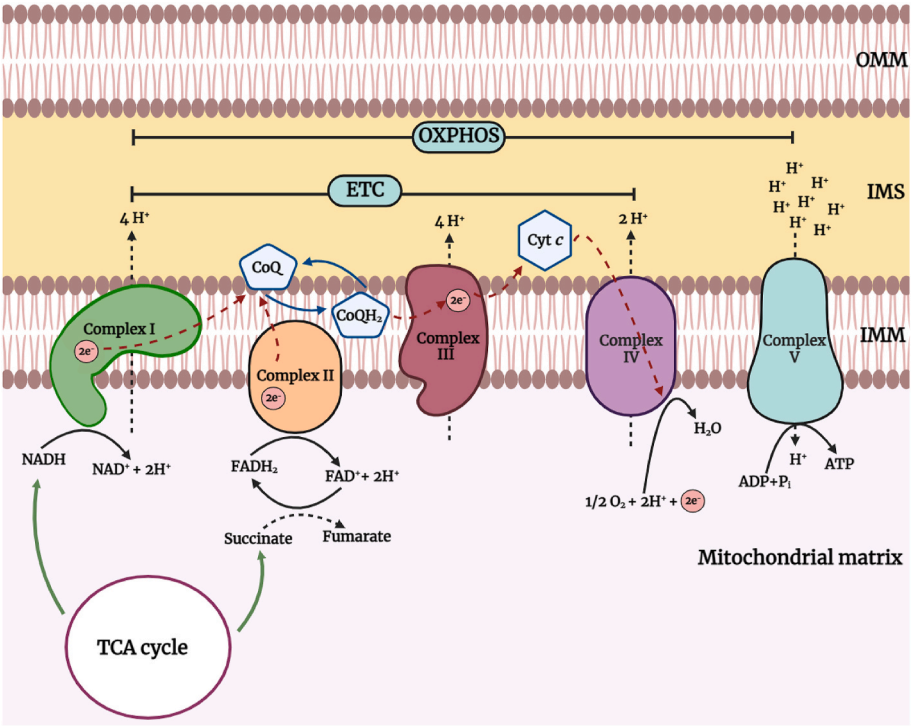

Fig. 2. Arrangement of the mtETC-OXPHOS system in macrophages. The illustration is self-explanatory, and Section 2 describes additional details and all acronyms. In brief, the OXPHOS system, comprising four proton-pumping protein complexes, including the ATP synthase and the two free electron carriers (CoQ and Cyt *c*) are as shown. The diagram also shows the reducing equivalents, NADH and $FADH_2$, contributed by the TCA cycle, and the flow of electrons. Of note, Complex II is unique for not pumping H^+ into the IMS. At the end of the ETC, Cyt *c* transfers electrons to complex IV where O_2 acts as the terminal electron acceptor and is reduced to H_2O. The pumping of H^+ from the mitochondrial matrix into IMS in response to electron transfer results in the generation of proton motive force (Δp), which is proton concentration (pH) combined with electrochemical proton gradient ($\Delta\mu\sim_{H+}$), known as mitochondrial membrane potential (ΔΨ). Complex V pumps H^+ back into the mitochondrial matrix from IMS, which is coupled to the production of ATP from ADP phosphorylation, known as oxidative phosphorylation. The broken red arrows indicate the movement of electrons across the mtETC components, the blue solid arrows indicate the Q cycle, the broken black arrows indicate the movement of H^+, the black solid arrows represent the oxidation of NADH, $FADH_2$, H_2O formation and ATP synthesis, and the green solid arrow represent the contribution of TCA products in the mtETC complex. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

translocation (Fig. 1).

Complex III transfers the electrons from $CoQH_2$ to Cyt *c* by the Q-cycle. $CoQH_2$ is oxidized to semiubiquinone ($CoQH^-$). The highly reductive $CoQH^-$ swiftly transfers second electron to Cytochrome b_L, and ultimately to the Cytochrome b_H. Electrons from the reduced cytochrome b_H are transferred to the Qi site (Fig. 3a). The second $CoQH_2$ molecule is oxidized at the Q_O site while displacing the other two H^+ in order to complete Q-cycle. Similarly, one electron is transferred to the 2Fe–2S cluster, and another electron to the Cytochrome b_H and finally to $CoQH^-$ to produce $CoQH_2$ (Fig. 3a).

Complex IV transfers electrons from Cyt *c* to O_2 to generate H_2O (Fig. 2). Four molecules of reduced Cyt *c* moves along the IMM surface, and simultaneously transmit electrons from heme of each Cyt *c* to the Cu_a^{2+} followed by heme in the cytochrome *a*, and then to the binuclear center i.e, Cu_b^{2+} and heme in the cytochrome $_{a3}$, and finally transfer to O_2 (Fig. 3a). In total, four electrons at a time are almost instantaneously transported from Cyt *c* to O_2; and eight H^+ are transported out from the matrix, of which four are used to form two molecules of H_2O, and the other four are pumped into the IMS.

2.1. Accessory proteins involved in mtETC

Various proteins in the matrix and IMM are involved in electron transfer *via* reduction of CoQ and thus, are involved in mtETC (Fig. 4). One such enzyme is electron transfer flavoprotein:ubiquinone oxidoreductase (ETF:QO) which catalyses generation of acetyl CoA eventually generating a molecule each of $FADH_2$ and NADH through TCA cycle. Electrons are then subsequently transferred from $FADH_2$, bound to acyl-CoA dehydrogenase, *via* ETF to ETF: QO reducing CoQ to $CoQH_2$ (Fig. 4).

Mitochondrial glycerol 3-phosphate dehydrogenase (mG3PD) catalyses the oxidation of glycerol 3-phosphate to dihydroxyacetone phosphate (DHAP) and during this process FAD is reduced into $FADH_2$ and then $CoQH_2$ ultimately the electrons are shuttled into the respiratory chain. mG3PD serves as the source of electron leakage and generates mtROS at the G_Q site (Mráček et al., 2013) (Fig. 3b).

Dihydroorotate dehydrogenase (DHODH) receives two electrons from dihydroorotate (DHO) and transfer them to CoQ, linking DHODH to OXPHOS *via* CoQ (Fig. 4). DHODH contributes to mtROS production either directly or indirectly (Fig. 3b). In direct mode, mtROS is generated at the CoQ-binding site of DHODH itself (Hey-Mogensen et al., 2014) whereas in indirect mode, it is produced at Complex II from electrons supplied to the CoQ pool *via* DHODH when Complex III is inhibited, and CoQ pool is reduced (Hey-Mogensen et al., 2014).

3. Assembly of mtETC as super-complexes and their biological implications

Growing evidence suggests that mtETC complexes are assembled as super-complexes required for performing OXPHOS according to cellular needs (Cogliati et al., 2016). The ability of free and assembled super-complexes to respire raises an interesting question of whether such alterations in the structural arrangement have any explicit physiological roles and confer functional advantages.

Five significant roles have been ascribed for the assemblage of

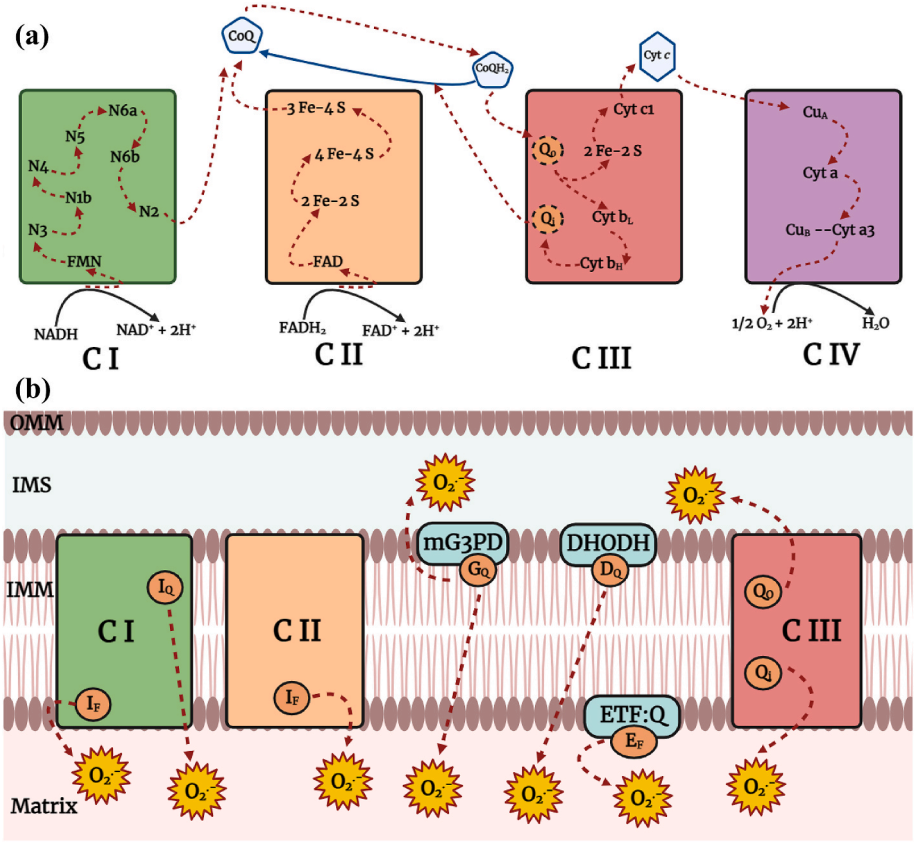

Fig. 3. (a) Flow of electrons in the mtETC of macrophages. The Complexes of the ETC were already presented in Fig. 2, which are shown in finer detail here (abbreviated as C I, C II, C III, and C IV), depicting the electron-transporting Fe–S clusters. The electron transfers are indicated by broken red arrows, and the hydrogen transfers by solid arrows. The flavin mononucleotide (FMN) cofactor of Complex I first accepts electrons from NADH generated by the TCA cycle in the mitochondrial matrix. These electrons are conducted *via* a series of 4Fe–4S or 2Fe–2S type clusters in C I (designated as N3, N1b etc.). In a similar fashion, FAD of C II receives electrons from $FADH_2$, generated from the oxidation of succinate. The last cluster of the two Complexes, i.e. N2 of CI and the 3Fe–4S cluster of C II, transfer the electrons to CoQ, which then gets reduced to $CoQH_2$. The latter donates the electrons at the Q_O in Complex III, which are transferred to Cyt *c via* 2Fe–2S cluster proteins and various cytochromes, resulting in partial oxidation of $CoQH_2$ to $CoQH^-$ (not shown for simplicity). The latter is formed at the Q_O site, and transfers the second electron rapidly to Cyt b_L and Cyt b_H at the Q_i, which is ultimately transferred to CoQ to form $CoQH^-$. Another $CoQH_2$ is oxidized at Q_O for the completion of Q-cycle (shown by blue solid arrow). Finally, Cyt *c* transfers the electrons to molecular O_2 *via* Cu_A, Cyt *a*, Cu_S-Cyt a3, thus completing this series. **(b) Sites of mtROS generation in the mtETC of macrophages.** The broken red arrows indicate the sites of mtROS production in mitochondria. In complex I, superoxide production occurs at the FMN site (I_F), and quinone binding site (I_Q) of 4Fe–4S cluster N2 in the mitochondrial matrix. In complex II, flavin site (I_F) is the sole producer of superoxide generation in mitochondrial matrix. In complex III, superoxide is produced during Q cycle due to auto-oxidation of ubisemiquinone ($CoQH^-$). $CoQH^-$ at Q_O site leads to superoxide production in IMS due to electron leakage, and at Q_i site leads to superoxide production at mitochondrial matrix. Accessory proteins that transfer electrons to mtETC also produce mtROS; the CoQ-binding site of mG3PD (G_Q) generates superoxide at IMS and in the mitochondrial matrix, whereas those of DHODH (D_Q) and ETF:QO (E_F) generate superoxide in the mitochondrial matrix. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mtETC complexes into super-complexes. First, super-complexes increase the efficiency of electron flux by channelling substrates (Berndtsson et al., 2020). Second, super-complexes prevent electron leakage and, thus, reduce mtROS production (Maranzana et al., 2013). Third, super-complexes stabilize individual respiratory complexes structurally (Moreno-Lastres et al., 2012), providing a structural environment that allows the assembly and activation of Complex I (Enríquez, 2016). Fourth, super-complexes prevent aggregation of proteins in the IMM (Blaza et al., 2014), and fifth, super-complexes optimize the catalytic activity of individual complexes (Moreno-Lastres et al., 2012). The super-complexes are formed by the assembly of different complexes in different stoichiometry (Fig. 5).

For example, the largest super-complex consists of one copy of Complex I, two copies of Complex III and one copy of Complex IV together forming the N-respirasome (Fig. 5). It functions as a single functional unit transferring electrons to O_2 with the concomitant establishment of an electrochemical gradient. Diverse cellular fuel produces different $FADH_2$/NADH (F/N) ratios; a high F/N ratio also results in an increase in $CoQH_2$/CoQ ratio, which facilitates reverse electron transfer (RET)-induced mtROS in conjunction with high $\Delta\Psi_m$ (Cogliati et al., 2021) (Fig. 6).

Enhanced mtROS disrupts the super-complexes, leading to a deleterious effect on Complex I and IV stability (Diaz et al., 2012) (Fig. 6). ER-stress is reported to impact the assembly of super-complexes. It drives mitochondrial bioenergetics *via* activation of protein kinase R-like ER kinase (PERK), which, together with eIF2α and ATF4 (PERK-eIF2α-ATF4 axis), promotes the assembly of super-complex *via* super-complex assembly factor 1 (SCAF1) and cristae formation (Balsa et al., 2019). Intracellular Ca^{2+} levels also influence OXPHOS activity (Dejos et al., 2020) reinforcing the super-complexes in adapting to the altered metabolic demands of the mitochondria. Furthermore, the pertinence between mitochondrial dynamics and the assembly and function of the mtETC complexes has also been demonstrated (Cogliati et al., 2013). A large protein complex termed mitochondrial contact site and cristae organizing system (MICOS) plays crucial role in the organization of IMM architecture (Pfanner et al., 2014). The core proteins of this complex include Mic10 subcomplex (Mic10, Mic12, Mic26, and Mic27) and Mic60 subcomplex (Mic60 and Mic19), which interacts with

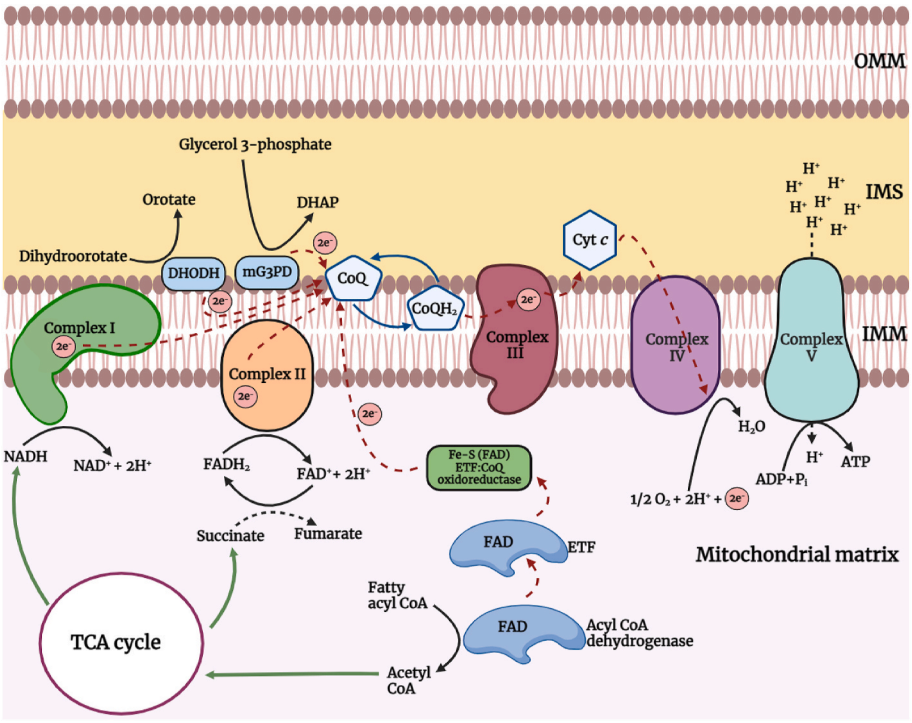

Fig. 4. Accessory proteins involved in the transfer of electrons to mtETC in macrophages. Three accessory membrane-embedded proteins are involved in electrons' transfer to mtETC. First, DHODH at outer leaflet of IMM, catalyses the conversion of DHO to orotate. DHODH accepts two electrons from DHO, which are then transferred to CoQ. CoQ, therefore, links DHODH to the mtETC. Second, mG3PD is a ubiquinone-linked flavoprotein localized at outer membrane of IMM. This enzyme catalyses the conversion of glycerol 3-phosphate to dihydroxyacetone phosphate (DHAP), during which electrons are transferred to CoQ resulting in the reduction of CoQ. Third, electron transfer flavoprotein:ubiquinone oxidoreductase (ETF:QO) catalyses the reoxidation of ETF. ETF:QO is localized at the inner leaflet of the IMM. ETF receives electrons from $FADH_2$ bound with acyl-CoA dehydrogenase. Fe–S cluster in ETF:QO accepts electrons from ETF, and ultimately transfers them to CoQ. Thus, CoQ integrates DHODH-derived electrons, ETF:QO-derived electrons, and mG3PD-derived electrons to mtETC. The broken red arrows represent the flow of electrons, the blue solid arrows represent Q cycle, the black solid arrow represent the oxidation of NADH, $FADH_2$, H_2O formation and ATP synthesis, and green solid arrow represent the movement of acetyl CoA in the TCA cycle, and NADH, succinate from the TCA cycle. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

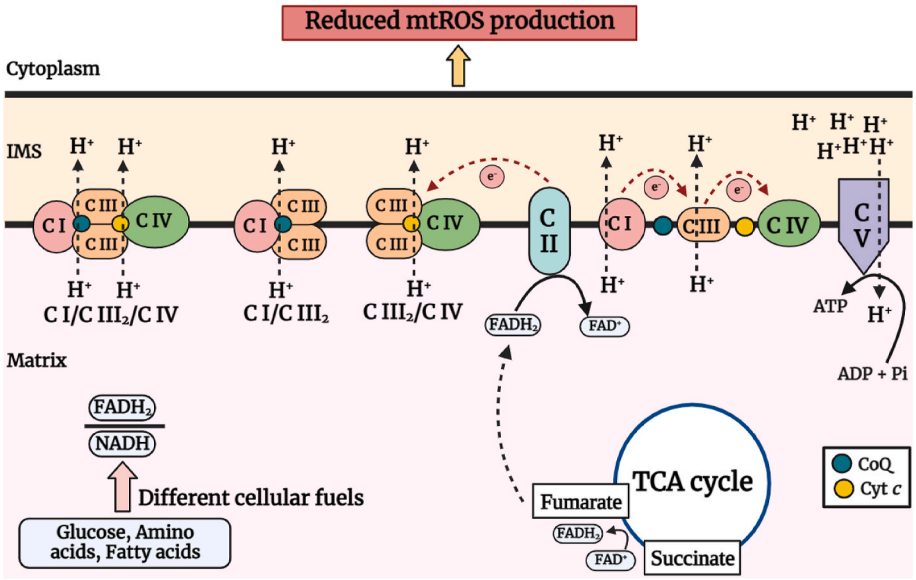

Fig. 5. Organization of mtETC complexes and super-complexes in the resting macrophages. The respiratory complexes I (C I) to IV (C IV) can organize into super-complexes as shown. The generally accepted composition of these super-complexes are: C I + C III_2 + C IV (N-Respirasome), C I + C III_2, and C III_2 + C IV (Q-Respirasome). Some fractions of mtETC may move freely in IMM but reside as a discrete component. The arrangement of the complexes reduces the leakage of electrons, ensuring efficient electron flux and reduced mtROS generation at resting conditions. The flow of electrons has been described in the main text. The broken red arrows represent the flow of electrons from C II, C I and C III, the black solid arrow represent $FADH_2$ oxidation and ATP synthesis, and the broken black arrow represent the flow of H^+ and $FADH_2$ from TCA cycle. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

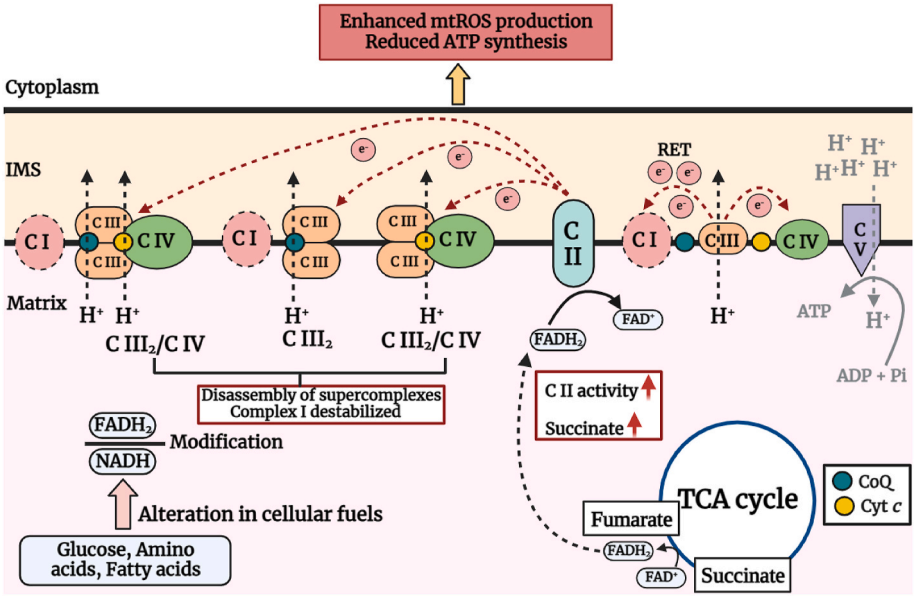

Fig. 6. Organization of mtETC complexes and super-complexes in activated macrophages. Macrophages are activated in response to pro-inflammatory stimulation and microbial infection, and the cellular fuels feeding the mtETC are altered, which modifies the $FADH_2$/NADH ratio. Changes in the cellular fuel are correlated with disruption of the organization of mtETC super-complexes. The activity of complex II is enhanced due to upregulation in succinate levels (shown by red solid arrows), which overloads $CoQH_2$ that are then transferred to complex I known as RET. RET-induced oxidation of Complex I results in its destabilization, and Complex I eventually dissociates from the C I + C III_2 + C IV (N-Respirasome), and C I + C III_2 (compare with Fig. 5). Such changes shift the function of mtETC from ATP production to enhanced mtROS generation which contributes to macrophage-mediated antimicrobial immunity. The broken red arrows represent the flow of electrons from C II and C III, the black solid arrow represent $FADH_2$ oxidation, the broken black arrow represent the flow of H^+ and $FADH_2$ from TCA cycle, the broken gray arrow and the gray solid arrow represent the reduced H^+ flow and ATP synthesis respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mtETC complexes and mutations in the core subunits of MICOS alters the IMM architecture impairing mitochondrial bioenergetics (Friedman et al., 2015). Mitochondrial fission in M1 macrophages leads to expansion of cristae that diminishes mtETC efficiency and augments aerobic glycolysis (Wang et al., 2021). TLR signaling promotes mitochondrial fission by regulating FAM73b (also known as Miga2, an outer mitochondrial membrane protein) levels, which plays a crucial role in switching mitochondria from fission to fusion (Gao et al., 2017). FAM73b and mitofusins (MFN1 and MFN2) have similar effects on the expression of inflammatory genes (Sloat et al., 2019), clearly indicating association between mitochondrial dynamics and mtETC in regulating macrophage polarization. Bacterial infection triggers ER-stress altering intracellular Ca^{2+} flux, mitochondrial dynamics, and metabolism (Kumar et al., 2022; Escoll and Buchrieser, 2018; Sharma et al., 2023), which may enhance or reduce the assembly of super-complexes in macrophages. Comprehensive research is needed to understand how super-complexes adapt to innate immune signaling and the physiological consequences of such adaptations in controlling pathogenic infections. Additionally, there is very little information on how alterations in the structure and functions of the mtETC complexes trigger macrophage activation or M1/M2 differentiation in response to pathogenic infection. Future work will undoubtedly provide valuable information on these matters.

4. mtETC as regulator of innate immunity

Bacterial infection alters OXPHOS in macrophages (Russell et al., 2019). However, how changes in mtETC components regulate bacterial infection is only starting to be characterized. Numerous reports suggested that pathogenic bacteria targets OXPHOS (Escoll and Buchrieser, 2018; Russell et al., 2019) but why these bacteria reduce OXPHOS remains a matter of debate. The probable mechanism could be that intracellular bacteria acquire energy resources by redirecting cellular fuel such as TCA cycle or glycolytic intermediates to sustain their replication and proliferation, and it has been demonstrated that the alteration of OXPHOS might be advantageous for these pathogens (Escoll and Buchrieser, 2018; Russell et al., 2019). On the other hand, metabolic alteration in macrophages also impact intracellular bacterial pathogens (Galli and Saleh, 2021), suggesting regulation of cellular metabolism might be a critical determining factor in the outcome of an infection. TLR-signaling induces changes in OXPHOS (Lachmandas et al., 2016). TLR-signaling triggers the switching of mitochondria from ATP- to mtROS-producing factories in murine BMDMs, which exhausts NAD^+ levels, and impedes mitochondrial respiration (Galli and Saleh, 2021; Tannahill et al., 2013) while promoting glycolysis. In order to replenish NAD^+ levels, and endure GAPDH activity in glycolysis, TLR4 induces NAD^+-salvage pathway *via* upregulation of nicotinamide phosphoribosyl transferase (NAMPT) enzyme (Cameron et al., 2019). Compelling evidences suggest that the metabolic status of mitochondria regulates additional sub-organellar functions, while immune signaling can also regulate mitochondrial metabolic functions (West et al., 2011; Mills et al., 2016). Hence, the importance of metabolic interactions is increasingly considered in host-pathogen studies, and therefore, the role of mtETC becomes quintessential in innate immunity. Interestingly, bacterial infection also leads to alterations in the composition, and activity of mtETC complexes, and super-complexes in macrophages (Garaude et al., 2016; Escoll et al., 2019). Thus, it has been general wisdom that during infections, mtETC might serve other roles than sustaining metabolic fluxes and generating ATP. Therefore, adaptations in the mtETC usually occur to meet the metabolic adjustments and strong energetic requirements for activation of the macrophages in response to bacterial infection.

4.1. Role of mtETC complexes in regulating anti-bacterial response in macrophages

4.1.1. Role of complex I

Complex I is one of the major sites of mtROS generation. It produces

mtROS by forward (FET) and reverse electron transport (Fig. 3b and . 6). During FET, leakage of electrons at I_F site reduces O_2 to mtROS (Fig. 3b) independent of both CoQ redox state and proton motive force (Δp) whereas RET depends upon redox status of CoQ and Δp (Scialò et al., 2017). The role of mtROS as signaling molecules and anti-bacterial agent is well established in bacterial infection (West et al., 2011; Kumar et al., 2022). Inhibition of Complex I activity impairs mtROS generation (West et al., 2011; Kelly et al., 2015; Mills et al., 2016; Carneiro et al., 2018) thereby dysregulation in complex I highlights its role in the onset of inflammatory responses (Fig. 7). GRIM19/NDUFA13 (Gene associated with retinoid–IFN–induced mortality 19/NADH dehydrogenase 1α subcomplex subunit 13) is a Complex I subunit, which interacts with NOD2 (Nucleotide Oligomerization Domain 2) critical for recognizing bacterial peptidoglycan (Guryanova, 2022). NOD2-GRIM19 axis triggers NF-κB activation inducing anti-microbial response against *S. typhimurium* infection (Barnich et al., 2005). The activity of Complex I and GRIM19 expression is enhanced in *Staphylococcus saprophyticus*-infected macrophages, and $GRIM19^{-/-}$ macrophages failed to clear the bacterial load due to impaired pro-inflammatory cytokine production (Chen et al., 2012). Furthermore, ligand recognition by TLR-1, -2, -4 leads to activation of TRAF6 (Tumour necrosis factor receptor-associated factor 6) which then translocate to the mitochondria and interacts with ECSIT (Evolutionarily conserved signalling intermediate in Toll pathways). ECSIT forms part of the MCIA (mitochondrial Complex I assembly) complex in macrophages (Carneiro et al., 2018) by interacting with Complex I assembly factors ACAD9 (Acyl CoA dehydrogenase family member 9) and NDUFAF1 (NADH dehydrogenase [ubiquinone] 1 α subcomplex assembly factor 1). LSP-stimulated $Ecsit^{-/-}$ bone marrow derived macrophages (BMDMs) failed to produce mtROS (West et al., 2011; Carneiro et al., 2018) and displayed reduced antibacterial activity in *S. typhimurium* infection (West et al., 2011). RET-induced mtROS production is also crucial in controlling intracellular *Mycobacterium avium* population in macrophages (Røst et al., 2022) (Fig. 7).

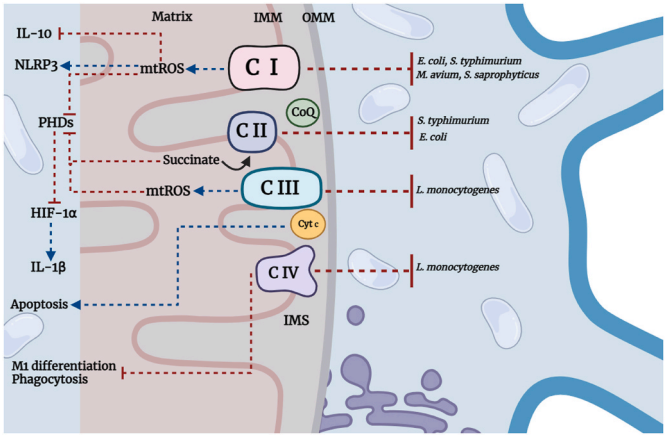

Fig. 7. Role of mtETC in regulating bacterial infection. The inhibited bacterial species are shown the right. C I triggers mtROS production, which induces NLRP3 activation leading to the production of HIF-1α-mediated IL-1β production; on the other hand, mtROS inhibits IL-10 production which prevents polarization of macrophages into M2 macrophages. C I-mediated mtROS and pro-inflammatory cytokines production inhibit *E. coli*, *S. typhiumurium, M. avium*, *and S. saprophyticus* infection. C II also induces RET-induced mtROS through C I. C II contributes to the inhibition of *E. coli*, and *S. typhiumurium* infection, and succinate, which is a substrate of C II, acts as a positive regulator of HIF-1α, leading to pro-inflammatory cytokine production. C III is the major producer of mtROS, both in the IMS and the mitochondrial matrix, thus restraining *L. monocytogenes* infection. C IV inhibits *L. monocytogenes* infection by maintaining high mitochondrial respiratory activity, however, C IV also negatively regulates M1 polarization and phagocytosis. The broken blue arrows represent the enhanced production of mtROS and/or activation of signaling pathway; the broken red lines represent the suppression of the signaling pathways and the type of bacterial infection controlled by respective mtETC complex. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Complex I-induced mtROS through RET induces necrosis of *Mycobacterium*-infected macrophages (Roca et al., 2022). It has also been observed that following infection with live bacteria the macrophages temporarily halt the assembly of Complex I and its constituent super-complexes (Garaude et al., 2016) impacting mitochondrial respiration and antibacterial responses (Fig. 7). Collectively, these findings suggest 1) the differential activation of Complex I in response to different pathogenic bacterial agents and 2) altered mtETC affects macrophage-immunometabolism and helps in fine-tuning antibacterial responses. Thus, it can be surmised that Complex I plays an important role in regulating inflammatory response in macrophages against bacterial infection.

Furthermore, Complex I is a metastable multiprotein complex, which is influenced by oxidative environment within the mtETC (Enríquez, 2016). The alteration in substrates that fuel the TCA cycle tweaks the $FADH_2$/NADH (F/N) ratio affecting the oxidation capacity of the coenzyme Q pool and induces RET (Fig. 6). This in turn enhances the levels of superoxide ions that oxidize the Complex I proteins, and thereby promotes destabilization of the complex (Guaras et al., 2016). F/N ratios are thus crucial for mtROS formation. The redox state of one of the electron carriers, namely ubiquinone, also seems to play a decisive role in mtROS formation. An increase in the levels of $CoQH_2$ at the expense of CoQ, results in a high $CoQH_2$/CoQ ratio.

Stimulation of TLRs (such as TLR4) induces mtROS at Complex I (West et al., 2011) through an unknown signaling pathway. Recently, a report suggested a mechanism by which mtETC integrates the signals emerging from TLR-signaling (Mills et al., 2016). Elevation in the $\Delta\Psi_m$, and enhanced succinate dehydrogenase (SDH) activity in LPS-stimulated murine macrophages incites mtROS production at Complex I. This rewiring of mitochondria from ATP production to mtROS generation establishes an inflammatory state in macrophages (Mills et al., 2018). However, nitric oxide (NO) produced during inflammation, impedes Complex I activity by damaging the Fe–S centers resulting in further production of mtROS (Brown and Borutaite, 2004). This is consistent with a previous study that reported the inhibition of Complex I *via* metformin-attenuated RET-linked mtROS production (Vial et al., 2019), which in turn suppressed the inflammatory activity of murine macrophages (Kelly et al., 2015). These reports clearly highlight the role of Complex I as a major regulator of inflammation in macrophages.

4.1.2. Role of complex II

The Complex II is the only integral constituent of the mitochondrial respiratory chain that participates in both TCA cycle and mtETC (Fig. 2). Although it has long been assumed that OXPHOS-derived mtROS are only produced at Complexes I and Complex III, recent studies demonstrated Complex II-mediated mtROS production in pathological infection as well (Garaude et al., 2016; Bezawork-Geleta et al., 2017) (Fig. 3b and . 6). Complex II plays a key role in IL-1β production *via* the RET/mtROS/HIF-1α-axis (Mills et al., 2016) (Fig. 7). Thus, it plays an indispensable role in antibacterial defence, leading to M1 macrophage activation *via* oxidation of succinate. Complex II activity is stimulated in macrophages in bacterial infection on the suppression of Complex I activity (Garaude et al., 2016), which suggests that a compensatory activation of another mtETC Complex to maintain ATP production is a pre-requisite for the survival of macrophages for counteracting bacterial infection. Consequent to the increase in Complex II activity, mitochondrial $FADH_2$-dependent enzyme activity also undergoes a transient increase (Garaude et al., 2016), which facilitates movement of the electrons to Complex III. *E. coli*-infected BMDMs have been shown to display high glycolytic activity that increased the maximum respiration rate (Garaude et al., 2016) despite a decrease in Complex I function. In this situation, mtETC receives electrons from the NADH that is generated

during glycolysis, which occurs without the utilization of Complex I. This is achieved by shuttling of cytoplasmic NADH electrons to coenzyme Q *via* mG3PD (Mráček et al., 2013) (Fig. 4), and in fact, enhanced activity of mG3PD has been reported in *E. coli*-infected BMDMs, and LPS-stimulated BMDMs (Garaude et al., 2016; Langston et al., 2019). Surprisingly, heat-killed bacteria and LPS did not induce the above-mentioned mtETC adaptations in macrophages as pharmacological inhibition of Complex II during infection with live bacteria led to induction of cytokine levels similar to those of dead bacteria-stimulated macrophages (Garaude et al., 2016), indicating that the alterations in mtETC occur in response to live bacterial infection only. Additionally, macrophages stimulated with TLR-3 agonist poly (I: C) and TLR7 agonist R848 induced Complex II activity, whereas stimulation with CpG (TLR9 agonist) and LPS (TLR4 agonist) did not (Garaude et al., 2016). TLR adaptors, TRIF and MyD88, were required for the commencement of alterations in the mtETC composition during *E. coli* infection (Garaude et al., 2016), and these amendments are regulated by phagosomal RNA-sensing TLRs (Blander and Sander, 2012), further corroborating that mtETC adaptations are associated with bacterial viability. Finally, bacteria-induced activation of phagosomal NADPH oxidase (phox) (Sharma et al., 2023) triggers phosphorylation of succinate dehydrogenase subunit A (SDHA) of Complex II at tyrosine 604 by the ROS-dependent tyrosine kinase, Fgr (Acín-Pérez et al., 2014), which demonstrated that phagosomal ROS produced during bacterial infection directly enhances Complex II activity. Super-complex formation was maintained in $Fgr^{-/-}$ BMDMs, which preserved Complex I-dependent ATP production and failed to enhance Complex II activity (Garaude et al., 2016), further highlighting the important role of Fgr kinase as a crucial regulator of mtETC adaptations in bacteria-infected macrophages. Inhibition of mtROS and phagosomal ROS-attenuated Complex II activity in BMDMs during *E. coli* infection (Garaude et al., 2016) also implicated the involvement of mtROS and phox in Complex II functioning. Another report demonstrated how Complex II triggers Complex I-induced mtROS production in pathological conditions (Mills et al., 2016). In another study, LPS-TLR4 interaction resulted accumulation of succinate in macrophages (Tannahill et al., 2013), and as a consequence, succinate oxidation enhanced Complex II activity, which supplied CoQ with electrons, leading to RET-induced mtROS generation *via* Complex I (Fig. 6). Succinate is transported into the cytosol, where it impairs prolyl hydroxylase (PHD) activity, stabilizing and activating HIF-1α (Selak et al., 2005) and triggering pseudo-hypoxia. LPS-induced TLR activation of murine BMDMs leads to accumulation of glycolytic and TCA intermediates including succinate due to the expression of dimeric PKM2 (Pyruvate kinase M2) (Tannahill et al., 2013; Palsson-McDermott et al., 2015). Dimeric PKM2 has lower catalytic activity, and therefore, cannot produce pyruvate at a steady rate, which eventually shifts the pathway towards lactate metabolism to meet the energetic demands, leading to interruption of TCA cycle (Z. Liu et al., 2021). Inhibition of Complex II activity using 3-nitropropionic acid (NPA) rendered mice more susceptible to infection by Gram-negative bacteria (such as *S. typhimurium* and *E. coli*) (Garaude et al., 2016) (Fig. 6). In addition, Complex II has been directly linked with apoptosis as this Complex acts as a pH sensor of apoptosis (Grimm, 2013). Early biochemical changes during apoptosis result in significant drop in the intracellular pH, leading to dissociation of succinate dehydrogenase A/B (SDHA/B) subunits from Complex II, which accumulates in the mitochondrial matrix but its SDH activity remains intact, while its Succinate Coenzyme Q Reductase (SQR) activity is attenuated due to loss of downstream electron acceptors (SDHC/SDHD). This results in the leakage of electrons from Complex II, leading to mtROS generation which ultimately instigates oxidative stress-induced apoptosis (Lemarie et al., 2011; Grimm, 2013). However, its direct involvement in bacteria-induced apoptosis is yet to be addressed; in fact, such a mechanism might not occur, as the Complex II activity increases in bacterial infection. Complex II triggers inflammasome formation (Fig. 7), evidenced by the finding that its inhibition results in attenuation of IL-1β production (Mills et al., 2016). These reports implicate Complex II as a contributor in respiratory functions, required for anti-bacterial response in macrophages.

4.1.3. Role of complex III

Complex III is another source of mtROS production (Fig. 3b and . 6) that has been reported to influence the effector functions of pro-inflammatory macrophages (Cameron et al., 2019; Kumar et al., 2022; Sharma et al., 2023) (Fig. 6). The leakage of electrons from Complex III results in partial reduction of O_2 to superoxide ions. The production of mtROS at the Qo site of Complex III (Fig. 3b) is of profound interest as it is produced at both IMS and mitochondrial matrix. mtROS has been recognized as a major activation signal for HIF-1α (Kumar et al., 2022). HIF-1α is a crucial transcription factor in the regulation of cellular metabolism and functions of macrophages (Palsson-McDermott et al., 2015; Kumar et al., 2022). However, no studies have directly investigated the role of Complex III or its subunits in bacterial infection of macrophages, even though its role in anti-bacterial immune response in T-cells has been demonstrated. Complex III-induced mtROS was essential for the expansion of antigen-specific T-cells in *Listeria monocytogenes* infection (Weinberg et al., 2019) and Rieske iron-sulphur protein (RISP) of Complex III was also required to generate memory response (Sena et al., 2013), highlighting the role of Complex III in mounting adaptive response against *L. monocytogenes* (Fig. 7). Additionally, structural integrity of Complex III dimers are essential in maintaining stability of Complex I (Acín-Pérez et al., 2014) which implicates the importance of Complex III in maintaining Complex I functioning.

4.1.4. Role of complex IV

Complex IV is not involved in mtROS generation. But it is imperative for apoptosis due to release of Cyt *c* into the cytosol, and ultimately decides the fate of infected macrophages. The role of Cyt *c* in apoptotic cell death has been reviewed extensively; therefore, it is not discussed further in this review. The Tyr kinases of the Src-family appear to play a role in mitochondrial energy metabolism (Acín-Pérez et al., 2014). As mentioned earlier, the Fgr tyrosine kinase phosphorylates Complex II, whereas another report has shown that c-Src enhances the activity of subunit II of the Complex IV (Miyazaki et al., 2003). The knockdown of cytochrome *c* oxidase (Cox) triggers increased production of IL-6, TNF-α, and IL-1β which favours M1 polarization and enhance phagocytosis (Angireddy et al., 2019) (Fig. 7), implicating that loss or deficiency in the functioning of Complex IV attributes inflammatory phenotype to macrophages. In addition, deletion of assembly protein of Complex IV i.e., SURF1 (surfeit locus protein 1) led to increased intracellular *L. monocytogenes*, and $SURF1^{-/-}$ mice display mitochondrial fragmentation, and triggers UPR^{mt} (Pulliam et al., 2014; Pharaoh et al., 2016) suggesting a functional link between Complex IV and mitochondrial fission in regulating the outcome of *L. monocytogenes* infection. $SURF1^{-/-}$ cells display low mitochondrial respiratory activity and enhanced bacterial growth (Spier et al., 2021). The underlying mechanism in SURF-mediated anti-bacterial immune response is that it impairs O_2 consumption in macrophages leading to alteration in recycling of receptors (such as c-Met) to the plasma membrane, resulting in decreased *L. monocytogenes* burden (Spier et al., 2021). Increased mitochondrial respiration slows down the receptors' recycling leading to reduction in cell surface receptors which facilitate *L. monocytogenes* entry in the cell (Spier et al., 2021). These findings highlight the involvement of Complex IV in controlling bacterial infection. In summary, the mtETC occupies a central position in cellular energy metabolism, regulating NADH, $FADH_2$, and ATP levels in conjunction with the TCA cycle, amino acid oxidation, fatty acid oxidation, and pyrimidine biosynthesis pathway, and we are just beginning to explore the involvement of mtETC in regulating macrophage functions in bacterial infection. Future studies will continue to untie the knot of mtETC functions in inflammation, resolution of inflammation which might explore novel therapeutic possibilities for treating bacteria-induced inflammatory conditions.

5. Functional consequences of mtETC adaptation in macrophages

The alterations in mtETC complexes and consequently the metabolic processes differ among M0, M1, and M2 macrophages. Alteration in mtETC eventually regulates metabolic reprogramming in M1 and M2 macrophages (Figs. 1 and 8).

Transcriptome analysis of M1 and M2 macrophages displayed remarkable changes in the expression of chemokines and cytokines, but very few were related to mtETC adaptations and metabolic activities. U–^{13}C-labeling analysis revealed that aerobic glycolysis, fatty acid (FA) synthesis, and pentose phosphate pathway (PPP) are the predominant metabolic pathways in M1 macrophages (Jha et al., 2015), whereas in case of M2 macrophages, enhanced OXPHOS, fatty acid oxidation and reduced glycolysis are the predominant pathways (Wang et al., 2018) (Fig. 1).

5.1. M1 macrophages

As in most mammalian cells, glycolysis in M1 macrophages results in the formation of cytosolic pyruvate, which can then be metabolized into lactate by the action of lactate dehydrogenase (LDH), regenerating NAD^+ from NADH, or into alanine *via* alanine transaminase to meet the energy requirements in the absence of O_2; alternatively, pyruvate can enter into mitochondria through the use of mitochondrial pyruvate carrier (MPC) (Fig. 9).

Glycolytic formation of pyruvate, and its shuttling into mitochondria, contributes directly in controlling *Mycobacterium avium* infection (Røst et al., 2022). Pyruvate kinase M2 (PKM2) has been implicated in inflammasome activation for IL-1β release (Xie et al., 2016) (Fig. 9), and inhibition of glycolysis by 2-deoxyglucose attenuated IL-1β release in macrophages stimulated with LPS (Tannahill et al., 2013). Pyruvate can be converted into acetyl-CoA *via* pyruvate dehydrogenase complex (PDC) in the mitochondrial matrix, and feed into the TCA cycle, generating reducing equivalents for OXPHOS (Fig. 9). During infection, pyruvate is involved in the establishment and/or maintenance of high proton motive force (Røst et al., 2022). Additionally, pyruvate influx into mitochondria triggers RET-induced mtROS (at Complex I), which controls intracellular bacterial burden (Røst et al., 2022). However, various biological processes have been described where even in the presence of O_2, pyruvate is preferentially metabolized into lactate, a process known as aerobic glycolysis (or glycolytic reprogramming). Glycolytic reprogramming is characterized by increased glycolytic flux, upregulation of glycolytic enzymes, and reduction in OXPHOS (Figs. 1 and 9). Glycolytic reprogramming allows M1 macrophages to synthesize enough ATP, and glycolytic intermediates essential for enhanced phagocytosis (Kelly and O'Neill, 2015; Soto-Heredero et al., 2020) (Figs. 8 and 9). This metabolic reprogramming appears to be a decisive factor in the development of M1 phenotype. Stimulation of TLRs triggers glycolytic reprogramming in M1 macrophages in response to *Salmonella typhimurium*, *Bordetella pertussis*, and *Mycobacterium tuberculosis* infection (Tannahill et al., 2013; Palsson-McDermott et al., 2015; Gleeson et al., 2016). The involvement of mTORC1 has been implicated in glycolytic reprogramming; however, the molecular mechanism of TLR-induced glycolytic reprogramming has not been fully elucidated. Furthermore, TLR stimulation has been demonstrated to suppress carbohydrate-kinase like protein (CARKL) which is an inhibitor of pentose phosphate pathway [79] (Fig. 9). M1 macrophages demonstrate enhanced PPP activity (Haschemi et al., 2012) (Fig. 1). In M1 macrophages, OXPHOS is limited due to the interruption in the TCA cycle at two major positions (Figs. 1 and 9). The first interruption is at the conversion of isocitrate to α-ketoglutarate (α-KG) step, which eventually leads to accumulation of isocitrate (Jha et al., 2015) and citrate (Tannahill et al., 2013; Jha et al., 2015) due to reduced expression of isocitrate dehydrogenase 1 (Fig. 9). Accumulated isocitrate or citrate can be withdrawn from TCA cycle and redirected for synthesis of itaconate (an anti-microbial molecule), fatty acids (synthesis of arachidonic acid, prostaglandins, membrane lipids), and oxaloacetate (generates NADPH which triggers NADPH oxidase-mediated ROS). Inhibition of citrate carrier negatively impacts M1 macrophage activation (Haschemi et al.,

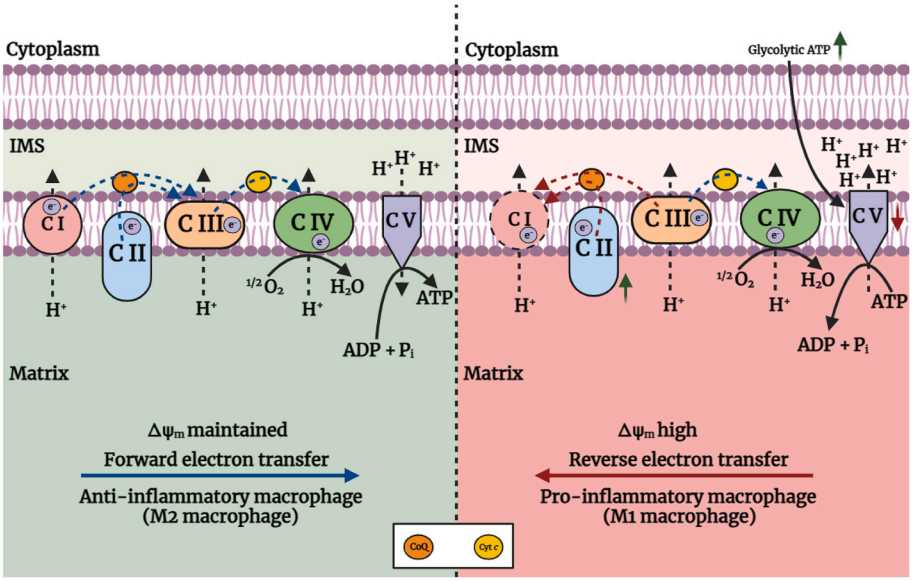

Fig. 8. Schematic of electron flow in mtETC of M1 and M2 macrophages. In M2 macrophages, the flow of electrons is maintained in a forward and sequential manner as indicated by broken blue arrows. This results in reduced leakage of the electrons, and eventual low production of mtROS. Pumping of H^+ (shown by broken black arrows) remains intact, $\Delta\Psi_m$ is maintained, and C V efficiently generates ATP (shown by black solid arrow in the left panel). In M1 macrophages, in contrast, mtETC is disrupted due to the enhanced activity of C II, which eventually results in the destabilization of C I. This results in changes in the flow of electrons within the mtETC; electrons flow backward to C I from C II and C III due to overaccumulation of $CoQH_2$ (shown by red dashed arrows). Disruption in mtETC subsequently interrupts OXPHOS, and therefore, the activity of C V is reduced, which derives ATP from glycolysis for pumping of H^+ in the IMS (shown by black solid arrow in the right panel). Excessive accumulation of H^+ in the IMS increases $\Delta\Psi_m$. Green solid arrows indicate increased levels or increased activity and red solid arrows indicate reduced activity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

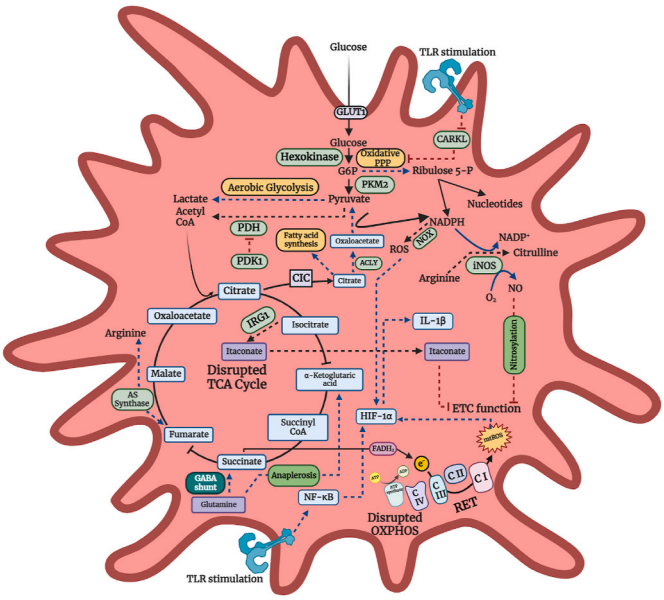

Fig. 9. Role of mtETC in M1 macrophage polarization. LPS, IFN-γ and/or TNF-α (not shown here) stimulate reprogramming of macrophages toward the pro-inflammatory phenotype. During metabolic reprogramming, the TCA cycle is disrupted at two points (at the steps of α-ketoglutarate and malate conversion) resulting in the accumulation of citrate and succinate, disrupting the feeding of electrons to the mtETC. The interruption activates the "Warburg effect" and shifts the cellular metabolism towards aerobic glycolysis to meet the energy demands. This results in the upregulation of glycolytic enzymes, and lactate production. M1 macrophages upregulate PKM2, catalyzing in the conversion of PEP into pyruvate and promoting glycolysis. Succinate synthesis is promoted in M1 macrophages *via* the channeling of glutamine into the TCA cycle through GABA shunt and through anaplerosis. Oxidation of succinate to fumarate *via* C II during low OXPHOS conditions results in the transfer of electrons in the opposite direction toward C I, leading to RET that induces mtROS production, which activates HIF-1α. HIF-1α, a signaling molecule characteristic of the M1 phenotype, drives the production of pro-inflammatory cytokines, such as IL-1β. In M1 macrophages, oxidation of G6P is enhanced due to an increase in oxidative PPP which generates NADPH that is required for NOX-induced ROS (shown by broken black arrow), synthesis of fatty acids, and anti-oxidant defense mechanisms, and ribulose-5P that is required for nucleotide biosynthesis, and this occurs due to suppressed activity of CARKL. M1 macrophages show upregulation in the PDK1 enzyme, a regulatory enzyme that phosphorylates PDH that catalyses the conversion of pyruvate to acetyl coA. Pyruvate-derived acetyl CoA (shown by broken black arrow) enters into the TCA cycle that generates NADH which fuels mtETC. Reduction in IDH1 levels led to an increase in the synthesis of antimicrobial molecule i.e., itaconic acid (or Itaconate) *via* IRG1 from decarboxylation of aconitate. However, increased production of itaconate inhibits SDH activity. Furthermore, citrate is exported from the mitochondria *via* CIC, and broken down into oxaloacetate and acetyl CoA *via* ACLY. Acetyl CoA can be used as a substrate for fatty acid synthesis. Oxaloacetate is metabolized to pyruvate accompanied by NADPH and NO production. In M1 macrophages, iNOS activity is upregulated, resulting in the catabolism of arginine to citrulline and NO (shown by broken black arrow), the latter playing a crucial antimicrobial role in M1 macrophages. Citrulline is metabolized along with aspartate to generate argininosuccinate *via* ASS, which is further catalyzed into arginine and fumarate. Further, NO-induced nitrosylation of Fe–S proteins in C I, and C IV results in their inactivation resulting in low OXPHOS. The black solid arrows represent the movement of substrates, the broken blue arrows represent the activation of enzymatic and signaling pathways in M1 macrophages, and the broken red line represent the inhibition of the enzymes/pathways in M1 macrophages. Abbreviations used are: GLUT1, glucose transporter 1; CARKL, carbohydrate kinase-like protein; PPP, pentose phosphate pathway; G6P, glucose-6-phosphate; PKM2, pyruvate kinase M2; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; CIC, citrate carrier; IRG1, immune responsive gene 1; ASS, argininosuccinate synthase; GABA, γ-amino butyric acid; NOX, NADPH oxidase; mtROS, mitochondrial ROS; CIC, citrate carrier; ACLY, ATP-citrate lyase; NO, nitric oxide; iNOS, inducible NO synthase; ASS, argininosuccinate synthase; RET, reverse electron transfer; PEP, phosphoenolpyruvate. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2012). The second interruption takes place at the succinate to malate conversion step due to the inhibition in the activity of succinate dehydrogenase, which is triggered by itaconate (Lampropoulou et al., 2016) resulting in the accumulation of succinate (Tannahill et al., 2013; Jha et al., 2015) (Fig. 7). Indeed, enhanced production of succinate and its oxidation regulates mtROS generation in M1 macrophages (Mills et al., 2016). In turn, succinate stabilizes HIF-1α subsequent to induction of IL-1β expression through the autocrine induction of succinate receptor 1 (SUCNR1) (Fig. 9). Additionally, succinate attenuates the activity of Complex I, resulting in enhanced mtROS production (Fig. 8). Reduction in OXPHOS is linked with accumulation of TCA intermediates (such as citrate, succinate, fumarate etc.), and triggers compensatory upregulation of glutamate anaplerosis and GABA shunt leading to the production of succinate that feeds the TCA cycle at α-ketoglutarate, and the aspartate-arginosuccinate shunt activity that feeds the TCA cycle at malate and fumarate (Tannahill et al., 2013; Jha et al., 2015). The aspartate-arginosuccinate shunt produces arginine and NO leading to enhanced transcription of iNOS and IL-6 in M1 macrophages (Jha et al., 2015) (Figs. 1 and 9). In addition, reduction in OXPHOS may be caused by NO-mediated nitrosylation of nearly all the mtETC complexes (Fernando et al., 2019), resulting in the inhibition of mitochondrial function and reduction in the $\Delta\Psi_m$. NO-mediated inhibition of mtETC reinforces breakage point in TCA cycle, eventually resulting in heightened mtROS production along with accretion of succinate levels, thus enhancing a pro-inflammatory phenotype. Enhanced glycolytic ATP is indispensable to meet the energy demands (Everts et al., 2012). But in order to maintain sufficient ATP production and $\Delta\Psi_m$ while the H^+ pump (Complex I, III and IV) is impaired, the ATP synthase (Complex V) activity is reversed. This leads to the delivery of H^+ to the IMS, and this process is fuelled by glycolysis-derived ATP (Fig. 9).

5.2. M2 macrophages

M2 macrophages exhibit flexibility in terms of metabolic activity as these cells can utilize OXPHOS even in the absence of glycolysis by augmented β-oxidation of fatty acids and use of the TCA cycle (Huang et al., 2014) and glutaminolysis (Wang et al., 2018) (Fig. 10). AMP kinase facilitates the increase in fatty acid oxidation (FAO) (Steinberg and Schertzer, 2014). IL-4 and IL-13 stimulation impedes mTOR activation (Byles et al., 2013), averting glycolytic reprogramming, and resulting in

decreased HIF-1α levels (Takeda et al., 2010). Furthermore, IL-4 stimulation induces STAT6-dependent activation of peroxisome proliferator-activated receptor γ (PPARγ)-co-activator 1β (PGC 1β), which promotes FAO and arginase 1 activity (Vats et al., 2006) (Fig. 10).

Prostaglandin E2 (PGE2), a multitasking eicosanoid regulates the expression of genes encoding the malate-aspartate shuttle proteins, leading to reduction in the levels of the shuttle metabolites, and consequently decreasing $\Delta\Psi_m$ in IL-4-stimulated macrophages (Sanin et al., 2018). Reduced $\Delta\Psi_m$ increases ETS variant 1 (ETV1, a transcription factor) activity that promotes the expression of IL-4 inducible genes (Sanin et al., 2018). The regulation of $\Delta\Psi_m$ by PGE2 in turn affects OXPHOS, as discussed earlier, and therefore, modulates immunity, although this aspect of PGE2 has remained under-studied. M2 macrophages express few active forms of glycolytic enzymes, one exception being the selective expression of the glycolytic enzyme 6-phosphofructo-2-kinase B1 (PFKFB1) that catalyses efficient conversion of fructose-2,6-bisphosphate to fructose-6-phosphate, thus reducing the glycolytic rate (Mills and O'Neill, 2016) (Fig. 10). M2 macrophages express high levels of CARKL, which reduces the oxidative pentose phosphate shunt, leading to the formation of ribose-5 phosphate that is essential for nucleotide and UDP-GlcNAC synthesis (Haschemi et al., 2012) (Figs. 1 and 10). This is supported by studies where suppression of OXPHOS, or inhibition of ATP synthase in IL-4-stimulated macrophages reduced M2-specific genes (such as Arg*1*, *Mrc1*, CD206), and arginase 1 activity (Vats et al., 2006; Van den Bossche et al., 2016). In M2 macrophages, therefore, glucose is not utilized primarily for glycolysis, but is rather used for the glycosylation of mannose receptors *via* the nucleotide-sugar precursor, UDP-GlcNAC, which is synthesized using the carbon of glucose and nitrogen of glutamine (Everts et al., 2012; Jha et al., 2015) (Fig. 1).

5.3. Implications of metabolic reprogramming on the macrophage epigenome

Various metabolites act as substrates, activators, or inhibitors of the post-translational modifications of histones, DNA, and RNA, and metabolic enzymes that can directly regulate epigenetic reprogramming (Flavahan et al., 2017; Britt et al., 2020). The involvement of metabolites in regulating epigenetic reprogramming implicates the involvement of mtETC in the reprogramming of macrophage epigenomes though direct evidence has not been reported yet. Hence, it remains an emerging area of study in the context of infection biology.

Metabolites such as acetyl-CoA and S-adenosylmethionine can be used by histone acetyltransferases (HATs) for lysine acetylation and by histone methyltransferases (HMTs) for lysine methylation, respectively (Dancy and Cole, 2015; Perez and Sarkies, 2023). Acetyl-CoA is an important cofactor required by HATs. Acetylation of NF-κB subunit p65 at Lys310 site results in the suppressed NF-kB activity leading to attenuation of inflammatory response (Zhang et al., 2023). Acetylation of the Stat6 transcription factor suppresses M2 polarization (Yu et al., 2019). Thus, regulation of acetyl-CoA production may serve as a critical checkpoint for HAT activity regulating macrophage reprogramming.

Histone acetylation depends on increased acetyl-CoA production. ATP-citrate lyase (ACLY) converts citrate into acetyl-CoA, and the availability of substrates that can be converted into citrate affects histone acetylation in an ACLY-dependent manner (Wellen et al., 2009). In LPS-stimulated macrophages, acetyl-CoA is utilized for H4 acetylation leading to the activation of inflammatory genes such as IL-6, IL-12, IL-18, IL-27, and chemokines such as cxcl9, cxcl10 whereas inhibition of ACLY results in increased expression of the signatory M2 macrophage cytokine, IL-10 (Lauterbach et al., 2019). TLR signaling in mammalian macrophages also redirects metabolic fluxes to increase acetyl-CoA production from glucose, leading to enhanced histone acetylation. The accumulation of H3K9 acetylation in $ODC^{-/-}$ macrophages results in the enhanced M1 response which suppresses bacterial persistence (Hardbower et al., 2017). Increased histone acetylation at H3K18 and H4K16

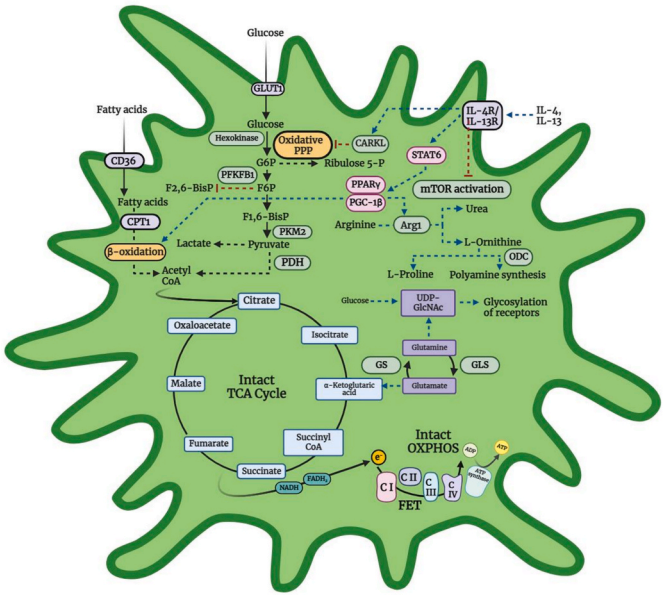

Fig. 10. Role of mtETC in M2 macrophage polarization. IL-4 and IL-13 stimulate reprogramming of macrophages toward the pro-inflammatory phenotype. During this metabolic reprogramming, the TCA cycle remains intact and supplies an ample number of electrons at mtETC, particularly at C I and C II. The electrons flow from C I to C IV in a sequential manner (known as FET; see abbreviations below), which results in ATP production by OXPHOS, and low mtROS production as well. M2 macrophages do not rely on glycolysis as long as OXPHOS is intact; these cells express PFKFB1 which efficiently catalyses fructose 2,6-BisP to fructose-6-phosphate, eventually reducing the glycolytic rate. In addition, the activity of oxidative PPP is also reduced due to enhanced expression of CARKL, and the generated products are utilized for nucleotide production and UDP-GlcNAc synthesis. UDP-GlcNAc is crucial for N-glycosylation for several cell surface proteins in M2 macrophages. Glucose is utilized for UDP-GlcNAc as well. Besides glutaminolysis, the synthesis of glutamine is also enhanced *via* the upregulation of glutamine synthetase, and glutamine is crucial for the synthesis of UDP-GlcNAc. M2 macrophages rely on fatty acid oxidation, and CD36 expression level is enhanced in M2 macrophages which facilitates increased uptake of fatty acids. CPT1 translocates fatty acids from the cytosol in the mitochondria for its oxidation which yields Acetyl CoA (shown by broken black arrow) that enters into the TCA cycle. IL-4 and IL-13 stimulation triggers PGC-1β and STAT6 phosphorylation that induces expression of nuclear receptor PPARγ. PPARγ stimulates the expression of CD36, and PGC-1β co-activates STAT6 transcription. Glutaminolysis is enhanced in M2 macrophages which elevates the levels of α-ketoglutarate *via* GLS. M2 macrophages express Arginase 1 that catalyses conversion of arginine to L-ornithine and urea. L-ornithine is further metabolized by ODC which yields L-proline and polyamines, the latter molecules are crucial for tissue repair. The black solid arrows represent the movement of substrates, the broken blue arrows represent the activation of enzymatic and signaling pathways in M2 macrophages, the broken red line represent the inhibition of the enzymes/pathways in M2 macrophages. The abbreviations used are: IL-4R, IL-4 receptor; CD36, cluster of differentiation 36; CPT1, carnitine palmitoyltransferase I, PFKFB1, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 1, PPARγ, peroxisome proliferator-activated receptor γ; PGC-1β, PPARγ coactivator-β; Arg1, Arginase 1; STAT6, signal transducer and activator of transcription 6; GS, glutamine synthetase; GLS, glutaminase; FET, forward electron transfer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was noted in M1 macrophages in response to *M. tuberculosis* infection (Khan et al., 2022). On the other hand, NAD^+-dependent deacetylase i.e., Sirt2 attenuates NF-κB acetylation that promotes M2-associated anti-inflammatory responses (Lo Sasso et al., 2014). mtETC plays a crucial role in maintaining the balance between NAD^+ and NADH. Pharmacological or genetic manipulation of NAD^+ synthesis in macrophages impaired phagocytosis and capability to resolve inflammation

(Minhas et al., 2019).

The epigenetic landscape is also regulated by demethylation, and deacetylation. α-ketoglutarate (α-KG) is an essential cofactor for the JMJD3 (Jumonji domain-containing protein D3) that orchestrates the polarization of macrophages to M2 phenotype through histone demethylase activity (Liu et al., 2017). JMJD3 induces H3K27 demethylation at the IRF4 locus, which is essential for the development of M2 macrophages (Satoh et al., 2010). Metabolites that are structurally similar to α-ketoglutarate such as succinate, and fumarate competitively inhibit demethylation. The succinate and fumarate levels are elevated in M1 macrophages (Fig. 6). Modification in the α-ketoglutarate/succinate ratio altered macrophage polarization in a dose-dependent manner, with decreasing α-ketoglutarate/succinate ratio promoting M1 polarization while increasing α-ketoglutarate/succinate ratio promoting M2 polarization (Liu et al., 2017). Lactate acts as a negative feedback signal in macrophages and prevents excessive inflammation (Zhou et al., 2022). It has been recently reported to regulate the epigenetic reprogramming of macrophages through a process known as lysine lactylation. Histone lactylation especially at H3K18 promotes the polarization of M2 macrophages and leads to the transcription of anti-inflammatory genes such as Arg*1* (Zhang et al., 2019). Remarkably, this type of epigenetic modification occurs later in the course of murine macrophage polarization as compared to histone acetylation.

DNA methylation is mainly associated with the repression of transcripts. Hypermethylation of SOCS1 which negatively regulates cytokine signaling, results in the enhanced expression of pro-inflammatory cytokines such as IL-6 and TNF (Cheng et al., 2014). Hypermethylation of PU.1, KIF4, and Notch1 at H3K9 promotes M1 polarization (Yan et al., 2018). Pharmacological inhibition or genetic manipulation of DNA methylation at PPARγ1 promotes M2 macrophages (Wang et al., 2016). In conclusion, these findings suggest that metabolic licensing of epigenetic landscape provides another layer of control in macrophage polarization.

6. Turning the tables: how bacteria rewire macrophage metabolism for survival and evasion of anti-bacterial response

Macrophage polarization induced by way of alteration in mtETC is crucial in restraining bacterial infection. To counter it, however, many bacterial pathogens have advanced their defence arsenals to survive within the menacing environment of the macrophages. Such mechanisms include rewiring of macrophage metabolism to attenuate the antibacterial response, exploitation of metabolic resources of macrophages, and secretion of bacterial effector molecules for subverting pro-host functions regulated by mitochondria.

There are several reports suggesting that pathogenic bacteria skew the mitochondrial metabolic process in favour of aerobic glycolysis to sustain their growth (Escoll and Buchrieser, 2018; Russell et al., 2019). The causative agent of Legionnaires' disease i.e., *Legionella pneumophila*, replicates inside human alveolar macrophages. *L. pneumophila* virulence essentially depends on energy and anabolic substrates usurped from the nutrient-limited intracellular milieu. *L. pneumophila* relies on a dual strategy depending on serine metabolism during exponential growth phase, which is replaced by glycerol and glucose at later phases of growth (Häuslein et al., 2017). The increased glycolytic fluxes induced by *L. pneumophila* sustain the production of serine which serves as the essential source of energy (Escoll and Buchrieser, 2018; Häuslein et al., 2017). However, the molecular basis of the switch between the two growth phases is not well established, and it is likely that the depletion of serine serves as the cue to initiate glycolysis and conversion of the bacterium to the mature intracellular form that is less energy-demanding. It has also been observed that *L. pneumophila* use phosphorylated hexose during the infective stage in murine macrophage (Price et al., 2018), further attesting to the plurality of infection strategies used by this bacterium. Currently, we do not know whether this is a host-specific response or a conserved virulence trait of the bacteria. *L. pneumophila* attenuate OXPHOS and increase glycolysis by triggering a Warburg-like metabolic state in the infected cells, which is achieved *via* the injection of the bacterial type IV secretion system (T4SS) effector proteins, particularly MitF, causing mitochondrial fragmentation (Escoll et al., 2017). The fragmentation of mitochondrial network reduces OXPHOS and causes glycolytic fluxes in the infected macrophages in a T4SS-independent manner, though the mechanisms remain largely unexplained (Galli and Saleh, 2021; Escoll et al., 2017). Enforcing a Warburg-like metabolic program in infected macrophages may help in generating essential resources for *L. pneumophila.* Nonetheless, the Warburg effect may also trigger antibacterial responses by elimination of the infected macrophages. To counteract, *L. pneumophila* reverse mitochondrial F_0F_1-ATPase activity to ATP hydrolase, this restores $\Delta\Psi m$ and delays cell death, effectively providing a window of growth in the macrophages (Escoll et al., 2021).

S. typhimurium shuts off OXPHOS, induces glycolysis, and increases accumulation of 2-phosphoglycerate, 3-phosphoglycerate and phosphoenolpyruvate *via* SPI-1 effector SopE2 which act as carbon source for the bacteria (Jiang et al., 2021), indicating that increased glycolysis creates a conducive intracellular niche for bacterial replication in the macrophages. *S. typhimurium* resides and replicates inside salmonella-containing vacuole (SCV) (Jennings et al., 2017), in which SPI-2 (*Salmonella* pathogenicity island-2) effectors play crucial role in converting anti-microbial phagosome into SCV (Jiang et al., 2021). Furthermore, accumulated lactate and pyruvate act as a cue in regulating bacterial SPI-2 genes (Jiang et al., 2021). In addition, SPI-2 effectors enable bacteria to acquire host nutrients in SCV (Liss et al., 2017). The disruption in mitochondrial metabolism and siphoning of energy resources favours inflammasome-induced cell death and removal of *S. typhimurium*. During active replication, *S. typhimurium* induces IFN-I-dependent and RIP3-mediated necroptosis in macrophages (Robinson et al., 2012). However, during chronic infection, inflammasome signaling is protracted to promote bacterial survival. *S. typhimurium* persists in M2 macrophages by sustaining fatty acid metabolism that helps bacterial survival inside the host; the persistent state, however, is not achieved in PPARδ$^{-/-}$ mice (Eisele et al., 2013). This is reminiscent of a similar role of PPAR in *Bacillus abortus*, as presented later, since *S. typhimurium* also depends on robust glycolysis for intracellular survival (Bowden et al., 2009). In apparent contrast, glycolysis is crucial for the anti-*Salmonella* response in macrophages, and is associated with apoptosis (Yu et al., 2020). A recent study suggested how bacteria maintain a balance in glycolysis levels to avoid host cell death and establish a chronic infection. It was shown that despite an increase in glucose intake during the early phase of infection, the glycolytic metabolites decline and glycolysis is reduced at later stages of infection to avoid glycolysis-mediated phagosomal maturation (Gutiérrez et al., 2021). Glucose is rerouted into bacterial vacuoles and becomes unavailable for the host cell (Eisele et al., 2013). Hence, it is conceivable that *S. typhimurium* blocks the up-regulation of genes that are required for regulating glycolytic flux (Gutiérrez et al., 2021). In another mechanism of immune evasion, *S. typhimurium* also augments leptin signaling to dodge lysosomal degradation in macrophages (Fischer et al., 2019). Recent studies have shown that SteE-mediated signals offset TNF-induced switching of M1 macrophages, promoting bacterial persistence (Pham et al., 2020). In this mechanism, SteE either acts as a cryptic kinase or associates with another kinase involved in phosphorylating the Y705 residue of STAT3 (Panagi et al., 2020), which facilitates M2 polarization. Additionally, SteE itself gets phosphorylated and acts as an endogenous receptor-binding site for recruitment and activation of STAT3 (Brodsky, 2020), eventually driving differentiation into M2 macrophages.

B. abortus is the causative agents of brucellosis. *B. abortus* increases aerobic glycolysis in macrophages in order to utilize lactate as a sole carbon and energy source (Czyż et al., 2017). The macrophage subpopulation in fact varies between acute and chronic *B. abortus* infection. It was observed that inhibition of lactate dehydrogenase or host

glycolysis inhibits the growth of *B. abortus* (Czyż et al., 2017) implying a role of altered OXPHOS in the virulence of this bacteria. *B. abortus* resides in M2 macrophages and stimulates PPARγ-mediated accumulation of glucose which is critical for bacteria survival during chronic infection (Xavier et al., 2013). Inhibition of the STING-HIF1α axis reduces succinate and IL-1β levels, resulting in enhanced bacterial growth (Gomes et al., 2021), which is likely a method by which the immunometabolism is altered, creating a favourable replicative niche for the bacteria. However, *B. abortus* does not induce differentiation of M2 macrophages (Xavier et al., 2013), suggesting a selective targeting of M2 macrophages. On the contrary, a recent report showed *B. abortus* regulates macrophage polarization *via* modulation of NF-κB signaling pathway (Zhao et al., 2023). This complex scenario warrants further study to determine whether the bacteria selectively infect M2 macrophages or induces a metabolic reprograming in macrophages.

OXPHOS is also deleterious for the infection and growth of *Listeria monocytogenes*, which relies upon glycerol and glucose-6-phosphate for energetic and anabolic demands respectively, rather than on lactate and pyruvate for energy source and intracellular survival (Grubmüller et al., 2014). The toxin, listerolysin O, released by the bacteria induces transient mitochondrial breaks that increase glycolysis (Stavru et al., 2011). However, this alteration in mitochondrial dynamics is transient, likely to ensure that permanent damages do not occur in this important organelle, which would otherwise restrict the anti-bacterial and pro-inflammatory responses (Stavru et al., 2011) and also reduce mitochondrial respiration that usually assists in bacterial internalization (Spier et al., 2021). Overall, this translates into activation of pro-survival molecular events in the infected cells. A recent report suggested that IFN-I signaling is also crucial in regulating OXPHOS, and that inhibition of fatty acid oxidation plays a host-centric role during *L. monocytogenes* infection (Demiroz et al., 2021).

Mitochondrial OXPHOS also plays a critical role in mycobacterial infection of macrophages. Infection by *M. tuberculosis* rewires macrophage metabolic program from OXPHOS toward glycolysis (Gleeson et al., 2016). The M1 macrophages are present in the early phase of *M. tuberculosis* infection whereas M2 macrophages are predominantly active in the later phase of infection (Shi et al., 2019). However, the host microenvironment shifts M1 macrophages (glycolytic macrophages) toward M2 macrophages (OXPHOS macrophages) *via* host lipids present in the TB-PE (Tuberculosis pleural effusion) fluid in the pleural space (Franco et al., 2020). *M. tuberculosis* remains in a non-replicative state in M1 macrophages in the early phase of infection (Shim et al., 2020). Enhanced glycolysis in this phase leads to increased *de novo* lipid synthesis due to defective mitochondrial respiration, leading to the development of foamy macrophages (Singh et al., 2015; Shim et al., 2020). Glycolysis with a disrupted TCA cycle supports an anti-mycobacterial response in macrophages (Huang et al., 2018), whereas excessive glycolysis is associated with enhanced lipid accumulation which serves as a pro-mycobacterial response (Singh et al., 2015). However, the initial switch to the Warburg-like phenotype initiated by *M. tuberculosis* ultimately diverts the energy resources toward the pathogen and helps it survive under the nutrient-starved condition in the macrophages which could be paramount in the transition from host-centric to pro-pathogenic role. In conclusion, glycolysis has contradictory roles in *M. tuberculosis* infection. However, the suppression of glycolytic pathway in macrophages by *M. tuberculosis*, *L. pneumophila*, and *S. typhimurium* during infection stages suggests the host-centric role of glycolysis in counteracting bacterial infection.

An interesting new development has demonstrated the molecular mechanism involved in reducing glycolysis by *M. tuberculosis*. Mycobacterial infection was shown to trigger upregulation of miR-21, which suppressed pro-glycolytic phosphofructokinase-M (PFK-M), thus reducing glycolysis (Hackett et al., 2020); this allowed the bacteria to bypass pro-inflammatory response fuelled by enhanced glycolysis at the initial stages (Gleeson et al., 2016; Braverman and Stanley, 2017). PFK-M contains a conserved miR-21 binding site in its long 3′ UTR in both mice and human. Mutation of the binding site relieves the negative regulation of miR-21 upon PFK-M (Hackett et al., 2020), highlighting the central role of miR-21 in limiting glycolysis. miR-21 also inhibits *bcl-2* and *il12p35* through binding at their respective 3' UTR (Wu et al., 2012), which enhances bacterial survival. Reduced glycolysis coupled with scaling down of OXPHOS and TCA cycle induces a "quiescent energy phenotype" in macrophages. Besides influencing the energy budget, these changes attenuate IL-1β production and hence, aid in the growth of *M. tuberculosis* in the macrophages (Hackett et al., 2020).

6.1. Effects of antibiotics in mtETC-based reprogramming of macrophages

Antibiotics are undoubtedly important in almost all therapeutic strategy used for the treatment of acute and chronic infectious bacterial diseases. However, the indiscrete usage of antibiotics has also increased antibiotic resistance bacterial pathogens. Furthermore, antibiotics may have unfavourable effects on the functionality of the immune cells, subsequently increasing the risk of secondary infection. Such adverse consequences may be mediated, at least in part, through effects on the mitochondria in macrophages. Mitochondria shares conserved features such as ribosomes (60S or 55S) with the bacteria due to their bacterial origin, making them exceptionally sensitive to antibiotics (Wang et al., 2015). Antibiotics can affect bioenergetic function of the mitochondria, which may compromise the macrophages' ability to counter the bacterial infection. Compelling evidence suggested that antibiotics induce oxidative stress and mitochondrial dysfunction (Kalghatgi et al., 2013). Intestinal macrophages became hyper-responsive and produce enhanced levels of inflammatory cytokines to bacterial stimulation after antibiotic treatment (Scott et al., 2018). Recolonization of microbiota in the colon of antibiotic-treated mice instigates prolonged macrophage-dependent increase in T_h1 $CD4^+$ T-cell response and dysbiosis. Antibiotic treatment severely disrupted the growth of microflora associated with the generation of short-chain fatty acids (SCFAs) including butyrate. Butyrate treatment enhances OXPHOS and lipid metabolism which are crucial for activation of M2 macrophages (Ji et al., 2016). It suppressed production of LPS-induced IL-6 and NO preventing M1 macrophage activation (Chang et al., 2014). The supplementation of antibiotics with butyrate restored hypo-responsiveness of intestinal macrophages and prevented T-cell dysfunction. It has been observed that butyrate induces upregulation of Arg*1* expression in macrophages that ultimately drive alternative activation of the macrophages and increases OXPHOS (Scott et al., 2018). Furthermore, stimulation of macrophages with butyrate increased H3K9 acetylation that induced STAT6-mediated transcription suggesting butyrate facilitates M2 macrophage polarization through inhibition of HDAC activity (Chang et al., 2014).

Oxazolidinones such as linezolid inhibits translation machinery in the mitochondria disrupting the mtETC (Priesnitz and Becker, 2018). Prolonged linezolid treatment triggers a range of toxic effects. It reduces enzymatic activity and protein levels of mtETC complexes, including enzymes as well (De Vriese et al., 2006). This causes reduction of mitochondrial mass, mtETC complex IV activity resulting in enhanced apoptosis (Garrabou et al., 2017). Gentamicin inhibits OXPHOS (Negrette-Guzmán et al., 2015) and kanamycin impairs mtETC activity (Kalghatgi et al., 2013). Vancomycin reduced complex I activity resulting in increased mtROS generation (Sakamoto et al., 2017). Ciprofloxacin attenuates mtETC complex I activity (Kozieł et al., 2006).

On the other hand, antibiotics also favored host cell to restrain bacterial infection. For e.g., antibiotics employed for the treatment of tuberculosis predominantly modulates mtETC complexes affecting mitochondrial function. Isoniazid inhibits mycolic acid synthesis in *M. tuberculosis*, and at the same time, it also impairs mtETC complex formation by inhibiting complex II activity (Ahadpour et al., 2016). This impairment is linked with autophagy in macrophages, which aid in the clearance of *M. tuberculosis* (Cahill et al., 2020). Ciprofloxacin and levofloxacin induce NO production which is crucial for anti-mycobacterial activity, and NO inhibits mtETC function by disrupting TCA cycle

(Poderoso et al., 2019; Yang et al., 2016; Palmieri et al., 2020) resulting in the M1 macrophages polarization which effectively controlled *M. tuberculsosis* infection. At low concentration, bedaquiline activates autophagy and induce innate immune resistance in hMDMs. Bedaquiline treatment resulted in killing of antibiotic-resistant bacterial strains including *S. typhimurium* and *S. aureus* through alteration of glycolytic activity of hMDMs (Giraud-Gatineau et al., 2020). Although, bedaquiline did not directly affect mtETC of hMDMs, the mechanism of modulating glycolytic activity remains unknown.

Understanding how antibiotics trigger unwanted effects in the host immune cells and also support anti-bacterial response through the modulation of mtETC would be advantageous to overcome infections with antibiotic-resistant bacterial pathogens and warrants further research.

7. Conclusion

The long-standing knowledge of metabolic pathways of ATP generation in mitochondria and the recent understanding of mitochondrial functions beyond ATP production highlight the importance of mitochondrial biology in macrophages. Thereafter, the metabolic machinery of the mitochondria has been recognized from latent to active players in regulating effector functions, specifically the fate and function of the macrophages in bacterial infection. This recent understanding has unlocked unanticipated perspectives, particularly in relation to the involvement of mtETC in physiological and pathological fluctuations in macrophages. Pathogenic agents, inflammatory signals, and apoptotic debris trigger differentiation of macrophages by rapidly modulating the expression of key metabolic genes for acquisition of new functions, befitting the need of the microenvironment. It is, therefore, of paramount importance that we understand the interplay between mtETC and immunity by dissecting the role of the mtETC complexes in immunity against tractable pathogens, in this case the bacteria. The mtETC complexes agglomerate in the form of super-complexes, yet the study of mtETC machinery during bacterial infection has been mainly circumscribed to the investigation of only a few individual mtETC components and their subunits, while the knowledge of the structural and functional adaptations of the super-complexes in the polarization of macrophages and their response during bacterial infection still remains scarce. For instance, only one recent study investigated the alterations in the assembly of mtETC super-complex in macrophages during *E. coli* and *S. typhimurium* infection (Garaude et al., 2016). Although the complete picture is still fuzzy, a dynamic pattern is already emerging, as we have presented in this review. Besides their value in basic science, the clinical importance of mtETC super-complexes is evident from the plethora of OXPHOS disorders that are well-known for years. However, the extent to which these defects affect immune functions remains unclear. Current progress in our understanding of the structural organization of mtETC super-complexes has only begun to unlock new opportunities for investigating the roles of their spatial organization, stability and catalysis in pathogenic infection.

Alteration of structural-functional activities of mtETC, and OXPHOS by pathogens may not only offer bioenergetic advantages, but also instigate or obstruct cell death pathways in macrophages. The optimal functioning of mtETC and Complex V represents usual cellular functions, whereas an altered, inept functioning of mtETC represents an exigent signal for the macrophages leading to alteration in their effector functions. In case of bacterial infection, relative contribution of the bacteria and macrophages to shifts in the respiratory chain remains a matter of debate, as host-driven and pathogen-driven alterations seem to co-exist during infection. Macrophages strive to avert an energy crisis due to reduced OXPHOS through the upregulation of glycolytic ATP production, whereas bacterial pathogens trigger a shift to glycolytic pathways coupled with other biosynthetic pathways to create a microenvironment that allows their replication within the infected macrophage. However, their roles in counteracting bacterial infection, or their targeting by pathogens to subvert mitochondrial metabolism during infection remain largely unknown. Extensive research in recent years has changed our view of the mtETC from mere ATP generation to a more integrated approach in regulating mitochondrial biogenesis, metabolic pathways, and immune response. In contrast, information regarding the role of mtETC in regulating the effector functions of M1/M2 macrophages is relatively limited. Nonetheless, the abundant literature on macrophage metabolism demonstrated several key discoveries in metabolite-mediated modulation of macrophage response in context of bacterial infection, and also suggested abnormalities in metabolic pathways in disease conditions. The mitochondria, discovered nearly two centuries ago, are considered prokaryotic endosymbionts of eukaryotic cells, where they harbour a large number of key pathways, such as TCA cycle, the urea cycle, β-oxidation of fatty acids, calcium homeostasis and apoptosis, to name a few. As a relatively newer area of research, their role in immunity is destined to garner greater attention.

8. Future directions

This review has revealed many deficiencies in our knowledge of mitochondrial regulation of macrophage antibacterial activity. In what follows, we summarize several of them in the hope that it will facilitate future research in this fascinating area.

(i) The mtETC super-complexes: The obvious role of the super-complexes is to optimize and galvanize the flow of electrons, regulate mtROS production, and modulate their activity in an autocrine manner; however, their regulation and dynamics in macrophages in response to bacterial infection need to be addressed, not only for mechanistic standpoint, but for the development of therapeutic approaches. In this approach, the assembly and disassembly of the mtETC super-complexes can be studied, including the molecular factors that are required to maintain or disassemble the super-complexes. Such studies will also highlight the bacterial proteins that may directly influence the super-complexes.

(ii) mtROS location: The mtROS is generated at multiple sites in the mtETC. It will be interesting to determine if site-specific mtROS production is a host-induced anti-bacterial strategy or a pro-bacterial strategy to subvert macrophage functioning. Hence, it can be explored if mtROS generation at specialized sites differentially regulates signaling and inflammatory response in macrophages. This knowledge may also provide insights in metabolic reprogramming and anti-bacterial response in the macrophages. Such studies may compare macrophages infected with wild-type bacteria and macrophages infected with mutant bacterial strains. By using pharmacological inhibitors, the involvement of site-specific mtROS in the activation/deactivation of signaling proteins and of inflammatory mediators can be studied. Also, alteration of site-specific mtROS generation on intracellular bacterial survival and replication by using knock-out or pharmacological inhibitors may provide some insights on the involvement of site-specific mtROS generation in anti-bacterial response.

(iii) Therapeutic potential: One approach is to target specific mtETC complexes (or specific electron feeders of mtETC) to regulate electron flux in macrophages to achieve a therapeutic effect in bacterial infection without causing adverse metabolic effects on the host cell. Conceivably, mitochondrial respiration, mtETC functioning, and electron flow in mtETC can be studied after depleting or over-expressing specific electron feeders such as NAD^+, FADH, and succinate, etc. Eventually this will help us gain insights about the involvement of these metabolic feeders in macrophage polarization, in turn allowing us to modulate macrophage phenotype to suppress bacterial infection. However, the targeted delivery of these molecules into macrophages will pose a problem that requires intensive research as virtually every

host cell including immune cells utilize these electron feeders. Furthermore, bacteria also use such resources, posing another additional problem in delivering those metabolites exclusively to the macrophages. A related query is whether the findings in macrophages also hold true for other immune cells, all of which have played shared roles in various forms of immunity.

(iv) "MITO-bolomics" of bacteria-infected macrophages: The majority of studies in the field of infection biology focus upon the type of interaction of bacterial toxins and anti-microbial proteins synthesized by macrophages. However, the study of the alterations that occurs at the level of genomic DNA, RNA transcripts and proteins synthesized in the mitochondria during bacterial infection could be identified to determine if the type of response activated in macrophages is specific to the type of infecting bacterial pathogen. This may open up several branches of new research, such as a novel class of non-coding mitochondrial RNA, specifically induced by subsets of bacteria.

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CRediT authorship contribution statement

Manmohan Kumar: Conceptualization, Writing – original draft, preparation. **Shagun Sharma:** Writing – original draft, figure preparation. **Jai Kumar:** Writing – original draft, preparation. **Sailen Barik:** Writing – review & editing, critical revision. **Shibnath Mazumder:** Supervision, Writing – original draft, preparation, critical revision, and suggestions. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known commercial, financial interests or personal relationships that could be construed as a potential conflict of interest.

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