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Mitochondria as central hub of the immune system

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ABSTRACT

Nearly 130 years after the first insights into the existence of mitochondria, new rolesassociated with these organelles continue to emerge. As essential hubs that dictate cell fate, mitochondria integrate cell physiology, signaling pathways and metabolism. Thus, recent research has focused on understanding how these multifaceted functions can be used to improve inflammatory responses and prevent cellular dysfunction. Here, we describe the role of mitochondria on the development and function of immune cells, highlighting metabolic aspects and pointing out some metabolic- independent features of mitochondria that sustain cell function.

1. Introduction

Cellular metabolism has been an extensively explored field during the past decade. The understanding of how cells use energy to perform their functions has attracted the attention of scientists, especially with regard to metabolic-related diseases such as obesity, diabetes, and cancer [1–4]. Classically, these conditions not only change whole-body metabolism, but also impair the inflammatory responses. Thus, immunometabolism has emerged as a potential new field of inquiry in order to investigate how the metabolic alterations affect immune cells.

The immune system comprises a family of heterogeneous cells with multiple roles during homeostasis and inflammation in a tissue-specific manner. Recent studies have shown that different immune cell subtypes use distinct metabolic programs to perform their functions. For instance, effector T cells prioritize aerobic glycolysis during anabolic metabolism to balance the synthesis of macromolecules and the generation of energy to support it [5]. Conversely, memory T cells, as well as regulatory T cells (Treg), prioritize fatty acid oxidation (FAO also called β-oxidation) to support the energy demand for survival and function [5].

As first hypothesized by Richard Altmann in 1890 [6], the generation of useful metabolic energy is provided mainly by mitochondria in virtually all eukaryotic cells. However, the concept that the mitochondria are only powerhouses of cells has changed due to a myriad of other roles this membrane-bound organelle can perform. In immune cells, for instance, mitochondria can also regulate cell development, activation, proliferation, differentiation, and death [7,8], which directly impact cell fate and fitness. Moreover, mitochondria are dynamic organelles and can change their morphology and position in the cells through coordinated cycles of fission and fusion to regulate their own and functions and cell metabolism [9]. This process is called mitochondrial dynamics. Another important aspect of the mitochondrial physiology is the local production of oxidants or more commonly referred to as “reactive oxygen species” (mtROS). They were first described as byproducts of the electron transport chain (ETC) and implicated in oxidative damage [10–13], but are also important signaling molecules for cell activation when produced in low quantities [10,14–16].

Thus, the importance of mitochondria goes beyond energy production. Mitochondria can orchestrate immunity by modulating both metabolic and physiologic states in different types of immune cells. In this review, we will focus on how mitochondria drive the development and function of immune cells, highlighting their main metabolic features and pointing out other metabolism-independent roles of mitochondria that sustain cell function.

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Abbreviations

$^{\bullet}NO_2$	Nitrogen Dioxide Radical
2DG	2-Deoxy-D-Glucose
APC	Antigen-Presenting Cell
ASCs	Antibody-Secreting Cells
ATP	Adenosine Triphosphate
Bcl-2	B-cell Lymphoma 2
BCR	B Cell Receptor
CARKL	Carbohydrate Kinase-Like Protein
CoA	Coenzyme A
CPT	Carnitine Palmitoyltransferase
CSR	Class-Switch Recombination
DAMPs	Danger-Associated Molecular Patterns
DCs	Dendritic Cells
Drp1	Dynamin-Related Protein 1
ETC	Electron Transport Chain
$FADH_2$	Reduced Flavin Adenine Dinucleotide
FAO	Fatty Acid Oxidation
FAS	Fatty Acid Synthesis
FFA	Free Fatty Acids
fMLP	N-Formylmethionine-Leucyl-Phenylalanine
FO	Follicular
GC	Germinal Center
GSH	Glutathione
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GPx	Glutathione Peroxidase
GSSG	Oxidized Glutathione
GTPases	Guanosine Triphosphatases
GLUT1	Glucose transporter 1
H_2O_2	Hydrogen Peroxide
HBP	Hexosamine Biosynthetic Pathway
HIF-1α	Hypoxia-Inducible Factor 1α
$HO^{\bullet}$	Hydroxyl
HOCl	Hypochlorous Acid
IFN-γ	Interferon-γ
IL	Interleukin
IMM	Inner Mitochondrial Membrane
KEAP1	Kelch-Like ECH-Associated Protein 1
LLPCs	Long-Lived Plasma Cells
MCU	Mitochondrial Calcium Uniporter
MiD	Mitochondrial Dynamics Proteins
MFF	Mitochondrial Fission Factor
MnSOD	Manganese-Dependent Superoxide Dismutase
MPO	Myeloperoxidase
Mfn	Mitofusin
mtDNA	Mitochondrial DNA
mtGSH	Mitochondrial Glutathione
mTOR	Mammalian Target of Rapamycin
mtROS	Mitochondrial Reactive Oxygen Species
MZ	Marginal Zone
NADH	Reduced Nicotinamide Adenine Dinucleotide
NETs	Neutrophil Extracellular Traps
NLRP3	Nucleotide-Binding Oligomerization Domain-Like Receptor Pyrin Domain-Containing-3
$NO^{\bullet}$	Nitric Oxide
NOS	Nitric Oxide Synthase
Nox	NADPH Oxidase
Nrf2	Nuclear Factor (Erythroid-Derived 2)-Like-2 Factor
O-GlcNAc	*O*-linked *N*-Acetyl Glucosamine
$O_2^{\bullet-}$	Superoxide Anion
OMM	Outer Mitochondria Membrane
ONO^-	Nitrite
$ONOO^-$	Peroxynitrite
Opa1	Optic Atrophy 1 Protein
OXPHOS	Oxidative Phosphorylation
ONOOH	Peroxynitrous Acid
PAMPs	Pathogen-Associated Molecular Patterns
PGC-1β	Peroxisome Proliferator-Activated Receptor γ–Coactivator-1β
PINK1	PTEN-Induced Putative Kinase 1
PMA	Phorbol 12-myristate 13-Acetate
PPP	Pentose Phosphate Pathway
PRRs	Pattern Recognition Receptors
RNS	Reactive Nitrogen Species
SDH	Succinate Dehydrogenase
SK3	Small Conductance Calcium-Activated Potassium Channel 3
SLPCs	Short-Lived Plasma Cells
SOD	Superoxide Dismutase
STAT6	Signal Transducer and Activator of Transcription 6
T4SS	Type IV Secretion System
TCA	Tricarboxylic Acid
TCR	T Cell Receptor
Th	Helper T Cell
TLRs	Toll-Like Receptors
Treg	Regulatory T Cell
UDP-GlcNAc	Uridine Diphosphate-*N*-Acetyl Glucosamine
xCT	Cystine/glutamate transporter

2. Immune cells metabolism: an overview

Immune cells need energy stored at adenosine triphosphate (ATP) to grow and perform their functions. Glucose is the most studied fuel for cells. After uptake, glucose undergoes a series of 10 consecutive reactions [reviewed in Ref. [17]] in the cytoplasm to be converted into pyruvate and in the process producing two molecules of ATPs [18]. Pyruvate can be either transported into the mitochondria by mitochondrial pyruvate carriers or converted into lactate in the cytoplasm by lactate dehydrogenase (LDH). During the latter reaction, the cofactor nicotinamide adenine dinucleotide (NADH) is oxidized, thus restoring cytosolic NAD^+ levels to allow continued glycolytic flux. Lactate production, first reported as a pathway that is activated only under anaerobic conditions, can also occur in the presence of oxygen [19]. In 1923, Otto Heinrich Warburg observed that glucose consumption by oxygen-exposed tumor cells was elevated compared to normal cells [20]. This event is called aerobic glycolysis or Warburg's effect. In general, aerobic glycolysis is the preferred metabolic pathway in activated immune cells [5].

Another important glucose-related metabolic pathway is the hexosamine biosynthetic pathway (HBP). This pathway regulates post-translational modifications (PTMs) such as phosphorylation, acetylation, glycosylation, ubiquitination, acetylation, and hydroxylation [21–23]. PTMs modify protein structure and function, modulating cell signaling pathways, for example [23–25]. HBP is linked to the glycolytic pathway by fructose-6-phosphate. In the presence of glutamine, fructose-6-phosphate generates glucosamine-6-phosphate [26]. Then, after subsequent enzymatic steps involving acetyl-CoA produced during fatty acid metabolism and from pyrimidine biosynthesis products, uridine diphosphate-*N*-acetyl glucosamine (UDP-GlcNAc) is generated [26]. UDP-GlcNAc is the substrate for *O*- and *N*-glycosylations, producing complex glycoconjugates, such as glycoproteins and proteoglycans [27]. These glycosylations are associated with conventional secretory pathways dependent on the Golgi apparatus and endoplasmic reticulum [23,28]. However, in 1984 Torres and Hart described a non-canonical glycosylation mechanism involving the association of *O*-linked *N*-acetyl

Glucosamine (*O*-GlcNAc) with cytoplasmic, nuclear and mitochondrial proteins [29]. This special form of glycosylation plays important roles in metabolic homeostasis, stem cell biology, signaling pathways, transcription factors function, and immune cell maintenance [29]. Defects in *O*-GlcNAc are linked to the development of cancer, diabetes, Alzheimer's disease, and other disorders [30–32].

As stated above, the pyruvate produced from glucose metabolism can be transported into the mitochondria and be oxidized by pyruvate dehydrogenase (PDH), generating acetyl-CoA that enters the tricarboxylic acid (TCA) cycle. In the TCA cycle, acetyl-CoA is further oxidized and generates key intermediates that can also be used as precursors of complex molecules. Some of the TCA reactions produce NADH and flavin adenine dinucleotide ($FADH_2$) to fuel the ETC, which in turn couples the electron transfer to the pumping of protons across the inner mitochondrial membrane (IMM) to create an electrochemical proton gradient. ATP synthase uses the potential energy from the protons when they move from high-to low-potential sides of the mitochondrial membrane to generate ATP [33,34]. This pathway is called oxidative phosphorylation (OXPHOS) and produces 36 ATPs per mole of glucose.

In addition, to provide intermediates for the biosynthesis of macromolecules, some TCA cycle-derived molecules can act as signaling molecules and even play microbicidal roles [35–37]. The TCA cycle in M1 macrophages and dendritic cells (DCs), for example, is not complete. The “broken TCA cycle” diverts some of its intermediates to other pathways. This can lead to the accumulation of citrate and succinate, for instance, in these cells [38]. When citrate accumulates in the mitochondrial matrix, it can be transported from the mitochondria to the cytosol by the mitochondrial citrate carrier [39]. Once in the cytosol, citrate is oxidized to acetyl-CoA, which leads to *de novo* lipogenesis. This is crucial to the expansion of organelles, such as the endoplasmic reticulum and Golgi, which is required for increased protein production and secretion. This is important during DCs activation [39,40]. To replenish the citrate in mitochondria and fuel the TCA cycle, the cells use other metabolites to produce more citrate through anaplerotic reactions [36]. For instance, glutamine metabolism can generate the intermediate α-ketoglutarate via glutaminolysis, allowing the TCA cycle to proceed [38].

Succinate is formed by the oxidation of succinyl-CoA via succinyl thiokinase (also called succinyl-CoA synthetase) and is oxidized to fumarate in complex II of the ETC by succinate dehydrogenase (SDH) and in the process FAD is reduced to $FADH_2$. $FADH_2$ can be oxidized again to FAD by the iron-sulfur (Fe-S) center of the SDH. This process produces both superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). A break in the TCA can occur during the conversion of succinate to fumarate by SDH, leading to succinate accumulation in the mitochondria and cytosol. Succinate has a well-established function in macrophage polarization [41]. Pro-inflammatory M1 macrophages are characterized by increased availability of succinate in the cytosol, where it acts to inhibit prolyl hydroxylases. Prolyl hydroxylases are responsible for the degradation of the hypoxia-inducible factor 1α (HIF-1α), leading to its stabilization [41]. Moreover, succinate stimulates DCs via succinate receptor 1 through the induction of intracellular calcium mobilization and enhancing DCs migration and cytokines secretion [35]. In order to restrain the pro-inflammatory role of succinate another TCA cycle-derived molecule, itaconate, is produced from cataplerosis of *cis*-aconitate [42]. Itaconate has anti-inflammatory properties and regulates both metabolism and effector functions of macrophages through the inhibition of succinate oxidation by SDH [42].

FAO is a catabolic pathway which converts long-chain fatty acids into acyl-CoA [43]. Mitochondria can import fatty acids from the cytosol through carnitine palmitoyltransferase I (CPT1), which is located in the outer mitochondria membrane (OMM), and carnitine palmitoyltransferase II (CPT2), located in the IMM [44]. In the mitochondrial matrix, acyl-CoA is oxidized into acetyl-CoA, fueling the TCA cycle. Moreover, FAO is not only important for energy production because it is also involved in T cell and macrophage fate decision, as will be discussed below [45–48]. To investigate FAO pathway, researchers widely use the CPT1 irreversible inhibitor etomoxir. However, caution is needed in interpreting results that use this drug since high doses of etomoxir also inhibit complex I of the ETC and deplete free CoA [49–51].

Further, amino acid metabolism, especially glutamine metabolism, is critical for immune cell development and response [38]. Leukocytes can use glutamine in the cytoplasm to divert glucose from glycolysis to HBP, as previously mentioned, or through glutaminolysis to fuel the TCA cycle [5]. Moreover, glutamine is used by immune cells to provide considerable amounts of NADPH [52]. Usually, the canonical pathway to generate NADPH is via the pentose phosphate pathway (PPP) [53,54]. This pathway can occur in parallel with aerobic glycolysis depending on glucose-6-phosphate (G6P) availability. When glucose is converted into G6P during glycolysis, carbon flux can be directed via glucose-6-phosphate dehydrogenase (G6PD) to the PPP, reducing $NADP^+$ into NADPH, and producing nucleotides, nucleic acids, and amino acids precursors [54–56].

Our understanding of the involvement and requirement of these different metabolic pathways in immune cells were facilitated by studies involving isotopically labeled metabolites by tracking the carbon flux into different intermediates under both physiological and pathological states.

3. Mitochondria: the central hub in innate and adaptive immune cells

Mitochondrion is a double-membrane-bound organelle. The OMM contains multiple proteins, such as mitochondrial voltage-dependent anion channel (VDAC), also called mitochondrial porin [57], mitochondrial antiviral signaling protein (MAVS) involved in virus recognition [58], regulators of mitochondrial dynamics, such as mitofusin1 and 2 (Mfn-1 and 2) [59,60], and antiapoptotic proteins, such as B-cell lymphoma 2 (Bcl-2) [61,62], among others [63,64]. The main role of VDAC is to facilitate the diffusion of small molecules and ions through the OMM, serving as a connection between the mitochondria and the cytosol. VDAC participates actively in necrotic and apoptotic cell death processes, which involve changes in mitochondria membrane permeability [57]. Thus, OMM can be seen as a signaling platform that directly connects the mitochondria with other molecules present in the cell. The IMM, in turn, is impermeable to the majority of small molecules and ions. It contains the ETC and other membrane transporters [41]. The mitochondrial matrix is enclosed by the IMM, has a viscous consistency and contains mtDNA, ribosomes, nucleotides and soluble enzymes [65].

Mitochondria are also involved in cell death. In addition to apoptosis and necrosis, mitochondria participate in pyroptosis, another inflammatory type of cell death that is dependent on inflammasome activation [66]. The inflammasome is a cytosolic high-molecular-weight protein complex that is able to sense pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). The most well-characterized inflammasome is the nucleotide-binding oligomerization domain-like receptor pyrin domain-containing-3 (NLRP3) [67]. mtDNA released into the cytosol is a DAMP that induces inflammasome oligomerization and activation in macrophages and T cells [68]. This activation leads to the cleavage of pro-interleukin (IL)-1β and 18 to their active forms, leading to pyroptosis [69]. mtDNA can also be released extracellularly as web-like structures by T and B lymphocytes, and neutrophils [70,71]. These webs boost the antiviral defense or trap and kill larger pathogens. The mechanisms by which these cells release mtDNA will be described below.

4. Mitochondrial dynamics

Mitochondria are dynamic organelles that are constantly

undergoing fission or fragmentation, fusion or elongation, and trafficking [72]. These processes are in accordance with cell energy necessity, in other words, low-proliferative cells and decreased the production of ATP by OXPHOS are associated with mitochondrial fission, and the opposite for mitochondrial fusion [73]. In contrast, M2 macrophages have elongated mitochondria [73]. A similar differential pattern is observed for memory T lymphocytes (fused mitochondria) and activated T lymphocytes (fragmented mitochondria) [74]. This indicates that mitochondrial dynamics can be regulated by and also regulates the polarization status of immune cells.

The four major proteins involved in mitochondrial dynamics are members of dynamin-like guanosine triphosphatases (GTPases), which include: Mfn1 and 2, optic atrophy 1 (Opa1) and dynamin-related protein 1 (Drp1) [75]. Mfn1 and Mfn2 are localized to the OMM, and Opa1 is localized to the IMM [75]. Mitochondrial fusion is mediated by the tethering of two mitochondria. Mfn 1 and 2 mediate the fusion of the two OMMs, followed by Opa1-dependent IMM fusion [76]. Mitochondria fusion occurs under starvation conditions or higher OXPHOS activity. This increases cristae formation and provides more surface area for OXPHOS and FAO.

On the other hand, nutrient excess, severe stress, and impaired OXPHOS lead to mitochondrial fission [74]. In such conditions, the cytosolic Drp1 is dynamically recruited to the OMM, where it binds to anchor proteins in the mitochondrial surface including mitochondrial fission factor (MFF) and the mitochondrial dynamics proteins 49 and 51 (MiD49 and MiD51) [72]. The binding of Drp1 with these proteins cause Drp1 oligomerization and the subsequent formation of a ring-like structure around the mitochondria, which leads to the narrowing of the membrane [76]. The mechanism by which each immune cell type trigger changes in mitochondria dynamics will be discussed later in this review.

The cell function is also influenced by the mitochondrial distribution within cells and this organization depends on their interaction with the cytoskeleton and molecular motors [75]. The movement in the cell depends on miro-anchoring proteins in the OMM that interact with molecular motors (kinesin or dynein) in the microtubules [72]. The trafficking of mitochondria increases the chances for random mitochondrial interactions [72]. Furthermore, we believe that the size of mitochondria may impact mitochondrial transport across the cell. In this sense, small mitochondria can be transported easier than larger ones.

5. Oxidants production: a double-edged sword

Oxidants in immune cells can be produced by NADPH oxidase (Nox), mitochondria and peroxisomes and are essential for antibacterial host defense and signaling pathways [77,78]. Nox is a multimeric protein complex present in phagocytes (neutrophils, macrophages, and DCs). After cell activation, cytosolic NADPH is recruited to the plasma or phagosome membranes, thus allowing Nox assembly and activation. An oxidative burst is produced when molecular oxygen is oxidized by activated Nox into the radical anion superoxide $O_2^{\bullet-}$. This is a crucial event for the elimination of engulfed pathogens by phagocytes [79]. There are several members of the Nox family [80]. Nox1 is most common in colon epithelial cells.

Nox2 is the most abundant and main isoform in immune cells, Nox4 is found mainly in fibroblasts and vascular cells, and Nox5 is regulated by Ca^{2+} and is expressed in spleen, kidney, vascular cells and lymphoid tissue [79].

Peroxisomes are ubiquitous organelles primarily involved with lipid metabolism, FAO and fatty acid synthesis (FAS) [81,82]. Peroxisomes are one of the main sources of oxidants, mainly H_2O_2, and are intimately related to mitochondrial physiology and structure through peroxisome-mitochondrial communication via vesicular trafficking, intraorganellar diffusion or physical contact [83,84]. At the same time, peroxisomes have the complete machinery to maintain cellular redox balance. Besides peroxisomes, mitochondria are also crucial sources of oxidants [85,86]. mtROS are produced from the leakage of electrons in the ETC, mainly at complexes I and III [10], although complex II can also produce $O_2^{\bullet-}$ [10]. Complex I and II produce mtROS only in the mitochondria matrix, but complex III produces mtROS in both the matrix and intermembrane spaces [10,87,88]. $O_2^{\bullet-}$ is mostly converted into H_2O_2, which can be further converted into hydroxyl radicals ($HO^{\bullet}$) [89]. In this reaction, transition metal ions, such as iron (Fe) or copper (Cu), can catalyze the formation of $HO^{\bullet}$. This reaction was proposed by the physical chemist, Fritz Haber and his student, Joseph Weiss, in 1934. The Haber–Weiss reaction (catalyzed by iron) describes how highly reactive $HO^{\bullet}$ is generated in live organisms [90]:

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^- \text{ (Fenton reaction)}$$

$$O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + HO^{\bullet} + OH^-$$

Hydroxyl radicals are very unstable and highly reactive [91] and can damage lipids, proteins, and DNA in the cells [92]. $O_2^{\bullet-}$ and H_2O_2 are more stable and less reactive compared to hydroxyl radicals but can also damage cellular components [92]. These oxidants directly kill pathogens by causing oxidative damage to biomolecules or by stimulating pathogen elimination via non-oxidative mechanisms, including the activation of pattern recognition receptors, autophagy, web-like structures formation in neutrophils (neutrophil extracellular traps – NETs), and T-lymphocyte responses [93].

Another important oxidant with a potential role as a microbicidal agent is hypochlorous acid (HOCl). Myeloperoxidase (MPO) promotes the formation of HOCl in phagosomes from H_2O_2 in the presence of chloride anion (Cl^-). MPO is a peroxidase enzyme present at high levels in primary granules of neutrophils [94–96]. HOCl is a potent oxidant and, is highly cytotoxic [94–96].

Since these reactive oxygen species are toxic to cells, antioxidant pathways have evolved to neutralize the molecules. The most common antioxidant machinery in immune cells is comprised of glutathione peroxidases (GPx), superoxide dismutases (SOD), and catalase [97]. First, $O_2^{\bullet-}$ is converted to H_2O_2 through copper zinc-dependent SOD (CuZnSOD) activity or manganese-dependent SOD (MnSOD) [97]. Likewise, the mitochondrial-produced $ONOO^-$ can be neutralized by MnSOD. Subsequently, H_2O_2 is converted into H_2O by catalase or in a GPx-dependent manner [97]. Due to the lack of catalase in the mitochondria, the conversion of H_2O_2 into H_2O is dependent on GPx and mitochondrial glutathione (mtGSH). Once GSH is the substrate to GPx, mtGSH is oxidized by GPx to generate oxidized glutathione (GSSG) [97,98]. At the same time, GPx neutralizes H_2O_2 generating H_2O. mtGSH also neutralizes $ONOO^-$/ONOOH and $^{\bullet}NO_2$. Later, GSSG is converted into its reduced form, GSH, by the NADPH-dependent GSSG reductase. GSH is a tripeptide synthesized in the cytosol, and can also be found in the ER, nucleus, and mitochondria [97,99]. Its concentration in the mitochondria is similar to that in the cytosol (10–14 mM) [97,99,100], allowing the detoxification of the oxidants produced during mitochondrial respiration and avoiding mitochondrial damage. In addition to its function as a major antioxidant, GSH can also play a role in immune cell reprogramming, proliferation, and activation [97,101–103]. A recent study showed that GSH can regulate glycolysis and support glutamine metabolism, essential to T cell proliferation [101].

Another class of oxidants involved in both cell damage and immune cell fate decision are the reactive nitrogen species (RNS). Important RNS are nitric oxide ($NO^{\bullet}$), peroxynitrite ($ONOO^-$) and nitrogen dioxide radical ($^{\bullet}NO_2$). $NO^{\bullet}$ is enzymatically synthesized during the conversion of L-arginine into L-citrulline by NO synthase (NOS) [104,105]. There are three distinct cellular isoforms of NOS: endothelial (eNOS), neuronal (nNOS), and inducible NOS (iNOS) ([106]. $NO^{\bullet}$ can react with $O_2^{\bullet-}$ and form $ONOO^-$, a strong oxidant and nitrating agent that can diffuse

from the cytosol to the mitochondria [107,108]. $ONOO^-$ can react with important mitochondrial components causing oxidation, nitration or nitrosation of proteins. This leads to caspase activation and, consequently, apoptosis [109]. In addition, $ONOO^-$ reacts with carbon dioxide, generating $^{\bullet}NO_2$ or spontaneously decomposes to $^{\bullet}NO_2$ and $HO^{\bullet}$. $ONOO^-$ also reacts with a hydrogen ion (H^+), generating peroxynitrous acid (ONOOH). Similar to reactive oxygen species, the RNS are toxic to the cells if produced in excess and must be neutralized by complex antioxidant machinery. One component of this machinery is an important antioxidant enzyme called peroxiredoxin-5 (Prx-5). This thiol-dependent monomeric peroxidase has an important role in inflammation [84,110]. Prx-5 has cytoprotective function against endogenous and exogenous peroxides, especially alkyl hydroperoxides and $ONOO^-$, and also H_2O_2 [110]. The expression of Prx-5 in macrophages and microglia increases after pro-inflammatory stimulation [111,112]. In microglia, oxidants are important agents for their activation and therefore have an important role in neurodegenerative diseases [111]. Park et al. showed that Prx-5 is a negative regulator of excessive mitochondrial fission because Prx-5 inhibits the dephosphorylation of Drp-1 that is induced by the Ca^{2+}/calcineurin pathway [111].

In this sense, the synthesis of oxidants in immune cells is crucial not only to host defense but also for cell signaling, function, and fate decision. Likewise, the neutralization of oxidants is vital to avoid mitochondria and cell damage.

6. Mitochondrial dysfunction and mitophagy

Mitochondrial dysfunction is commonly coupled with the loss of ETC efficiency, resulting in decreased ATP production [113]. However, abnormalities in any mitochondrial process, such as generation and detoxification of mtROS, apoptosis, and decrease in mitochondrial calcium uptake from the cytosol can cause mitochondrial dysfunction [113]. Mitochondrial dysfunction can activate the NLRP3 inflammasome in immune cells [114]. This activation is induced by mtROS or mtDNA, which promotes the secretion of IL-1β and cell death by pyroptosis [115]. Thus, impaired mitochondria function is closely linked to inflammation, neurodegenerative disorders and cancer development [116,117]. One mechanism by which cells prevent mitochondria dysfunction is through mitophagy [118]. This process is a specific form of macroautophagy and involves the removal of damaged mitochondria to maintain the overall mitochondria functionality [118,119]. Mitophagy is crucial because mitochondria are one of the main sources of oxidants and, consequently are overexposed to oxidant-mediated damage [120]. This specific type of autophagy is regulated by two main proteins, PTEN-induced putative kinase 1 (PINK1), localized on the OMM, and Parkin, an E3-ubiquitin ligase localized in the cytosol [121]. After activation, Parkin mediates the polyubiquitination of proteins associated with the OMM, such as Mfn-1 and 2. This results in mitochondria sequestration by the autophagosome [122–125]. Autophagosome engulfs the mitochondria and fuse with lysosomes to degrade damaged mitochondria [118]. This process prevents exacerbated activation of inflammatory signaling pathways [118,126]. The importance of mitochondria integrity and functionality is notable and can be seen by the variety of diseases related to mitochondria dysfunction, such as neurodegenerative (Parkinson, Alzheimer and Huntington's disease), cardiovascular (cardiomyopathy, cardiac hypertrophy) liver diseases (diabetes, fatty liver disease) and cancer [118,126]. Thus, mitophagy is a cell survival mechanism crucial to prevent non-functional mitochondria causing cellular disorders by eliminating dysfunctional mitochondria.

7. Neutrophils

Neutrophils are quick-responder cells during the inflammatory response and belong to the so-called innate immune system. They sense pathogens and quickly respond to stress situations [127]. Chemoattractants induce the migration of neutrophils into inflammatory sites by a series of molecular mechanisms that coordinate cell chemotaxis, involving cytoskeleton and cell membrane rearrangement [128]. Once in the site of inflammation, they recognize, phagocytose and kill microorganisms through different mechanisms, such as degranulation, oxidant production or releasing NETs [129].

Recently, the importance of neutrophils beyond the acute phase of inflammation has become evident. Neutrophils can modulate the function of cells of both innate and adaptive immunity by secreting soluble mediators or via cell-cell contact [128]. As a result, interest in neutrophil metabolic requirements has grown. However, so far, little is known regarding the metabolic profile of neutrophils as compared to other immune cells. A growing body of evidence suggests that neutrophils contain few mitochondria and are purely glycolytic [130–133]. In contrast, Riffelmacher and colleagues observed that autophagy is crucial for neutrophil maturation since it limits glycolysis and increases OXPHOS [134]. Autophagy is intimately related to neutrophil metabolism and differentiation [134–136]. By using genetically modified mice lacking Atg7 (an essential autophagy protein) in hematopoietic precursors, neutrophils showed an immature phenotype. During granulopoiesis, neutrophils undergo a metabolic shift from glycolysis to free fatty acids (FFAs)-dependent OXPHOS and this metabolic reprogramming was not observed in Atg7-deficient neutrophils [134]. Thus, since the autophagy can promote lipid breakdown through lipolysis to provide FFAs to feed OXPHOS, Atg7-deficient neutrophils accumulate lipid droplets and have reduced FFA levels, remaining immature (Fig. 1A) [134]. Although a previous study suggested that autophagy is not essential for neutrophil maturation [135], Riffelmacher and colleagues comment that difference in the results from the two studies may be related to the use of different mouse strains [134].

Another recent study reinforces that aerobic glycolysis is the preferable metabolic pathway for mature neutrophils [137]. Rice and colleagues showed that immature subsets of neutrophils ($Ly6G^{int}$, $c\text{-}Kit^+/CXCR2^-$) have a higher number of mitochondria and a higher OXPHOS rate after stimulation with phorbol 12-myristate 13-acetate (PMA) or after treatment with a competitive inhibitor of glucose, 2-deoxy-D-glucose (2DG). 2DG competes with glucose to enter into the cells and inhibits the hexokinase, the first enzyme in the glycolysis pathway [137]. Interestingly, cancer patients often have immature subsets of immune cells in the peripheral blood and their neutrophils have increased mitochondrial content and OXPHOS activity [137]. In this report, the authors also showed that oxidative neutrophils use mitochondria to maintain intracellular NADPH levels via FAO and, consequently, the increased production of oxidants by Nox2 (Fig. 1A) [137]. In this context, these oxidative neutrophils could promote tumor progression by inhibiting T cells due to excessive oxidant production, especially in a microenvironment where glucose content is limited [137].

Usually, glycolytic neutrophils produce $O_2^{\bullet-}$ by Nox2 via PPP [53,54]. In neutrophils, other oxidants generated in the phagosome, mainly H_2O_2 and HOCl, are crucial to kill phagocytosed microorganisms [138]. HOCl, as previously mentioned, is a highly reactive product generated by MPO during the oxidative burst. However, oxidants and MPO are not only related to the oxidative burst. They are also essential to NET formation [139]. This most recent neutrophil effector function was uncovered in 2004 by Brinkmann and coworkers when they observed that neutrophils died in a particular way after bacterial stimulation [139]. Classically, NETs are composed of chromatin fibers with intracellular enzymes/proteins - MPO, elastase, and histone (Fig. 1B) [139]. This effector function can immobilize and kill extracellular pathogens or prevent the spreading of microorganisms [139]. The release of NETs occurs by decondensation of nuclear chromatin and the disintegration of nuclear envelope culminating with cellular membrane rupture and extravasation of intracellular content of the neutrophil. This specific type of cell death in neutrophils is called NETosis [140]. It differs from apoptosis and necrosis by its caspase independence,

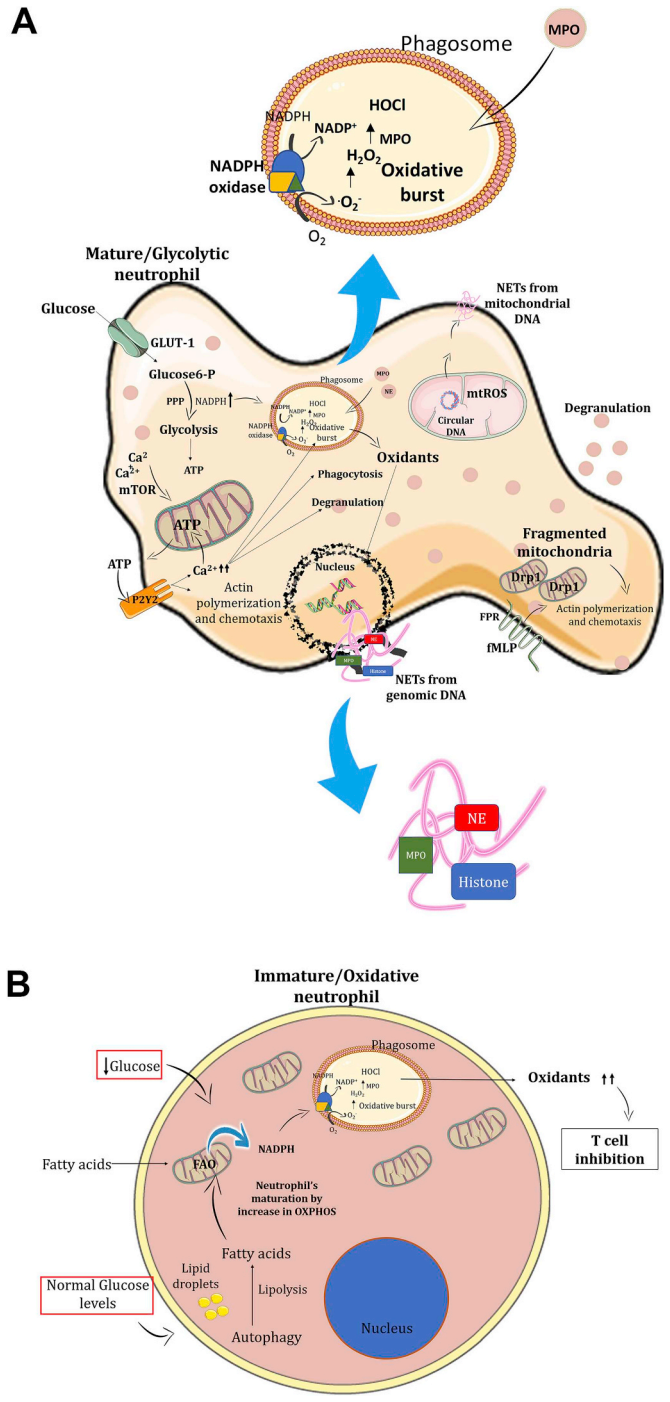

Fig. 1. *Immature and mature neutrophils have different metabolic requirements*. A. In the presence of normal glucose levels, immature neutrophils undergo autophagy to a proper maturation through FAO associated with OXPHOS. In the same way, during a low glucose levels context (tumoral environment), immature neutrophils prioritize FAO associated with OXPHOS to increase NADPH levels and consequently oxidants production. Excess of oxidants leads to T cell inhibition, contributing to tumor growth. **B**. Mature neutrophils prioritize aerobic glycolysis and generate NADPH from PPP. NADPH is the Nox2 substrate to produce oxidants inside the phagosome and crucial to eliminate phagocyted pathogens. Oxidants from Nox2 are also important to NETs formation from genomic DNA while mtROS are important to mitochondrial NETs production. Intracellular Ca^{2+} levels stimulate mitochondrial ATP production by the mTOR pathway. The ATP acts in an autocrine way by binding the P2Y2 receptors in the neutrophils stimulating intracellular Ca^{2+} mobilization and chemotaxis. Mitochondrial fission also contributes to neutrophil chemotaxis.

morphological characteristics (NETosis involves plasma membrane rupture, while apoptosis forms membrane blebs) and dependence on oxidants [139,141]. So far, two distinct types of NETosis have described: Nox-dependent and Nox-independent pathways [142]. Nox-dependent NETosis is the classical type of NETs release and is well described after PMA stimulation *in vitro* [143]. The process starts 1 h after PMA stimulation and requires oxidants production by Nox2. Nox-independent NETosis pathway requires mtROS generation [139,144,145] and an increase in intracellular calcium concentration [142,146,147]. Douda and colleagues observed that calcium ionophore-induced NETosis is rapid (occurs in less than 1 h), is NADPH-oxidase independent, is mediated by small conductance of calcium-activated potassium channel 3 (SK3) and relies on mtROS production [142]. Due to the exacerbated increase in intracellular Ca^{2+} concentrations (induced by calcium ionophores, for instance), mitochondria produce elevated mtROS levels, which trigger NET formation in the absence of Nox2-derived oxidants [148]. Importantly, in both types of NETosis described above, cellular membrane rupture and neutrophil death occur [139,141,142]. However, a different type of NETs release was suggested by Youssef and colleagues [71]. Using confocal microscopy, they showed that neutrophils stimulated with granulocyte-macrophage-colony-stimulating factor (GM-CSF) and complement component 5a (C5a) remain alive after NETs release [71]. They claim that it is because the chromatin source is not nuclear but mitochondrial [71]. They also demonstrate the dependence of oxidant production for generating mitochondrial NETs as well as in classical NETosis (Fig. 1B) [71]. Recently, the same authors showed that Opa1 is required for ATP production through aerobic glycolysis in neutrophils [149]. Mitochondria-derived ATP is important for microtubule network formation, which is crucial to NETs formation [149]. This suggests that Opa1 is required to release NETs [149].

Regarding the metabolic requirements for NETs release, several studies have shown that NET formation and release is an aerobic glycolysis-dependent process [150,151] and any manipulation that disrupts glycolysis inhibits NETs release. In 2014, Rodríguez-Espinosa et al. suggested a metabolic diversity to NET formation: the early phase, that comprises chromatin decondensation, is not strictly dependent on exogenous glucose. However, exogenous glucose and the aerobic glycolysis are necessary for the late phase that comprises the release of web-like structures [151].

Although mitochondria and cell metabolism play a role in NETs release, they are also important in well-described neutrophils functions, such as phagocytosis, degranulation, and chemotaxis. Recently, Bao and colleagues demonstrated that mitochondria-derived ATP is transported extracellularly and activates purinergic receptors, such as P2Y2, in an autocrine manner, resulting in neutrophil activation [152,153]. This activation is mediated by an increase in intracellular Ca^{2+} levels leading to an amplification of mitochondrial ATP production [152,153]. Increased ATP production provides positive feedback of ATP binding to P2Y2 and sustains the neutrophil oxidative burst, degranulation, and phagocytosis (Fig. 1B) [152,153]. Mitochondrial ATP burst can be regulated by the mammalian target of rapamycin (mTOR) signaling pathway, which controls mitochondrial Ca^{2+} uptake [153]. The inhibition of mTOR complex 1 (mTORC1) or both mTORC1 and mTORC2 limits mitochondria-derived ATP production and consequently neutrophil chemotaxis [153]. Recently, a study using a zebrafish model indicated that a mitochondrial network plays an indispensable role in the regulation of neutrophil motility *in vivo* [154]. Using a transgenic zebrafish lineage, they disrupted the mtDNA polymerase specifically in neutrophils and observed a reduced velocity in neutrophil interstitial migration [154]. One of the consequences of mtDNA polymerase dysfunction is the loss of the ETC proteins that are encoded by mtDNA. To demonstrate that this is due to the loss of ETC function they used specific inhibitors to disrupt the mtROS production, such as rotenone (complex I) and antimycin (complex III) and observed inhibition of neutrophil motility [154]. Consistent with the

participation of mitochondria in neutrophil chemotaxis, another research group suggested that mitochondrial dynamics are involved in neutrophil's actin polarization and chemotaxis [155]. They showed that the activation of the mitochondrial calcium uniporter (MCU), a well-known Ca^{2+} transporter located in the IMM, enhances the polarization and chemotaxis of neutrophils stimulated by N-formylmethionine-leucyl-phenylalanine (fMLP) or IL-8. In the same way, Ru360, an MCU inhibitor, decreases and even abrogates neutrophil polarization and chemotaxis [155]. Analysis of mitochondrial morphology using a MitoTracker® Red probe coupled confocal analysis showed that in fMLP-stimulated neutrophils the mitochondrial anatomy changed from elongated/fused (unstimulated) to fragmented [155]. The treatment of neutrophils with Ru360 prevented mitochondria fragmentation and, consequently, neutrophil polarization and chemotaxis [155]. Treatment with the Drp1 inhibitor MDIVI-1 abrogated neutrophil polarization and chemotaxis, confirming the role of mitochondrial dynamic in these mechanisms (Fig. 1B) [155].

Neutrophils modulate their own metabolism in accordance with environmental cues. Recent studies have highlighted the importance of mitochondria in several neutrophils functions, including chemotaxis. Certainly, there is much more to be discovered but these recent studies using novel technologies have enhanced our understanding of the immunometabolism of these cells.

8. Macrophages

Macrophages are immune cells derived from the yolk sac and fetal liver during the early stages of development and from bone marrow-derived monocytes after birth [156]. The majority of the adult tissue macrophages originate from embryonic precursors [156]. However, during immune responses bone marrow-derived monocytes can migrate from the blood into the tissues, where they acquire specific macrophage profiles and intermingle with resident macrophages to remove the foreign material, depending on the cytokine milieu found in the inflammatory microenvironment [157]. The majority of the studies focusing on macrophage immunometabolism have been done *in vitro* using macrophage colony-stimulating factor (M-CSF or CSF-1) to differentiate bone-marrow progenitor cells into macrophages (also known as M0). Differentiated macrophages can be divided into two important classes. (i) M1 or classically activated macrophages are associated with tissue proinflammatory responses and microbial killing. They are induced *in vitro* by treating M0 macrophages with LPS and interferon-γ (IFN-γ). (ii) M2 or alternatively activated macrophages are associated with resolution of inflammation, wound healing and resistance to helminth infections [158–160]. They are induced *in vitro* by IL-4.

8.1. M1 macrophages

During tissue injury, foreign molecules or pathogens (bacteria, virus or toxins) are recognized by resident innate cells (mainly dendritic cells and macrophages). This leads to the secretion of cytokines and chemokines to recruit additional inflammatory monocytes from the blood. These monocytes recognize infectious microorganisms-related molecules (e.g., LPS) and inflammation-related cytokines (TNF-α, IL-1β and/or IFN-γ) in the milieu, and then differentiate into M1 macrophages. These cells produce inflammatory cytokines (IL-1β, TNF and IL-6) and oxidants (NO and ROS). One important marker of M1 activation *in vitro* is an increase in iNOS and consequently production of NO (see Oxidants production: a double-edged sword). Another important source of oxidants is the mitochondria recruitment to phagosomes to promote the delivery of oxidants to augment the bactericidal activity of macrophages [161].

Although the proinflammatory profile of M1 macrophages was characterized decades ago, only recently have the metabolic pathways involved in this activation been examined. One of the firsts established findings was that M1 macrophages have increased glucose uptake, mostly by increasing the transcription and translocation of glucose transporter 1 (GLUT1) to the plasma membrane [162,163]. The uptake of glucose is associated with increased glycolytic flux. However, interestingly, M1 macrophages use glycolysis to produce lactate, a process named aerobic glycolysis [164]. The importance of glycolysis for M1 macrophages has been presented in several reviews [see Ref. [165]], therefore we will focus on the mitochondrial aspects involved in classical activation.

Although most of the pyruvate is converted to lactate, a portion goes to the mitochondria to feed the TCA cycle. However, in M1 macrophages the metabolic intermediates citrate and succinate accumulate due to two interruptions in enzymatic reactions resulting in breaks in the TCA cycle. The breaks are a consequence of downregulation of both isocitrate dehydrogenase (IDH) and SDH. The accumulation of citrate was identified as a consequence of downregulation of IDH, the enzyme that catalyzes the conversion of isocitrate to α-ketoglutarate [166]. This metabolite can be transported to the cytosol, where will be used for the production of oxidants, prostaglandins and fatty acids [42,167]. Alternatively, it can be used to synthesize itaconate [167], a metabolite with antibacterial properties described in the 1970s [168]. However, recently it has been shown that following LPS stimulation itaconate can leave the mitochondria and modify the Kelch-like ECH-associated protein 1 (KEAP1) via alkylation. This enables the nuclear factor (erythroid-derived 2)-like-2 factor (Nrf2) to initiate an anti-inflammatory program and induce the expression of anti-oxidant genes [169]. It is interesting to observe that itaconate acts as a negative-feedback loop in the inflammation induced by LPS, acting mainly in late time points. Furthermore, itaconate can also inhibit SDH, leading to decreased fumarate levels and succinate accumulation (the second break) [161]. It is important to point out that during M1 polarization, the main source of succinate is glutamine-dependent anaplerosis [41]. As previously discussed, when succinate is in the cytosol, it attenuates the degradation of HIF-1α by prolyl hydroxylases. Macrophages treated with succinate have increased HIF-1α levels, enhancing its migration into the nucleus resulting in increased transcription of IL-1β and glycolytic genes, such as the Ldh subunit a, pyruvate kinase isozyme M2 (Pkm2), hexokinases I and II, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 enzyme (Pfkfb3) [38,41,42] (Fig. 2A).

Upon M1 stimuli, the protons that are pumped out from complexes I, III and IV of the ETC are not used to produce ATP by ATP synthase [170]. Interestingly, the activity of complex I, II and IV are decreased during M1 polarization, leading to an increase in the $\Delta\Psi m$ [170,171]. The consequence of complex II (SDH) suppression is the accumulation of succinate along with high proton motive force that leads to a reversal on the electron flow at complex I, in a process referred to as reverse electron transport (RET) [37] (Fig. 2A). Succinate accumulation and RET leads to an increased production of oxidants, which is known to stabilize HIF-1α [170]. Similar to the effects observed with itaconate treatment, pharmacological inhibition of SDH using dimethyl malonate (DMM) blocks RET mtROS production, HIF-1α stabilization and induction of IL-1β, while increasing anti-inflammatory gene expression [170]. Furthermore, a study using M1 macrophages with a specific knock-out of immune responsive gene 1 (Irg1), a mitochondrial-associated enzyme that converts *cis*-aconitate to itaconate, observed more HIF-1α stabilization, and production IL-1β than in the M1 from WT mice [42]. These results raise the possibility that HIF-1α - IL-1β axis is a consequence of the efficiency and directionality of the ETC rather than signaling through succinate accumulation.

Although the majority of the literature has used macrophage cell lines or bone marrow-derived macrophages to describe the interplay between metabolism and macrophage function, the extent to which these findings translate into the diverse spectrum of macrophage phenotypes *in vivo* remains to be defined. Artyomov et al. presented an extensive comparison between M1 bone marrow-derived macrophages and peritoneal macrophages [69]. Both macrophages are highly glycolytic upon LPS treatment [69]. However, peritoneal macrophages

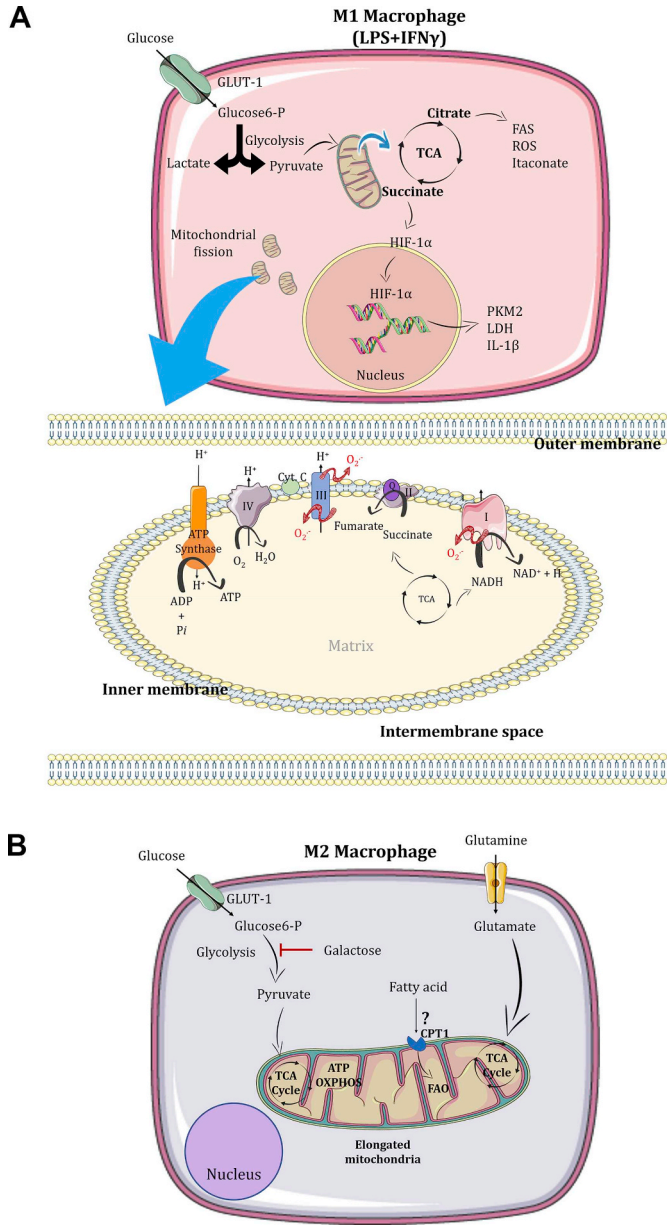

Fig. 2. *Metabolism of macrophages*. A. Under LPS and IFN-γ stimuli, macrophages preferentially use glucose to produce lactate. In the mitochondria, citrate can be exported and used to produce fatty acids and itaconate. The mitochondria increase the production of oxidants and succinate, which are capable to stabilize the transcription factor HIF-1α in the cytosol, triggering the transcription of HIF-1α-target genes. The M1 macrophage is characterized by mitochondria fission. B. *Metabolism of M2 macrophages.* Alternatively activated macrophages (M2) use glucose and glutamine to feed the TCA cycle and produce ATP by oxidative phosphorylation. These cells are known to have elongated mitochondria, and consequently more efficient energy production. The use of fatty acid oxidation and glycolysis by M2 macrophages are under review in the literature.

also display increased OXPHOS activity, using the high levels of acetyl-CoA available to supply the TCA cycle. This is not observed in bone marrow-derived macrophages [69].

8.2. M2 macrophages

The induction of M2 macrophages is observed under several conditions, such as helminthic infection and allergy. In addition, when a tissue injury occurs, these cells are responsible for the resolution phase. They mediate wound healing by the secretion of anti-inflammatory cytokines and by promoting the migration and proliferation of matrix cells, in a mechanism not totally understood [172]. In each response mentioned above, groups of immune cells (e.g. eosinophils, mastocytes, and T helper 2 cells) secrete type 2 cytokines, such as IL-4, and IL-13. It was recently established that IL-4 and M-CSF drive mTORC2 activation and phosphorylation/activation of the signal transducer and activator of transcription 6 (STAT6), which in turn induces interferon regulatory factor 4 (IRF4) and STAT6 M2 markers [173].

The initial metabolism studies described that M2 macrophages prefer FAO to fuel mitochondrial activity [174,175]. However, this concept was recently challenged by investigations describing that FAO is dispensable for M2 macrophage polarization [49,176]. Importantly, researchers frequently use 200 μM of etomoxir to inhibit FAO [49]. At this high concentration, etomoxir was shown to also affect adenine nucleotide translocase (an enzyme that catalyzes ADP/ATP exchange across the IMM) and complex I of the ETC. In addition, excess etomoxir leads to the formation of etomoxiryl-CoA, which depletes intracellular free CoA levels, thus blunting cell metabolism [49]. At low concentrations (< 3 μM), etomoxir still inhibits CPT-1 but does not affect these other metabolic processes. Divakaruni et al. also showed that in macrophages lacking both CPT1 and CPT2 proteins, etomoxir did not affect M2 polarization, indicating that FAO is dispensable for M2 polarization [49] (Fig. 2B). We speculate that M2 macrophages use FAO to maintain their metabolism after polarization. This recently published data started a new chapter in M2 macrophages polarization and metabolism and other groups will need to investigate the real necessity of FAO for M2 polarization and review the concentrations of classical modulators and their off-targets effects.

The second wave of studies showed that glucose can also fuel OXPHOS in M2 macrophages. Interestingly, after 16–24 h of stimulation with IL-4, the glucose consumption is increased in these macrophages. In 2016, two important studies showed that different subunits of mTOR are important for the upregulation of glucose. Covarrubias et al. showed that IL-4 induces an increase in glucose uptake in a time-dependent manner, in which AKT and mTORC1 act in parallel to support this change and are essential regulators of M2 markers. Huang et al. showed that mTORC2 and IRF4, but not mTORC1, are responsible to enhances glucose utilization in M2 macrophages [173,177]. To test this hypothesis, these two groups and the majority of the studies in the literature have used 2DG as a readout for glycolysis inhibition [173,177]. After these studies, Wang et al. showed that the doses of 2DG used also have important off-target effects, altering not just glycolysis, but also OXPHOS and ATP levels [178]. They showed that pretreatment of bone marrow-derived macrophages with 2DG blocks the STAT6 signaling pathway induced by IL-4, which is required for M2 macrophage polarization [178]. To better understand the effects of glycolysis inhibition, the authors used macrophages cultured with IL-4 in either glucose-free medium or containing galactose instead of glucose as the main carbon source (which render glycolysis functionally ineffective, with no ATP produced). They observed that the absence of glycolysis does not affect the expression of M2 markers, OXPHOS, and intracellular ATP levels [178]. It is important to point out that glutamine can fuel the TCA cycle, and it has been shown that under glucose depletion or the presence of galactose, M2 macrophages utilize this metabolite [178]. These results indicate that both aerobic glycolysis and glucose-derived acetyl-CoA are not required for M2 polarization (Fig. 2B). Further, they highlight the importance of cautious data interpretation with the use of 2DG in the literature regarding M2 polarized macrophages.

One of the most important functions of macrophages is the clearance of apoptotic cells. While the mechanisms related to phagocytosis of apoptotic cells are well known, Wang et al. demonstrated that mitochondrial fission occurs during this process. Drp1 is essential for degradation of the apoptotic cells in phagolysosomes [179]. Indeed, Escoll et al. used *Legionella pneumophila*, a Gram-negative intracellular

bacterium, to infect human macrophages and showed that the presence of type IV secretion system triggers mitochondrial fission in a mechanism that is dependent on Drp1 [180]. Although the range of stimuli in macrophages is extensive, the literature has shown that M1 phenotype accumulates fragmented mitochondria and M2 macrophages elongated mitochondria [73] (Fig. 2).

8.3. Resident macrophages

The milieu in which macrophages are found can directly impact their metabolism. In the context of a tumor microenvironment, cancer cells produce large amounts of lactate, as the final product of aerobic glycolysis. Lactate induces HIF-1α expression and M2 polarization of macrophages thus promoting tumor growth [181]. Moreover, during obesity, adipocytes release pro-inflammatory factors like C-C motif chemokine ligand 2 (CCL2), TNF and FFAs that induce the recruitment and activation of adipose tissue macrophages [182]. Adipose tissue macrophages acquire a "metabolically activated" phenotype after high-fat diet-induced obesity [183]. This is accompanied by increased glycolysis and oxygen consumption in comparison to lean adipose tissue macrophages [184]. The production of proinflammatory factors by macrophages amplifies an inflammatory pathway that blocks insulin action in adipocytes, and consequently contributing to insulin resistance [185,186].

Although the metabolism of these distinct phenotypes of macrophages has been extensively studied, the metabolism adaptation of tissue-resident macrophages is still poorly understood. However, mitochondria occupy a central place during macrophage polarization, controlling metabolism and signaling pathways in this cell. Future studies are necessary to delineate how mitochondria contribute to the metabolism of these cells during activation.

9. Dendritic cells

DCs are specialized cells that recognize pathogens via pattern recognition receptors (PRRs), phagocytose microorganisms, process antigens and present antigens via major histocompatibility complex to activate T lymphocytes. The efficiency of these processes depends on the ability of DCs to migrate from the tissue to the tissue-draining lymph nodes through the lymphatic vessels [39,187]. DCs can be generated *in vitro* by treating human peripheral blood monocytes with GM-CSF and IL-4 or treating mouse bone-marrow-derived cells with only GM-CSF(39). Although there is a variety of DCs in tissues, the majority of the studies on DCs differentiation have been performed by using these *in vitro* approaches [39].

The activation of DCs with TLR agonists leads to increased glucose consumption via aerobic glycolysis [164,188]. It is important to point that, as in macrophages, the majority of the literature uses high doses of 2DG to inhibit glycolysis and therefore this glycolytic dependency will likely be reassessed in the next few years. However, it is known that the acetyl-CoA derived from glycolysis induces the production of citrate in the mitochondria. In DCs citrate is exported to the cytosol via mitochondrial citrate carrier Slc25a1 [189]. Once in the cytosol, citrate accumulation together with higher levels of PPP-derived NADPH increases FAS [39] (Fig. 3). FAS is required to fulfill the requirements of the endoplasmic reticulum and Golgi to support the high demand for protein synthesis and secretion [40].

Although some of the glucose is used to produce FA, DCs also use OXPHOS in the early stages of activation [190]. It is known that TLR activation of DCs have increased expression of iNOS and consequently production of NO [191]. However, NO is a potent inhibitor of the complex IV of the ETC [191], consequently decreasing OXPHOS-generated ATP. Interestingly, 24 h after stimulation, DCs have increased NO production, reduced TCA cycle activity and lower levels of oxygen consumption, but increased glycolytic capacity (Warburg metabolism) [191]. A recent finding showed that DCs have intracellular glycogen stores that support the early steps of TLR (one PRR subtype) activation [192]. In the first 3 h of activation, the breakdown of glycogen stores increases the oxygen consumption rate (OCR) and citrate accumulation in the cytosol to fuel FAS. After this early stage of activation, DCs decrease OXPHOS activity [191,192] (Fig. 3). Thus, the changes in DCs metabolism are supported by intracellular stores of glycogen associated with glucose import. The increase in NO production is also an essential step for the degradation of pathogens by DCs.

Despite the fact that OXPHOS is important for the early stages of activation, mitochondria have been showed to be essential for DCs differentiation. The inhibition of mitochondrial respiration in monocytes by the ETC inhibitor rotenone blunts DCs differentiation induced

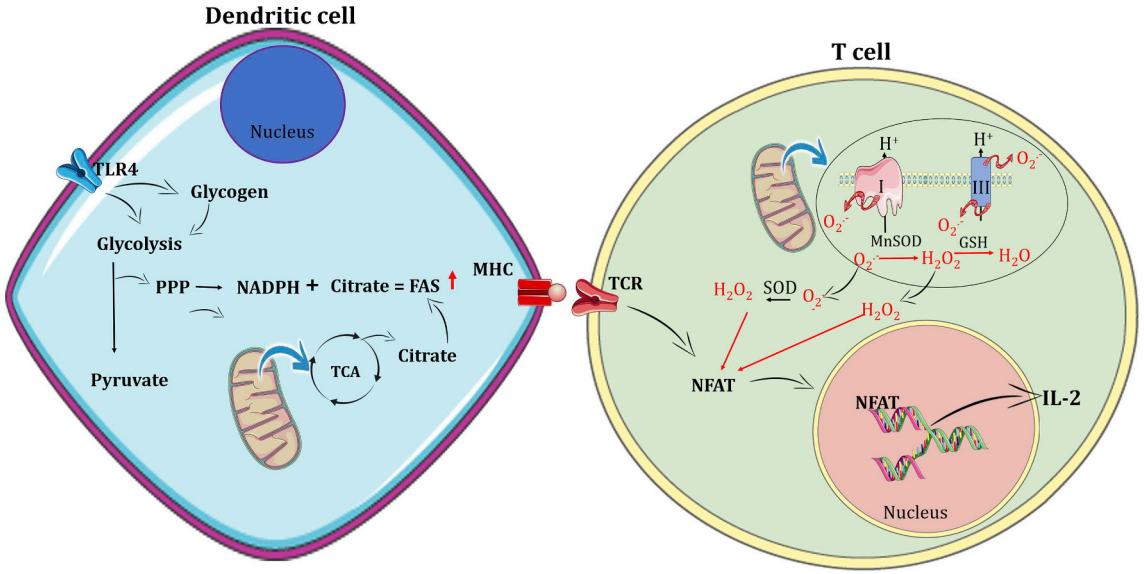

Fig. 3. *Metabolism of dendritic cells and T lymphocytes.* Inflammatory signals drive glycolysis, increasing glucose uptake and glycogenosis in DCs. The conversion from glucose to pyruvate increases citrate levels in the mitochondria, which can be exported to the cytosol. The PPP generates NADPH, used in the FAS. FAS are used to generate membranes to the endoplasmic reticulum and Golgi complex, in an NADPH-dependent manner, both necessary to increase protein synthesis. Dendritic cells process antigens and present them using the MHC at the cell surface where they can be recognized by the TCR. This event engages ROS production, which facilitates the activation of nuclear factor of activated T cell (NFAT) activation. This transcription factor induces IL-2, a T cell growth cytokine.

by GM-CSF [193,194]. One important aspect of DCs development is that during the initial stages (3 days after GM-CSF stimuli), DCs present lower levels of oxidants and are highly responsive to TLR stimulation as assessed by the upregulation of activation markers (such as CD80, CD88) and induction T cell proliferation upon antigen presentation [195]. In contrast, 6 days after GM-CSF treatment, DCs have higher levels of oxidants, increased release of H_2O_2 and decreased capacity to stimulate T cell [195]. This indicates that mitochondria have a central role during DCs differentiation [193,194].

In general, there are three major DCs subtypes: classical DCs, monocyte-derived DCs and plasmacytoid or tolerogenic DCs. These subtypes may have different metabolic demands [39,187]. Tolerogenic DCs, which are commonly found in tumors where they function to inhibit T cell function to favor tumor progression, use lipid metabolism to fuel OXPHOS and produce the necessary ATP [196,197]. In the same way, antigen presentation by plasmacytoid DCs is regulated by mitochondria through a ROS-dependent mechanism. The reduced mtROS levels in these cells decrease their cross-presentation capacity to generate $CD8^+$ T-cell responses *in vivo* [198].

Little is known about mitochondrial dynamics in DCs. Ryu et al. showed that GM-CSF-induced immature DCs have increased levels of Mfn2 and Opa1 [199], suggesting that before differentiation DCs have fused mitochondria. On the other hand, Del Prete et al. described that after LPS-induced differentiation and activation, DCs have an increased number of condensed mitochondria [194]. However, the mechanisms by which mitochondria dynamics influence antigen presentation is still not elucidated.

For us, it is clear that little is known about DCs metabolism, because the literature has focused on the effects of TLR activation. However, DCs present a great diversity and a range of different PAMPs are recognized by them. Even though mitochondrial metabolism is remarkably important for them, new studies are necessary to better understand how mitochondria morphology and metabolism influence the function of DCs during immune responses, particularly in the *in vivo* context.

10. T lymphocytes

T lymphocytes originate from bone marrow-derived hematopoietic stem cells and migrate to the thymus, where beta chain rearrangement and selection happens. After this stage, it is known that double-positive thymocytes have increased expression of Glut1, indicative of increased glycolysis [200]. During the early stages of development, T lymphocyte metabolism has been studied only after positive and negative selection checkpoints. After these maturation steps, T lymphocytes migrate to the periphery. These naïve T lymphocytes can be $CD4^+CD8^-$ (also called as helper T cells) or $CD4^-CD8^+$ (or cytotoxic T cells). Naïve T lymphocytes express low levels of the activation marker CD44 and high levels of the lymph node homing receptors CD62L and CCR7 [201].

APCs expressing processed peptides migrate to the lymph nodes, where they encounter naïve T lymphocytes. The interaction between the cells promotes T cell activation and a rapid metabolic shift from OXPHOS towards aerobic glycolysis in a mechanism dependent on increased activity of pyruvate dehydrogenase kinase 1 that inhibits pyruvate transport to the mitochondria [202]. TCR activation along with CD28-mediated co-stimulation leads to a rapid increase in Glut1 expression and consequent glucose uptake [47,203,204]. Indeed, it was shown that T cell activation using both anti-CD3 and anti-CD28 antibodies switches T lymphocytes metabolism from FAO and pyruvate oxidation via the TCA cycle to aerobic glycolysis, PPP and glutamine oxidation [205].

Activated $CD4^+$ T lymphocytes can be differentiated into distinct helper T cells. There are four major subsets of $CD4^+$ cells: Th1, Th2, and Th17 are effector T cells, while the Treg prevents autoimmunity and exacerbated immune responses. It is well accepted that Th1, Th2, Th17, and cytotoxic $CD8^+$ T lymphocytes use aerobic glycolysis to promote their effector function and differentiation [38]. Increased aerobic glycolysis generates metabolites that are substrates for nucleotide, amino acid, and lipid biosynthetic pathways to produce molecules required for cell division [206,207].

As presented above, glycolysis is essential for effector T cells action. However, it was shown that T lymphocytes can use glycolysis or OXPHOS to promote cell proliferation and survival of naïve T cell [209,210]. CD3/CD28 stimulation in glucose-free medium and using pyruvate or α-ketoglutarate to feed the TCA cycle was sufficient to induce T cell activation [211]. While Th2 and Th17 cells depend on glutamine metabolism to enrich TCA cycle intermediates and to promote differentiation, in Th1 cells it does not occur [212,213]. On the other hand, in Th1 cells, glutaminolysis is essential to promote IFN-γ production, which suggests the importance of the TCA cycle not in differentiation, but in effector function [210]. Furthermore, the blockade of ATP synthase is sufficient to abrogate the proliferation of T lymphocytes [210]. Therefore, the ATP produced by mitochondria is sufficient to support T cell metabolism [211]. It is also known that Treg depends on higher levels of OXPHOS and FAO, with decreased glycolytic flux, compared to Th17 cells [47]. t is interesting to observe that inhibition of FAO by etomoxir did not affect effector T lymphocyte proliferation but impaired Treg differentiation [48,208]. However, it is important to point out that, as in macrophages, the use of etomoxir in T lymphocytes is under review in literature. Raud et al. used genetic animal models with CPT1a specifically deleted in T lymphocytes to show that FAO was not necessary for effector and memory T lymphocytes or Treg development [50].

The formation of membranes are essential for T cell proliferation and immunological synapses between Th cells and APCs. In this context, FAS is also important for T cell development. At the immunological synapse, a cytoskeletal-dependent mitochondrial redistribution occurs to allow for immunological synapse formation. Calcium influx increases in these mitochondria close to immunological synapse, allowing calcium-dependent activation and clonal expansion in Th lymphocytes [211,212]. The deletion or inhibition of acetyl-CoA carboxylase 1, the enzyme that catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA impairs Th17 and $CD8^+$ T cell induction and proliferation [213–216]. However, to our knowledge, the role of FAS in other T subsets is not clear. Interestingly, FAO is preferentially used by non-inflammatory and tolerogenic immune cells, whereas FAS is predominantly observed in inflammatory adaptive cells.

Although adaptive immune cells do not use ROS for their effector functions, these molecules can act as signaling messengers in several pathways. During TCR cross-linking mtROS are generated within 15 min [210,217]. During T cell activation, low, physiologically relevant levels of ROS are generated, i.e., an H_2O_2-mediated oxidative signal, which facilitates activation of the ROS-dependent transcription factors NF-kB and NFAT, both essential for T cell activation [12,218,219]. This oxidative signal is indispensable for T cell activation [12,217,220]. Different enzymatic sources, such as the respiratory chain [218–221], lipoxygenases [222], Nox2 and dual oxidase 2 [223,224], have been described as participating in T cell activation-triggered ROS production. In a series of important studies, Kaminski et al. showed that TCR-induced PKC activation drives ETC complex-I-mediated mtROS production. The $O_2^{\bullet-}$ released into the mitochondrial matrix is converted into H_2O_2 by MnSOD. This H_2O_2 diffuses into the cytoplasm to act as an oxidative signal mediator that is essential for T cell activation-induced gene expression (e. g., IL-2, IL-4, CD95 ligand) [218–220,225] (Fig. 3). The production of mtROS from complex III of the ETC is also important for T cell signaling in $CD8^+$ and $CD4^+$ T lymphocytes *in vivo* [210]. Even though the role of mtROS has been explored, future studies will be needed to clarify the targets of mtROS during T cell activation.

Following activation of an immune response, a large number of lymphocytes can be found secreting high quantities of cytokines and chemokines. This can be dangerous to the host tissues if these cells are

not finely regulated. The reduction in the antigen load leads to apoptosis of effector T cell by two mechanisms: (a) the “extrinsic pathway” that is dependent on death receptors (e.g. TNF receptor family) and leads to cleavage of caspases 8 and 10. (b) or the “intrinsic pathway” that is triggered through damage to mitochondrial membranes, endoplasmic reticulum stress and initiator caspase 9 [226]. The majority of the effector T lymphocytes undergo apoptosis, while memory T lymphocytes survive and restore their lipid oxidation metabolism [201]. Memory $CD8^+$ and $CD4^+$ T lymphocytes display increased mitochondrial number and spare respiratory capacity, which is the maximal mitochondrial respiratory capacity available to a cell to produce energy under conditions of increased work or stress [48,227,228]. Interestingly, $CD8^+$ memory T lymphocytes do not take up external FA. Rather, these cells synthesize FA by using glucose-derived carbon, which is incorporated into triacylglycerol TAG in the ER and stored as neutral lipids in lysosomes. The lysosomal lipolysis can be used to generate FA for FAO [229]. This work was the first to describe the futile metabolic cycling of FAS and FAO in immune cells [229].

One important aspect of T lymphocyte differentiation is mitochondrial dynamics. T cell activation increases the mitochondrial mass and $\Delta\Psi m$ [230]. Buck et al. showed that the mitochondria of memory $CD8^+$ T lymphocytes were elongated [74]. The same article showed that the deficiency of OPA1 causes defective development of memory T cell *in vitro* and *in vivo* and that this is accompanied by decreased OXPHOS [74]. One important aspect of mitochondria fusion is that even in effector T lymphocytes culture conditions, the induction of fusion confers a memory T cell phenotype. In contrast, effector T lymphocytes show fission of mitochondria [74]. Based on these observations, we speculate that mitochondrial dynamics can define T lymphocyte fate. Regarding the mitochondrial dynamics during $CD4^+$ T cell development, more studies are necessary to determine the underlying mechanisms. For example, little is known about T cell mitochondrial dynamics *in vivo* and how the immune response modulates the metabolism of these cells.

The metabolism of the most studied $CD4^+$ and $CD8^+$ cells have many questions to be solved. Another important aspect is that T lymphocytes are plastic, therefore understanding the metabolism of these various T lymphocyte subsets is essential to the next steps in the treatment of several diseases.

11. B cells

B cells are components of the adaptive immune response. In addition to being derived from lymphoid-committed precursors in the bone marrow, B cells share other features with T cells such as antigen recognition through individual antigen receptors (B cell receptor or BCR), coordinated developmental progression, costimulatory requirements for activation and the generation of memory cells [231]. However, the stages of B cell development, mechanisms of activation, effector functions and transcriptional profile are quite different from those found in T cells. B cells can act as APCs and respond to infectious pathogens earlier than T lymphocytes since they have functional PRRs (e.g, TLRs and Nod-like receptors), although the subtypes of these PRRs can differ between mice and humans [232,233]. More recently, human B cells have been described as capable of releasing mtDNA into the extracellular environment after treatment with the TLR9 agonist class C CpG in a dose-dependent manner (Fig. 4A) [70]. This B cell-derived webs have different morphology, composition, and function from those produced by neutrophils and are essentially responsible for activating antiviral responses via induction of type I IFNs. Interestingly, the web releasing process is independent of the BCR, TLR9, cGAS/STING, AIM-2 and oxidant signaling pathways [70].

Although B cells perform a variety of functions as stated above, they are well known for their ability to produce and secrete polyreactive and antigen-specific antibodies after differentiating into plasmablasts and plasma cells. They are collectively referred to as antibody-secreting cells (ASCs) [234]. There are five major antibody isotypes (IgM, IgG,

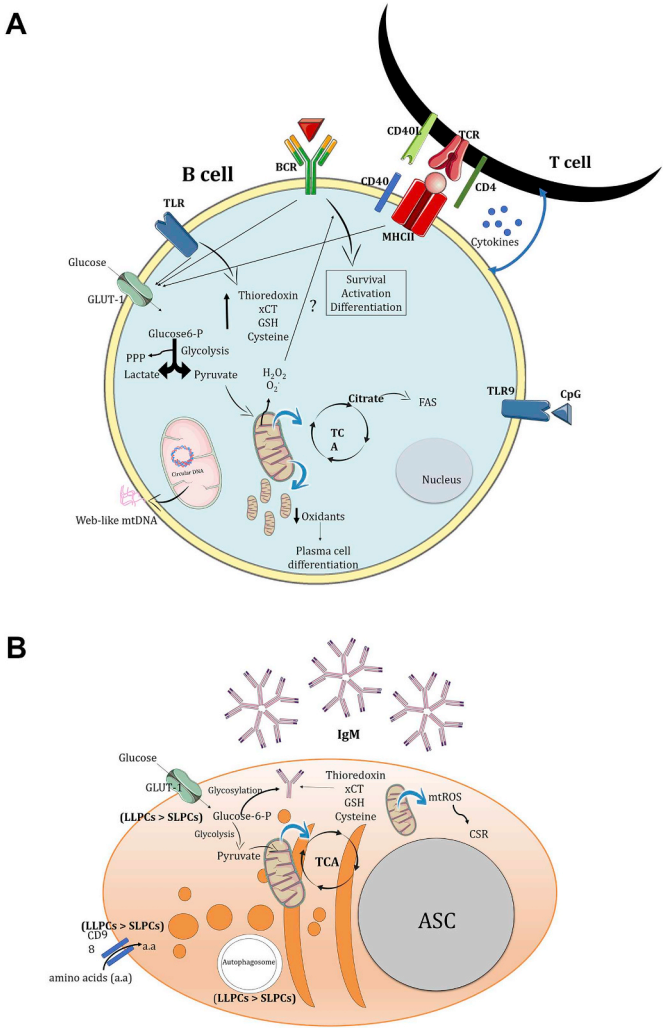

Fig. 4. Essential *metabolism and mitochondrial regulation on B cells (A) and Antibody-Secreting Cells (B)*. A. B cells are activated through T cell-independent or -dependent mechanisms. B cell activation increases both aerobic glycolysis and OXPHOS rates, enhancing the expression of the glucose transporter Glut1 and mitochondrial mass. Part of the TCA cycle fueling is used to *de novo* lipid biosynthesis via citrate transport from the mitochondria into the cytoplasm. B cell activation promotes both oxidants and antioxidants production, which the balance regulates B cell survival, effector functions and cell fate. Higher mtROS levels are important for sustaining both mitochondrial activity and mass, activating the CSR. On the other hand, lower mtROS levels were directly associated with plasma cell differentiation. Moreover, B cell activation also increases the mitochondria number and change their anatomy from an elongated to a rounded shape. Recently, CpG-stimulated TLR9 in B cells causes mtDNA releasing into the extracellular environment and its associated in stimulating antiviral responses via type I IFN production. **B.** ASCs have high metabolic demand in order to produce antibodies. Part of the glucose is used to glycosylate antibodies and intracellular membrane expansion. Higher antioxidant activity is associated with increased IgM secretion by ASCs. Different subtypes of plasma cells show different metabolic profiles. LLPCs show increased glucose uptake, CD98 amino acid transporter expression and autophagosome mass compared to SLPCs. a.a: amino acids.
ASC: Antibody-Secreting Cell, BCR, B Cell Receptor, CSR, Class-Switch Recombination, FAS, Fatty Acid Synthesis, GSH, Glutathione, LLPCs, Long-Lived Plasma Cells, OXPHOS, Oxidative Phosphorylation, PPP, Pentose Phosphate Pathway, SLPCs, Short-Lived Plasma Cells, TCR, T Cell Receptor, TLR, Toll-Like Receptor, TCA, Tricarboxylic Acid Cycle, xCT, Cystine/glutamate transporter.

IgA, IgD, and IgE), whose structures and functions have been reviewed elsewhere [235].

B cells are activated through T cell-independent or -dependent mechanisms. The former includes the participation of pleiotropic cytokines (IL-6, TNF, IL-1) and the recognition of PAMPs via PRRs (e.g., TLR4, TLR7 or TLR9). T-dependent B cell responses occur when B cells obtain help from $CD4^+$ T cells, generating more specific antibody responses. T cells are primed by recognizing antigens presented by B cells, which in turn, further activate the cognate B cell [234]. Activation of the PI3K-Akt-mTORC1 axis is important to generate ASCs in a process that is also dependent on mtDNA replication, mitochondrial remodeling, and metabolic switching. The β isoform of protein kinase C (PKCβ) and the R-Ras2 (a Ras superfamily GTPase) are essential to activate mTORC1 after BCR triggering [236,237].

B cell subsets include B1 and B2 cells. The B1 cells (also subclassified into B1a and B1b B cells) populate the pleural and peritoneal cavities, and the mucosal system. They secrete polyreactive antibodies and are primarily derived from fetal liver precursors, although the bone marrow can produce some B1 cells with less efficacy throughout life [238,239]. The limited *de novo* production of B1 cells may be a putative consequence of their evolutionary advantage of self-renewal in the periphery by mechanisms partially dependent on autophagy [240].

B2 cells are subdivided into follicular (FO) and marginal zone (MZ) B cells and are continuously produced in the bone marrow [239,241]. Committed B cell lineage precursors in the bone marrow (e.g. early pro-B cells, late pro-B cells, large pre-B cells, and small pre-B cells) have high metabolic rates but show some differences at each developmental stage. This includes distinct glucose uptake rates, oxidant production and mitochondrial mass [242]. These metabolic dynamics are tightly regulated by pre-BCR signaling (an important checkpoint at the pro-B/pre-B transitional stage), which its activation causes the highest glucose uptake and oxidants production, but no change in mitochondrial mass [242]. Furthermore, it has also been shown that any metabolic disturbance during the earliest stages impairs B cell development [242]. After leaving the bone marrow, immature B cells reach the secondary lymphoid tissues to complete their maturation process.

FO B cells are recirculating cells expressing monoreactive BCRs and home to the follicles in sites close to T cell zones in both the spleen and lymph nodes [241]. This location facilitates the bi-directional communication between B and T cells and, consequently, specific and sustained immune responses. The MZ B cells, however, inhabit the splenic marginal sinus, have polyreactive BCR and play mixed functions by performing T cell-independent and -dependent responses through rapid antibody production after recognizing PAMPs or via antigen presentation to $CD4^+$ T cells localized in the splenic follicles, respectively [243].

Following cross-talk between B and T cells, B cells may become blasts and undergo class-switch recombination (CSR) to differentiate into ASCs (short-lived plasma cells – SLPCs) without forming a germinal center (GC) reactions in a process called “extrafollicular response” [244]. On the other hand, antigen-activated B cells may enter the follicles and initiate GC responses to yield high proliferative rate, high-affinity antibody production, inducing CSR and generating both memory B cells or long-lived plasma cells (LLPCs) [231]. GC reactions have a high energy demand and mitochondrial biogenesis is needed to support these activation steps. GC B cells have high HIF-1α-dependent glycolytic activity [245] that is sustained by the hypoxic microenvironment in the light zone of GC [246]. Moreover, the metabolic sensor glycogen synthase kinase 3 (GSK3) was described as an important regulator of oxidant production during the GC reactions to prevent the overproduction and consequent activation of apoptosis signaling pathways [245]. In addition, the CSR increases both mitochondrial mass and activity, and ROS production [247].

The investigations into the metabolic profiles of B cells subtypes have only recently started. In general, the metabolism of total splenic B cells is distinct from T cells in both the quiescence and activation states. Caro-Maldonado et al. showed that B cells have higher glycolytic activity than T cells in the resting state [248]. After activation, both glycolysis and OXPHOS rates increase proportionately in B cells, without the metabolic shift observed in T lymphocytes. B cell activation also increases the expression of the glucose transporter Glut1 and mitochondrial mass (Fig. 4A) [248]. Uptake of extracellular glucose is essential for B cell proliferation, intracellular membrane network expansion (required for antibody production) and for ASC marker expression (CD138 and Blimp-1) [249]. In fact, mice with B cells deficiency of *Glut1* failed to produce total and specific IgM or IgG 7 days after immunization with NP-ovalbumin [248]. However, a study using RNAseq and isotopic labeling approaches concluded that glycolysis is dispensable for B cell activation, whilst biosynthesis of ribonucleotides, OXPHOS and mitochondrial dynamic remodeling are required [250]. Naïve B cells have a low number of mitochondria and they have an elongated shape. On the other hand, activated B cells have rounded shape mitochondria (Fig. 4A). This indicates that activated B cells may prioritize mitochondria number instead of the most efficient metabolism-related anatomy in order to be transmitted to daughter cells during clonal expansion. Moreover, the same study showed that mtDNA remains unchanged even with the increased number of mitochondria in activated B cells [250].

A more recent study has shown that aerobic glycolysis, TCA, PPP, and FAS are more active in B1 cells than B2 cells [240]. This high metabolic activity may be associated with the rapid response of B1 cells. Corroborating this hypothesis, IgA-producing plasma cells also have high metabolic activity (predominantly glycolytic) in the gut, since they are continuously producing antibodies as frontline surveillance at mucosal surfaces [251]. Additionally, B1a cells with impaired autophagy function due to deficiency of *Atg7* show decreased metabolic activity and mitochondrial dysfunction [240]. Although there are some indications that MZ B cells have higher glucose uptake rates than FO B cells [245], the understanding of which metabolic pathways support the activation of B cells and their subtypes still require further investigation. In addition, no study has described the contribution of HBP to support the development, differentiation and activation of B cells.

B cells produce regulated amounts of H_2O_2 to amplify and maintain the BCR signaling pathway (Fig. 4A) [252]. This is particularly important for GC reactions. In fact, one study demonstrated that GC B cells are more sensitive to H_2O_2 compared to other B cell subtypes (mature B cells, memory B cells and plasma blasts) as determined by higher levels of phosphorylated proteins associated with BCR activation [253]. Furthermore, the source of oxidant production following BCR activation changes over time. In the first 2 h of stimulation, the production is mediated by Nox2, whereas after this period the main source of oxidants is mitochondrial respiration [254]. Oxidant production during these both stages is important to regulate the BCR-activated B cell proliferation since the treatment with N-acetylcysteine impairs their proliferation rate. However, the use of gp91-deficient B cells, which lack of functional Nox activity, showed that $O_2^{\bullet-}$ and other ROS-derived oxidants constrain the proliferation of BCR-stimulated B cells and limits T cell-independent B cell responses [255]. One recent study also described that Duox1 (an H_2O_2 producer)-deficient B cells had unchanged antibody production but increased proliferative rate [256]. Thus, additional studies are necessary to confirm the impact of oxidant production on B cell proliferation.

T cell-independent B cell activation also increases oxidant production as observed in LPS-stimulated B cells [257]. At the same time, B cells upregulate the antioxidant response, including the production of thioredoxin, the cystine transporter xCT and nonprotein thiols, mainly GSH and cysteine [257]. This redox balance seems to be essential to regulate B cell development and function. Oxidant production is required for their differentiation (Fig. 4A), whereas the higher antioxidant activity increases IgM secretion in ASCs (Fig. 4B) [257]. However, one study showed that OXPHOS, but not ROS production, is essential for antibody synthesis [258].

Increasing efforts have been made to understand the metabolic

profile of plasma cells (mainly LLPCs) as well as the factors that impact GC responses and memory generation [246]. These cells are potential therapeutic targets since they have increased high-affinity antibody production capacity and long-lasting responses. Lam et al. showed that LLPCs take up more glucose than SLPCs [259]. It was further confirmed that this glucose is fundamental for pyruvate generation and its consequent use by the mitochondria for maintenance of LLPCs, since the absence of a functional mitochondrial pyruvate carrier decreased the frequency of LLPCs in the bone marrow. Moreover, a recent study emphasized the importance of immunometabolism in describing ASC subpopulations and other immune cells. It has been shown that LLPCs and SLPCs have very similar transcriptional programs, but different metabolic profiles [260]. The former showed higher glucose uptake, greater expression of the CD98 amino acid transporter, and higher autophagosome mass compared to the latter (Fig. 4B). Thus, it becomes necessary to investigate which non-transcriptional factors regulate the longevity of plasma cells. The understanding of mitochondrial dynamics of memory B cells and plasma cells remain undefined and future investigations must describe if mitochondria anatomy can define the fate of these populations.

Fine-tuning the regulation of oxidant production is also required to coordinate plasma cell fate. Higher mtROS levels are important for sustaining both mitochondrial activity and mass and activating the CSR (Fig. 4A). On the other hand, lower mtROS levels were directly associated with plasma cell differentiation [254]. Interestingly, increased oxidant production inhibits the synthesis of the heme group by preventing the addition of ferrous ion to the protoporphyrin IX group. Heme is iron-prophyrin complexes that modify protein function through conformational modifications and have been shown to regulate both the CSR and differentiation of B cells into plasma cells by directly and indirectly modulating the transcription factors associated with these processes (Bach2 and Blimp-1, respectively) [261].

Altogether, these results highlight the paucity of studies describing both B cell metabolism and the impact of redox status and mitochondrial milieu on B cell development and function. Future studies are needed to reveal whether mitochondrial dynamics can dictate B cell fate as is observed in T lymphocytes.

12. Future perspectives

The concept of mitochondria as being more than the powerhouse of immune cells is relatively new. Recent studies have shown that mitochondrial metabolites and mtROS are important regulators of signaling pathways and cell fate in both innate and adaptive immune cells. Furthermore, mitochondrial dynamics modulate not only cell metabolism, but also cell fate and function. The many roles of mitochondria in different immune cells have been uncovered, but this is only a small portion of the different aspects that mitochondria can orchestrate in immune cells. Understanding cell metabolism *in vivo* is one of the challenges in immunometabolism, but great progress has been made in recent studies. Thus, modulating mitochondria in immune cells may be a future approach to treat a wide spectrum of diseases, such as inflammatory diseases and cancer.

Conflicts of interest

The authors declare no conflicts of interest.

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