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The emerging role of immune dysfunction in mitochondrial diseases as a paradigm for understanding immunometabolism

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Abstract

Immunometabolism aims to define the role of intermediary metabolism in immune cell function, with bioenergetics and the mitochondria recently taking center stage. To date, the medical literature on mitochondria and immune function extols the virtues of mouse models in exploring this biologic intersection. While the laboratory mouse has become a standard for studying mammalian biology, this model comprises part of a comprehensive approach. Humans, with their broad array of inherited phenotypes, serve as a starting point for studying immunometabolism; specifically, patients with mitochondrial disease. Using this top-down approach, the mouse as a model organism facilitates further exploration of the consequences of mutations involved in mitochondrial maintenance and function. In this review, we will discuss the emerging phenotype of immune dysfunction in mitochondrial disease as a model for understanding the role of the mitochondria in immune function in available mouse models.

Keywords

Immunity; metabolism; mitochondria; bioenergetics; mitochondrial disease; oxidative phosphorylation; mouse models; patient studies

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AUTHOR CONTRIBUTIONS

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1. INTRODUCTION

“The human system [...] comes equipped with an astonishingly broad array of characterized mutant phenotypes; thousands of human genes have been identified by virtue of the phenotype they confer when inherited in mutant form.”

Ray White and C. Thomas Caskey, *The Human as an Experimental System in Molecular Genetics* [1]

Mitochondria arose only once in evolution, approximately 2.5 billion years ago, via endosymbiosis during the evolution of eukaryotic cells [2]. The persistence of this arrangement is due to the mutual benefit derived from the ability of mitochondria to convert organic molecules from the environment to energy. Over time, the mitochondria developed an ultrastructure characterized by a double membrane composed of phospholipids and protein with an intermembrane space between the outer and inner membranes. Residing in the center is the matrix which is surrounded by the inner membrane. The inner membrane has numerous folds, or cristae, that increase its surface area and house the chemiosmotic machinery that facilitates cellular respiration.

The matrix of each mitochondrion contains multiple copies of a double-stranded, circular mitochondrial DNA (mtDNA). Approximately 17kb, each mtDNA contains 37 genes: 22 transfer RNAs, and 2 ribosomal RNAs, and 13 protein-coding genes. During evolution, many genes required for mtDNA replication and maintenance, and other functions, were transferred to the nucleus [3]. To date, >1100 nuclear-encoded genes have been identified to be involved in sustaining mitochondrial function [4], further emphasizing the importance of coordination between the nuclear and mitochondrial genomes.

Mitochondrial diseases (MDs) are a group of clinically heterogeneous disorders that can arise from heritable mutations in either mtDNA or nuclear DNA (nDNA). Mutations in genes involved in mitochondrial function lead to disorders of oxidative phosphorylation (OXPHOS), mtDNA integrity, and mitochondrial maintenance, among others. MDs due to mtDNA mutations are inherited in a matrilineal fashion, whereas MDs arising from mutations in nuclear genes are inherited in an autosomal dominant, autosomal recessive, or X-linked manner. For MDs due to mtDNA mutations, the proportion of mutant mtDNA molecules within a tissue determines both the penetrance and severity of the phenotype in that tissue. This phenomenon of mixed populations of mtDNA (i.e. mutated and wildtype), known as heteroplasmy, serves to modify the phenotypic expression of MDs. In addition, the phenotype of mtDNA and nDNA mutations in MDs may also be modified by tissue specific expression of the gene, modifier genes, and epigenetics [5].

Since most cells contain mitochondria, the clinical effects of mitochondrial disease are potentially multisystemic, and involve organs with large energy requirements including the heart, skeletal muscle, and brain [6]. The burgeoning field of immunometabolism aims to define the role of intermediary metabolism in immune cell function [7], cells which also have potentially large energy requirements during innate and adaptive responses to insult or injury. Based on recent medical literature and advances in profiling technologies, there has been renewed interest in the bioenergetics of immune cells, with the mitochondria and

OXPHOS taking center stage. In addition to performing the critical function of energy production, which is the basis of life, additional roles of mitochondria include heat production, calcium storage, apoptosis, cell signaling, and biosynthetic roles (e.g. heme, sterols), all important for cell survival and function [3, 8–10]. In the spirit of the seminal article by White and Caskey [1], extolling the virtues of the human as an experimental system, here, we explore a patient centered approach to understand the role of mitochondria in immune cell function using MDs as a guide (Figure). In the sections to follow, we review the emerging clinical phenotype of immune dysfunction in MDs, which is generally absent from textbooks and reviews on MD, and discuss existing mouse model systems for mitochondrial disease genes that have expanded, or have the potential to expand, our understanding of multiple aspects of mitochondrial function in the context of MDs.

2. METHODS

In this review, we provide a blueprint for studying mitochondrial function in immune cells. Patients with MD serve as the starting point. Clinical and experimental findings in patients are then followed up in physiologically relevant mouse models. Overall this approach is critical for revealing the interplay between the mitochondria and immune cell function. Research was performed by employing combinations of the terms ‘mitochondrial disease’, ‘mitochondria, immune’, ‘immunodeficiency’, and ‘mouse models’, in PubMed.

3. IMMUNE DYSFUCTION IN PATIENTS WITH MITOCHONDRIAL DISEASE

The toll infection takes on patients with MD is readily appreciated by clinicians: ~30-50% of MD patients may experience recurrent upper respiratory tract infections [11, 12] with ~50% of those resulting in life-threatening or neurodegenerative sequelae [11]. In a cohort of 221 pediatric patients with mitochondrial disease, the global mortality rate was 14%, with sepsis (55%) and pneumonia (29%) being the two most common causes of death [13]. Despite studies documenting the effects of infection in this medically vulnerable population, immune dysfunction remains an under-recognized phenotype in MDs.

Evidence that the immune system is affected by an energetic deficit can be found in multiple reports where patients with MD have similar presentations to patients with primary immunodeficiencies, including unusual infections not generally seen in immunocompetent populations. Generally speaking, leukopenia, which may increase the risk of infection, has been reported in a number of MDs, including Barth syndrome, Pearson syndrome, Leigh syndrome, and other nonsyndromic forms of MD [14]. In patients with Pearson syndrome, a mitochondrial deletion syndrome that causes pancytopenia, invasive aspergillosis and cutaneous zygomycosis have been described [15]. Along with unusual infections, clinical signs of immunodeficiency have also been observed in MD case reports. Mutations in *FBXL4*, a gene encoding an orphan F box protein believed to be important for E3 ubiquitin ligase complex activity, cause a mitochondrial depletion syndrome. Besides central nervous system symptoms, patients with *FBXL4* mutations also experience neutropenia, lymphopenia, and frequent infections [16, 17]. In a similar case of neonatal onset mitochondrial depletion syndrome without a molecular diagnosis, infectious history was significant for episodes of bronchopneumonia, gastroenteritis, and bouts of septicemia.

Repeated vaccination with *S. pneumoniae* failed to elicit a protective immune response. Recurrent hypogammaglobulinemia and reductions in natural killer (NK), total CD8⁺ T cells, and CD8⁺ memory T cells were also noted [18]. These findings are most likely due to the depletion of mtDNA encoded components of OXPHOS which are critical for immune cell function (Section 4.4.).

Beyond single case reports, there have been a small number of studies examining infection risk in patient cohorts with MD. In a retrospective study of 92 patients with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), an adult onset mitochondrial disease, 7.6% were noted to have a history of infections that provoked worsening of symptoms [19]. Carnitine palmitoyl transferase 1A (CPT1A) deficiency, although not a MD in the strictest terms, eventually affects the mitochondrial bioenergetics chain via reduced β -oxidation of long chain fatty acids. In response to recent data regarding the role of CPT1A and fatty acid oxidation (FAO) in memory T-cell formation [20–22], a study of Native Alaskan children carrying a hypomorphic variant of CPT1A demonstrated either an increased association of lower respiratory tract infection or acute otitis media compared to heterozygous or non-carrier children [23]. Although there were multiple confounders in this study, this represents an example of how further immunophenotyping of this population could be beneficial to defining the molecular mechanism by which long chain fatty acids specifically modulate memory T-cell function.

One of the largest retrospective studies performed to date on MD and infection involved 97 patients with “probable” or “definite” MD (Walker criteria) [24] and related disorders of mitochondrial metabolism (e.g. short chain hydroxyl acyl coA dehydrogenase deficiency) [25]. Patients with MD and related disorders (42%) experienced recurrent or serious infections, with the majority of cases being bacterial infections alone or in combination with other microorganisms. Sepsis or Systemic Inflammatory Response Syndrome (SIRS) occurred in 13% of the population studied, and, on average, at a rate of once every 10 years. Patients ranged from newborn to 68 years, making it somewhat difficult to draw conclusions regarding infectious susceptibility for pediatric populations. Despite this limitation, immune phenotyping revealed T lymphopenia, low switched memory B-cells, hypogammaglobulinemia requiring replacement therapy, impaired vaccine responses, and decreased T-cell mitogen responses in 5 pediatric patients with OXPHOS abnormalities. Concerning pediatric patients specifically, our group recently described a cohort (mean age = 7.4 years) of 62 patients with MD [12]. The most common finding was recurrent or severe infections (89%) involving patients with contemporaneous symptoms of upper (47%) and lower (40%) respiratory tract infections, otitis media (24%), and sinusitis (32%). Immunoglobulin replacement therapy for clinical immunodeficiency was documented in 21% of patients. Further immunophenotyping in a subset of pediatric patients expanded the immune phenotype to include memory T-cell deficits and leukopenia following the bioenergetic stress of infection. One explanation for the immune phenotype observed in our cohort could be due to reduced viability of T-cells. Using a mouse model of T-cell cytochrome c oxidase (COX) deficiency, we showed increased apoptosis following T-cell activation (Section 4.4) and recapitulated the clinical phenotype observed in patients [12].

Together, this medical literature and anecdotal reports on patients with MD suggest an emerging phenotype that includes immunodeficiency, which may be due to cell intrinsic defects in mitochondrial function. Indeed, the doubling time of a T cell may be as short as 4.5 hours, potentially giving rise to 10^{12} cells in a week, suggesting a high bioenergetic requirement [26]. Similarly, B cell activation can lead to plasma cell differentiation that requires extensive cellular enlargement to accommodate secretion of thousands of antibodies per second [27]. Overall, the clinical findings of immunodeficiency in MD suggest that reductions in components of oxidative phosphorylation are critical for immune cell function and homeostatic maintenance. Indeed, various different types of immune cells require oxidative phosphorylation for cellular activation and differentiation (Section 4.4). Although immune activation after pathogen exposure is an energy demanding process, the loose organization of the immune system has oftentimes precluded the conceptualization of this system as energy demanding. This may explain why none of the reviews and textbooks on MDs include a section on the immune system.

4. MOUSE MODELS OF MITOCHONDRIAL DISEASE GENES HAVE EXPERIMENTAL LINKS TO IMMUNE CELL FUNCTION

Over 50 gene-edited or transgenic mouse models for studying mitochondrial biology have been reported to date, including a wide variety of inbred strains (Table). Many of these models have been elegantly reviewed elsewhere [28–31]. Based on the core mitochondrial functions outlined in our mitochondrial morbidity map (Figure), we next discuss selected examples of MD-associated genes and existing mouse models of mitochondrial biology in which immune dysfunction or dysregulation has been reported that may be useful in further exploring immune-related phenotypes (also found in the Table). When considering mouse models, it should be noted that C57BL/6J mice have a spontaneous deletion in the nicotinamide nucleotide transhydrogenase (*Nnt*) gene which leads to absence of mature NNT protein and impacts mitochondrial function and redox (i.e. oxidation and reduction reactions) [32].

4.1 Mitochondrial calcium flux

Long before the discovery that the endoplasmic reticulum was the major Ca^{2+} -storing organelle in eukaryotic cells, mitochondrial Ca^{2+} was known to be important for buffering cytosolic Ca^{2+} [33–35], as well as regulating mitochondrial metabolism. For example, mitochondrial Ca^{2+} plays a role in regulating isocitrate and alpha-ketoglutarate dehydrogenases through direct binding to these enzymes, increasing NADH levels and thus modulating ATP synthesis [36–39](and reviewed in [40]). However, the molecular details of the transporter(s) responsible for mitochondrial Ca^{2+} uptake and release remained elusive until 2010, when a new candidate was finally identified in a siRNA screen: the 54kDa mitochondrial Ca^{2+} uptake 1 (MICU1) protein. MICU1 downregulation in HeLa cells led to significant reductions in repeated IP_3 -induced mitochondrial Ca^{2+} uptake [41]. However, with only a single transmembrane domain and no evidence of oligomerization, it was suspected that MICU1 was likely not the Ca^{2+} channel itself, but instead an important player in the regulation of Ca^{2+} transporter function. In 2011, two groups simultaneously identified a 40kDa protein with oligomerization capability, now known as the mitochondrial Ca^{2+}

uniporter (MCU) [42, 43]. While the exact topology of MCU is still unknown, identification of the MCU and its regulatory component, MICU1 [44, 45], has revived interest in the role of mitochondrial Ca^{2+} flux in multiple cell types.

Experimental evidence for a role of MCU/MICU1 (Figure, panel A) in myeloid lineage-derived immune cell function is accumulating. Using a series of inhibitors, Takekawa et al. showed that mitochondrial-mediated Ca^{2+} uptake plays an important role in cytosolic Ca^{2+} buffering in mast cells [46]. In MCU KD mast cells, antigen-induced beta-hexaminidase release was suppressed, suggesting a role for MCU-mediated mitochondrial Ca^{2+} flux in mast cell degranulation [47]. This dysfunction can lead to altered immune responses as mast cells often represent one of the first cell types to interact with environmental antigens or invading pathogens, and play immunomodulatory roles during specific innate or adaptive immune responses via the secretion of cytokines, small molecules and extracellular enzymes [48].

In humans, two different homozygous truncating mutations in *MICU1* were recently identified using whole exome sequencing. While mitochondrial membrane potential and OXPHOS were similar in fibroblasts from patients compared with controls, confocal imaging revealed a fragmented mitochondrial network in patient cells [49]. Although immune-related phenotypes were not reported, it is interesting to note that morphological changes in mitochondrial networks (“fissed” versus “fused,” discussed in detail in the next section) in T lymphocytes are associated with different metabolic profiles [7]. Examining whether T cells from MICU-deficient patients also exhibit a fragmented mitochondrial network, and how that affects activation, may reveal insight into links between mitochondrial-mediated Ca^{2+} flux, morphology and energetics, and T cell function. Mitochondria have also been reported to localize to the immunological synapse in T lymphocytes in conjugates with antigen-presenting cells [8, 9, 50]. At the synapse, lytic granule fusion machinery in CD8^+ T cells is dependent on localized Ca^{2+} flux for secretion and target killing. However, whether or not mitochondrial-mediated Ca^{2+} import plays a role in this critical secretory step is still unclear. Recent reports of newly generated *MCU^{fl/fl}* [51] and MICU1 KO mouse models [52], and one using CRISPR-Cas technology [53], will most certainly help address related outstanding questions such as these (Table).

Ca^{2+} -dependent transcription is also essential for regulating a number of immune cell responses. Mitochondria serve as Ca^{2+} “sinks” when receptor-triggered Ca^{2+} concentrations increase too rapidly. To examine the interplay between cytosolic and mitochondrial Ca^{2+} flux and gene regulation, Shanmughapriya et al. showed that in the absence IP3R, STIM1, and ORAI, critical mediators of cytosolic Ca^{2+} flux, MCU abundance and CREB phosphorylation were decreased in chicken DT40 B lymphocytes. ChIP assays in HeLa cells revealed CREB bound the MCU reporter, and restoration of cytosolic Ca^{2+} signals using pharmacological means rescued both MCU expression and mitochondrial metabolism [54]. This work raises the question of whether defects in mitochondrial function also contribute to diseases with phenotypes ascribed to deficiencies in STIM or ORAI proteins, such as immunodeficiency, autoimmune hemolytic anemia, and muscular hypotonia [55, 56].

4.2 Mitochondrial fission and fusion

Maintenance of mitochondrial quality and quantity are dependent on the processes of fusion and fission, particularly under stressful metabolic or environmental conditions [57, 58]. Fission, or division, is critical during proliferation to ensure dividing cells receive adequately distributed mitochondria. Fusion, or merging, allows cells to build large interconnected mitochondrial networks that promote complementation between damaged mitochondria, and increased oxidative capacity. Fusion and fission machinery also play a role in apoptosis through cytochrome c-mediated caspase activation.

Mitochondrial fission and fusion dynamics are regulated by dynamin family member guanosine triphosphatases (GTPases) that are conserved among yeast, *Drosophila*, and mammals. This conversation emphasizes the evolutionary importance of these processes (reviewed in [59]). The core machinery in mammals mediating fusion are the mitochondrial membrane-anchored proteins, mitofusins (MFN1, MFN2), and mitochondrial dynamin like GTPase (OPA1), which regulate outer and inner membrane fusion, respectively (Figure, panels B–C). During mitochondrial division, recruitment of cytoplasmic DRP1 to sites of fission is mediated through complex interactions with the endoplasmic reticulum that define division sites [60]. Tight regulation of these proteins allows cells to fine-tune metabolic activity by building large, interconnected mitochondrial networks or distinct small organelles.

In response to infection, T lymphocyte activation triggers massive clonal expansion of antigen-specific cells, a contraction phase, and the development of a long-lived memory population. In activated T cells, mitochondria translocate to the immune synapse, regulating Ca^{2+} levels and generating ATP for cellular processes [8]. Renewed interest in activation-induced metabolic reprogramming that occurs in the differentiation of T cell subsets [12, 20, 22] has revealed distinct differences in mitochondrial morphology between effector T and memory T lymphocytes. Short-lived effectors, which rely mostly on glycolysis to provide the building blocks required to fuel their proliferation, exhibit fragmented mitochondrial morphology associated with increased fission. In contrast, memory T cells have increased mitochondrial mass, tight cristae, and a generally more “fused” mitochondrial morphology [20, 61]. Consistent with these data, although effector T cell activation was intact in *Opa1*-deficient CD4-Cre mice (Table), *Opa1*^{-/-} T cells were unable to undergo efficient mitochondrial fusion and failed to produce memory T cell populations in response to bacterial infection [61]. Similarly, promoting mitochondrial fusion improved adoptive cellular immunotherapy against tumors [61, 62]. Together these data suggest that mitochondrial morphology plays an important role in the generation of memory T cell populations, possibly by facilitating super complex formation leading to increased respiratory complex efficiency, which memory T cells rely on to meet their metabolic demands. Based on the phenotypes observed in the aforementioned mouse models, we would propose that studying patients with autosomal dominant optic atrophy (i.e. *OPA1* mutations, OMIM 16550) and Charcot Marie Tooth Type 2A (i.e. *MFN2* mutations, OMIM 609260) would be critical for understanding the role of mitochondrial fusion/fission in immune function

4.3 Mitochondrial DNA maintenance and replication

In mammalian cells, mtDNA is packaged into higher order structures called nucleoids that can contain more than one mitochondrial genome, as well as several proteins involved in replication and transcription. The nuclear-encoded mitochondrial transcription factor A (TFAM) is one of the most abundant components of nucleoid structures (Figure, panel D). TFAM is a member of the high-mobility group box (HMGB) domain family of proteins capable of unwinding and bending DNA sequences [63, 64]. The importance of TFAM for the regulation of mtDNA copy number and mtDNA preservation during development is underscored by the fact that global deletion of the gene is embryonic lethal in mice [65, 66]. However, tissue-specific deletion of TFAM has recapitulated a number of phenotypes associated with human mitochondrial diseases [67, 68]. There have been numerous elegant studies examining the role of TFAM in skeletal muscle [69–71] and the central nervous system [72–74], where tissue-specific loss of TFAM resulted in respiratory chain deficiency in mice, leading to cardiomyopathy and diabetes mellitus (reviewed in [75]).

To investigate the mechanisms by which mitochondria influence T cell responses, Baixauli et al. deleted *Tfam* in CD4⁺ T cells of mice via *cre*-mediated excision [76]. As expected, CD4⁺ *Tfam*^{−/−} T cells displayed reduced mtDNA and OXPHOS, impaired fatty acid β -oxidation, and a compensatory increase in glycolysis. Along with mitochondrial dysfunction, CD4⁺ *Tfam*^{−/−} T cells also displayed abnormal lysosomal Ca²⁺ regulation and lysosomal degradation with skewed differentiation toward proinflammatory T cell subsets leading to an exacerbation of the inflammatory response *in vivo*. These data suggest that mitochondrial function may not be necessary for the development of certain T cell subsets, or that T cells may have an inherent metabolic flexibility to employ alternative metabolic pathways. Of interest, restoration of NAD⁺/NADH balance improved lysosomal function and abrogated the inflammatory phenotype. Overall, these data provide a link between mitochondria and lysosomal function, the disruption of which leads to perturbations in T cell differentiation and effector function.

Studies in TFAM-deficient mice have also shed light on other aspects of inflammatory pathophysiology. Due to its bacterial origins, mtDNA contains several unmethylated CpG DNA repeats. When released by ruptured or dying cells during injury or infection, these bacterial-associated molecular motifs are recognized as damage-associated molecular patterns (DAMPs) by the cytoplasmic Toll-like receptor 9 (TLR9) and initiate innate immune responses in cells [77]. Recent studies have shown that cytoplasmic mtDNA activates the Nod-like receptor pyrin domain containing 3 (NLRP3) inflammasome in macrophages [78, 79]. Analogous to TLR9 signaling during traumatic injury and NLRP3 activation during cell death, perturbation of mtDNA maintenance, as is the case in TFAM-deficiency and other mitochondrial depletion syndromes, also induces gene expression important for innate immune responses. Using *Tfam*^{+/-} mice as a model of mtDNA depletion, West et al. showed that aberrant mtDNA packaging in the absence of sufficient TFAM promoted escape of mtDNA into the cytoplasm. There, mtDNA activated complex signaling pathways to induce interferon-stimulated gene expression, which likely evolved to help dampen intracellular bacterial and viral propagation [80]. Regarding other diseases associated with inflammation, mutations in TFAM and/or defective mtDNA maintenance are

observed in cases of systemic lupus erythematosus [81, 82] and in some cancers (reviewed in [83]).

mtDNA replication is carried out by a protein complex involving the mtDNA polymerase γ (POLG), a helicase (Twinkle), topoisomerase I, and single strand binding proteins [84] (Figure, panel D). It is interesting to note that in *Polg*^{D257A/D257A} mice (i.e. Mutator Mice), a model of aging due to the accumulation of mtDNA mutations from absent exonuclease activity in POLG, induction of apoptosis in cells was significant only in tissues with rapid cellular turnover or under stressed conditions [85]. In the hematopoietic system of Mutator Mice, mtDNA mutations interrupt early differentiation events and lead to perturbations in downstream hematopoietic progenitor cells [86]. This impaired differentiation results in abnormal myeloid lineage development and is directly associated with the aging phenotypes of anemia and lymphopenia [87]. Although the Mutator Mouse is not a model of mitochondrial disease, per se, the model does demonstrate a critical point: immune phenotypes may be passed via mtDNA mutations.

Patients with mutations in *TFAM* or *POLG* suffer from mitochondrial depletion syndrome (OMIM 617156 and OMIM 613662, 203700, 258450 respectively). Although immune phenotypes have not been specifically reported, we have described T cell memory dysfunction in three patients with mtDNA depletion syndrome [12]. Given a clinical report documenting increased susceptibility to sepsis and SIRS in patients with MD [25], the inflammatory phenotypes associated with mtDNA depletion as described above are of particular interest. Overall, further investigation of immune cell function in mitochondrial depletion syndromes will almost certainly shed light on how mitochondria contribute to immune-related human disease pathogenesis.

4.4 Oxidative phosphorylation

Nicknamed the “powerhouse of the cell,” mitochondria generate adenosine triphosphate (ATP) via respiration. Mitochondrial respiration, or OXPHOS, is a process through which electrons are donated by reducing equivalents (e.g. NADH and FADH₂) produced from metabolism, and eventually transferred to a final electron acceptor: oxygen (Figure, panel E). The transfer of electrons is facilitated by membrane bound protein complexes: NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome b-c₁ (Complex III) and cytochrome c oxidase (Complex IV), and their mobile intermediaries: coenzyme Q (CoQ) and cytochrome c (cytC). These large protein complexes leverage redox reactions to transport protons (H⁺) into the intermembrane space, thus generating an ion gradient. ATP synthase (Complex V) is a H⁺-transporting phosphohydrolase. By dissipating the proton gradient via translocation through a “channel” (F₀), the world’s tiniest motor (F₁) turns, generating ATP from ADP and inorganic phosphate. To maximize the efficiency of mitochondrial respiration, OXPHOS complexes may form supercomplexes containing Complexes I, III and IV via cristae remodeling [88] (Figure, panel E).

Activated and effector T cells undergo metabolic reprogramming that includes the adoption of a glycolytic (Warburg) metabotype: enhanced glycolysis with lactate production despite the availability of oxygen [89, 90]. The glycolytic metabotype, although energy inefficient, is necessary for the synthesis of intermediates and macromolecules to support T cell

proliferation. We and others have shown that activated T cells are dependent on both pathways, and are unable to function without intact OXPHOS [12, 21]. Although the role of OXPHOS in T cell activation is still under active investigation, mitochondrial ATP generation in naïve T cells is necessary for the upregulation of glycolysis, via hexokinase [21]. The requirement for mitochondrial ATP was shared between naïve and memory T cells, suggesting related bioenergetic requirements for activation amongst the many T cell subsets.

In conjunction with supporting ATP production, individual OXPHOS complexes play unique roles in T cell activation. This question was first addressed for Complex III in a seminal paper using a model of T cell intrinsic Complex III dysfunction (*T-Uqcrrs*^{-/-} mice). Sena et al. demonstrated that Complex III was necessary for the production of reactive oxygen species (ROS) for nuclear factor of activated T cells (NFAT) signaling and the downstream production of IL-2 [91]. As a result, *Uqcrrs*-deficient T cells showed proliferation defects; they remained poised during activation due to a lack of ROS production [91]. Restoration of mitochondrial ROS was able to rescue IL-2 production, but not proliferation. *In vivo*, Complex III deficiency in T cells translated into defects in antigen specific expansion. To date, MD due to *UQCRRS1* mutations has not been reported. Nonetheless, these data suggest that mutations in proteins integral to Complex III function may produce an immune phenotype. As seen in our morbidity map, there are three genes (*UQCRB*, *UQCRQ*, and *BCS1L*) that, when mutated, produce mitochondrial disease (Figure, panel E). *UQCRRS1* and *UQCRQ* interact with *UQCRRS1* [92], suggesting that patients with mutations in these MD-associated genes would be good candidates for exploring the role of Complex III in T cell function.

Cytochrome c oxidase (COX), or Complex IV, is the terminal enzyme in the electron transport chain, catalyzing the electron transfer from reduced cytochrome c to oxygen. In mammalian cells, over 30 proteins are required for efficient COX assembly and function, many of which have clinical implications in humans (Figure, panel E) [93, 94]. COX10 is a heme A farnesyltransferase found in the inner mitochondrial membrane, and the only enzyme in the respiratory chain that requires heme as a cofactor. Thus, the critical role of COX10 in heme biosynthesis makes it particularly critical for both COX expression and function [95]. *COX10* mutations result in COX deficiency that can lead to MDs in humans with heterogeneous clinical characteristics, including Leigh disease, sensorineural hearing loss, anemia, and hypertrophic cardiomyopathy [96–98].

While work understanding the contribution of COX activity to skeletal and neural tissue has shown that *Cox10*^{-/-} mice recapitulate many of the characteristics of human MDs, it was not until recently that interest in the potential role of Cox10 and COX-deficiency in immune cell function has piqued. We recently reported that pediatric patients with MDs may display T cell memory dysfunction [12], suggesting a role for COX activity in immune cell function. We and others have now shown that COX10 activity is critical for T cell activation of the TH1, TH2, and TH17, but not TREG, subsets [12, 99], and that the dependence of T cells subsets on functioning COX was related to bioenergetics requirements [12]. In support of this, CD8⁺ T cells, which have especially high metabolic demands to accommodate their proliferative responses to infection, were particularly susceptible to loss of COX activity. T-

dependent primary and secondary immune responses were impaired in Cox10-deficient mice, as was their ability to mount efficient T cell responses to both influenza [12] or *Listeria* infection [99].

During OXPHOS, protons are pumped by the respiratory chain out of the mitochondrial matrix into the intermembrane space. Generation of this ion gradient establishes the membrane potential that drives ATP production. OXPHOS is uncoupled from ATP production by dissipation of this proton gradient through a family mitochondrial uncoupling proteins 1-5 (UCPs) located in the inner mitochondrial membrane. UCP2 is found in the mitochondria of skeletal muscle, adipose tissue, liver, and lung, as well as in lymphocytes and macrophages [100], making it the most ubiquitously expressed UCP family member. Since its discovery in 1997, intensified research has established a role for UCP2 in fatty acid metabolism [101], glucose metabolism and metabolite transfer [102, 103], and in regulating ROS production [104].

Mitochondrial respiration is the primary source of ROS production in cells, and multiple groups have shown a correlation between malfunctioning mitochondria and oxidative stress (reviewed in [105]). Increased mitochondrial membrane potential leads to higher ROS production; thus, UCP2 activity reduces potentially harmful ROS in cells. Given its important function in regulating energy metabolism, it is perhaps not surprising that the dysregulation of UCP2 has been described in a number of pathological conditions, including diabetes [106], cancers [107–110], neurodegenerative diseases [111–113], and inflammatory diseases [114, 115].

An important link between UCP2 activity and inflammatory immune responses has been established, although the conditions under which UCP2 promotes or reduces inflammation are still being investigated. For example, Ucp2-deficient mice exhibit accelerated development of streptozotocin induced diabetes associated with increased macrophage recruitment to pancreatic islets [116]. On the other hand, Ucp^{-/-} mice exhibit resistance to *Toxoplasma gondii* infection, in which increased ROS in the absence of Ucp2 leads to significantly increased toxoplasma activity [104]. Similarly, delayed death of Ucp2-deficient mice in response to *Listeria monocytogenes* infection has been observed. Delayed death was associated with increased macrophage recruitment to secondary lymphoid organs and upregulation of proinflammatory cytokines [117].

Comparable to macrophages, Ucp2 has also been shown to play a role in T lymphocyte activation and trafficking. Following antigen stimulation, CD8⁺ T cells undergo clonal expansion accompanied by Ucp2 upregulation and metabolic reprogramming. Knockdown of Ucp2 leads to reduced glycolysis, fatty acid synthesis, ROS production, and survival during CD8⁺ T cell *in vitro* activation [118]. During experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis, UCP2 was found to be upregulated in T lymphocytes recruited to the spinal cord during periods of neuroinflammation [119]. Whether Ucp2 expression exacerbates autoimmune disease or helps fight infection likely depends on the role of ROS in dealing with specific pathogens or pathological condition. Nonetheless, these data suggest a role for UCP2 in regulating inflammation and lymphocyte activation through modulation of ROS production.

5. CONCLUSIONS

MDs are clinically heterogeneous multisystemic disorders due to mutations in genes involved in mitochondrial maintenance and function. Rare genetic diseases like MDs have always served as a critical starting point for understanding human biology. This top-down approach to investigating both diseased and healthy states has the advantage of an abundance of genetic defects and complex phenotypes, as well as social relevance.

While many existing mouse models of mitochondrial disease genes potentially could be, or already are, useful for exploring the intersection of mitochondrial biology and immune cell function, it is important to note potential current limitations. Emerging evidence suggests a pivotal role for signaling from the mitochondria to the nucleus in regulating cell function, and many of current MD mouse models are on different nuclear backgrounds. This, combined with the emerging availability of mitochondrial replacement therapy to women at risk of transmitting mitochondrial disease to their children, makes understanding the nuances of nuclear:mitochondrial interactions even more relevant (reviewed in [120]). Furthermore, knockout mutations leading to MD in humans are rare [121], and many of the mouse models for studying MDs currently exist as complete knockouts. For example, Charcot Marie Tooth Type 2A (i.e. *MFN2* mutations, Figure, panel C) is inherited in an autosomal dominant fashion, whereas the mouse model for *MFN2*-deficiency is a floxed allele which results in a complete loss of Mfn expression. However, with heightened interest in understanding the bioenergetics of immune cells and the advent of CRISPR-Cas gene editing techniques, we anticipate an exponential expansion of more physiologically relevant mouse models of MD in the coming years.

The laboratory mouse continues to be a powerful tool for exploring phenotypes, and developing a deeper understanding of human disease pathophysiology and basic biology. As such, we propose that understanding the role of the mitochondria in immune function begins with patients with MD. Leveraging the tools of model organisms will result in a deeper understanding of mitochondrial biology.

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ABBREVIATIONS

MD(s)	mitochondrial disease(s)
OXPHOS	oxidative phosphorylation
FAO	fatty acid oxidation
SIRS	systemic inflammatory response syndrome

KD	knockdown
ChIP	chromatin immunoprecipitation
CD4-Cre	CD4 <i>cre</i> recombinase
DAMPs	damage associated molecular patterns
TH1	T helper 1 cells
TH2	T helper 2 cells
TH17	T helper 17 cells
TREG	T regulatory cells
ROS	reactive oxygen species
KO	knockout
EAE	experimental autoimmune encephalomyelitis
MNGIE	mitochondrial neurogastrointestinal encephalopathy
SANDO	sensory ataxic neuropathy dysarthria, and ophthalmoparesis)
SCAE	spinocerebellar ataxia with epilepsy
CRISPR	clustered regularly interspaced short palindromic repeats
PS	phosphatidylserine
PEO	progressive external ophthalmoplegia
IOSCA	infantile-onset spinocerebellar ataxia
STAT	signal transducer and activator of transcription
PRR	proline-rich region
RIPK3	receptor-interacting serine/threonine protein kinase 3
NFAT	nuclear factor of activate T cells
NKT	natural killer T cell
BMDC	bone marrow-derived dendritic cell
MEF	mouse embryonic fibroblast

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169. MITOMAP.

Figure. Human mitochondrial morbidity map as a guide for identifying mitochondrial disease patients that may have defects in innate or adaptive immune responses

Cartoon provides a list of known mtDNA or nDNA-encoded mitochondrial disease-associated genes, and in which key mitochondrial function that gene product is known to play a role: (A) mitochondrial calcium transport, mitochondrial membrane (B) fission and (C) fusion, (D) mtDNA replication and stability, or (E) the respiratory chain. Genes listed were compiled from MitoMap [169].

Table
Existing mouse models of mitochondrial dysfunction

Listed in this table are selected examples of human mitochondrial disease-associated genes, pathologies, and the existing mouse models of the disease genes. Included for each are any experimental links to immune dysfunction, either in the mouse model, cell culture systems, or clinical reports.

MOUSE MODELS OF MITOCHONDRIAL DISEASE				
mtDNA REPLICATION, TRANSCRIPTION, TRANSLATION, AND MAINTENANCE				
Human Gene	Name & Function	Human Disease (OMIM)	Mouse model	Experimental links to immune dysfunction
<i>POLG</i>	Polymerase γ mtDNA polymerase, replication	Mitochondrial depletion syndromes Alpers and MNGIE, Mitochondrial ataxia syndromes SANDO and SCAE (174763)	Transgenic mice expressing common human <i>POLG</i> ^{Y955C} mutation [122]	Increased apoptotic markers in tissues with high turnover (ie. thymus) isolated from aged <i>POLG</i> mice [85]
			Mutator mouse, mutant <i>POLG</i> knock-in with defective proofreading 3'–5' exonuclease activity [85, 123]; model for ageing	
<i>TFAM</i>	Transcription factor A mtDNA maintenance, packaging, replication, transcription	Mitochondrial depletion syndrome type 15 (600438)	Tissue-specific <i>Tfam</i> deletion of two terminal exons achieved in <i>Tfam</i> ^{loxP} mice via <i>cre</i> -mediated excision [65]; model for mtDNA depletion	TFAM deletion in CD4+ T cells exacerbates inflammatory responses through complex interplay between respiration and lysosomal function [76]
				mtDNA released into cytoplasm associated with TFAM deletion primes the antiviral innate immune response in MEFs and BMDMs [80]
<i>RNaseH1</i>	Ribonuclease H1 degradation of RNA/DNA hybrids	PEO w/muscle weakness, mtDNA deletions (604123)	RNaseH1 ^{flx} tissue-specific deletion via <i>cre</i> -mediated excision; model for mitochondrial liver dysfunction and regeneration [124]	None reported
<i>TWINK</i>	Twinkle mtDNA helicase	Mitochondrial depletion syndrome type 7, Perrault syndrome, PEO w/ mtDNA deletions, IOSCA (606075)	Deletor mouse, multiple patient PEO mutations [125]	None reported
			Knock-in mouse expressing c. 1526A>G IOSCA patient mutation (p.Y509C) [126]	
<i>TK2</i>	Thymidine kinase 2, mitochondrial dNTP synthesis for mtDNA replication	PEO w/mtDNA deletions, Mitochondrial depletion syndrome type 2 (188250)	<i>Tk2</i> KO mouse; deletion of exon 4 and part of exon 5 affecting binding domain of enzyme active site [127]	None reported
			Knock-in mouse expressing c.378–379CG>AA (H126N) PEO patient mutation (p.H121N) [128, 129]; model for	

MOUSE MODELS OF MITOCHONDRIAL DISEASE				
mtDNA REPLICATION, TRANSCRIPTION, TRANSLATION, AND MAINTENANCE				
Human Gene	Name & Function	Human Disease (OMIM)	Mouse model	Experimental links to immune dysfunction
			mitochondrial depletion syndrome	
<i>ANT1-3</i>	Adenine nucleotide translocator(s) ADP/ATP transporter, apoptosis	Mitochondrial depletion syndrome type 12; PEO (103220)	<i>ANT1</i> KO mice deficient in skeletal muscle and heart due to tissue-specific ANT1 isoform expression [130]; model for multiple mitochondrial depletion syndromes	Altered ANT isoform expression correlates with enteroviral infection and reduced CD8+ T cell infiltration in human heart tissue [131]
			<i>ANT2</i> hypomorphs, targeted deletion of exon 2 and 3 via <i>cre</i> -mediated excision [132]	ANT2 hypomorphs exhibit B lymphocytopenia, altered OXPHOS, and cell death [132]
				ANT3 expression in T cells is regulated by IL-4 and IFN γ via STAT signaling and influences <i>in vitro</i> T cell survival [133]
<i>LRPPRC</i>	Leucine-rich PRR motif-containing protein Post-transcriptional regulation of mt genes	Leigh syndrome, French-Canadian type (607544)	Targeted deletion of exons 3, 4, 5 via <i>cre</i> -mediated excision [134, 135]	None reported
<i>DARS2</i>	Aspartyl-tRNA synthetase 2 tRNA synthesis	Leukoencephalopathy w/brain stem and spinal cord involvement (610956)	Targeted deletion of exon 3 via <i>cre</i> -mediated excision [136]	None reported
<i>MTO1</i>	Mitochondrial translation optimization 1 mt tRNA modification	Combined OXPHOS deficiency 10 (614667)	Homozygous hypomorphic MTO1 gene trap mice [137]	None reported
ANTIOXIDANT DEFENSE				
Human Gene	Name & Function	Human Disease (OMIM)	Mouse model	Experimental links to immune dysfunction
<i>NNT</i>	Nicotinamide nucleotide transhydrogenase Redox regulation	Glucocorticoid deficiency (607878)	C57BL/6J mice have a spontaneous deletion in the <i>Nnt</i> coding region leading to absence of mature NNT protein	C57BL/6J, in contrast with NNT-expressing transgenic C57BL/6J, mice exhibit increased resistance to <i>Strep pneumo</i> infection, stronger macrophage-mediated inflammatory responses [138]
<i>GPX1</i>	Glutathione peroxidase H ₂ O ₂ production	Hemolytic anemia (138320)	<i>GPX1</i> mutant allele mice generated via homologous recombination [139]	Gpx1 KO mice exhibit reduced Th1 cytokine production in response to ConA treatment [140]
				DSS-induced colitis was attenuated in Gpx1 ^{-/-} mice associated w/ hyperfunctional Tregs and attenuated Th17 differentiation [141]
				Influenza challenged Gpx1 ^{-/-} mice exhibit increased macrophage & neutrophil lung infiltration, influenza-specific CD8+ splenic T cells [142]

MOUSE MODELS OF MITOCHONDRIAL DISEASE				
mtDNA REPLICATION, TRANSCRIPTION, TRANSLATION, AND MAINTENANCE				
Human Gene	Name & Function	Human Disease (OMIM)	Mouse model	Experimental links to immune dysfunction
<i>SOD2</i>	Superoxide dismutase 2 Free radical scavenging	Microvascular complications with diabetes (147460)	<i>Sod2</i> ^{m1Cje} on CD1 background [143]	Conditional loss of SOD2 in T cells (<i>Lck-Cre</i>) results in increased thymocyte apoptosis [144]
			<i>Sod2</i> ^{m1Leb} on B6 background [145]	
OXIDATIVE PHOSPHORYLATION				
Human Gene	Name & Function	Human Disease (OMIM)	Mouse model	Experimental links to immune dysfunction
<i>UCP2</i>	Uncoupling protein 2 Regulation of mt membrane potential/ROS	obesity susceptibility, type II diabetes (601693)	Targeted disruption of exons III and IV encoding N terminal UCP2 [104]	UCP-2 KO mice exhibit accelerated diabetes [116], resistance to Toxo infection [104], delayed Listeria-induced death [117] associated with altered ROS-producing macrophage recruitment and activation
				Possible role for UCP2 in T lymphocyte recruitment to spinal cord in EAE models [119]
				UCP2 expression in CD8+ T cells promotes differentiation and survival [118]
<i>COX10</i>	Cytochrome C oxidase assembly factor Complex IV assembly	Complex IV deficiency, Leigh syndrome (602125)	COX10 ^{flx} mice: tissue-specific exon 6 deletion via <i>cre</i> -mediated excision [146]; model for COX deficiency	COX10-deficient mice have impaired CD4+ T cell subset differentiation [12, 99] antibacterial [99], and anti-viral responses[12]
<i>SURF1</i>	Surfeit 1 Complex IV assembly	Charcot-Marie Tooth disease, Leigh syndrome (185620)	<i>Surf1</i> disruption via insertion of a <i>loxP</i> site in exon 7, results in prematurely truncated, unstable protein [147]; model for COX deficiency	None reported
<i>NDUFS4</i>	NADH ubiquinone oxioreductase Complex I subunit	Leigh syndrome, Complex I deficiency (602694)	<i>Cre</i> -mediated deletion of exon 2 generates frameshift and undetectable protein in <i>Ndufs4</i> ^{lox/lox} mice [148]; model for Complex I deficiency	Increased innate and inflammatory response in retinas of <i>Ndufs4</i> KO mice associated w/blindness [149]
				Global <i>Ndufs4</i> deletion in mice leads to spontaneous macrophage-mediated systemic inflammation associated w/elevated serum FAs, likely from Complex I-deficient hepatocytes [150]
MITOCHONDRIAL MEMBRANE DYNAMICS AND INTEGRITY				
Human Gene	Name & Function	Human Disease (OMIM)	Mouse model	Experimental links to immune dysfunction
<i>OPA1</i>	Mitochondrial dynamin like GTPase Mitochondrial membrane fusion	Mitochondrial depletion syndrome type 14, Behr syndrome, Optic atrophy plus syndrome (605290)	ENU-induced mutant mouse w/nonsense mutation (O285STOP). in	OPA1 KD in BMDCs resulted in reduced expression of chemokines

MOUSE MODELS OF MITOCHONDRIAL DISEASE				
mtDNA REPLICATION, TRANSCRIPTION, TRANSLATION, AND MAINTENANCE				
Human Gene	Name & Function	Human Disease (OMIM)	Mouse model	Experimental links to immune dysfunction
			exon 8, ~50% reduction in Opa1 protein in all tissues [151]	important for migration [152]
			<i>Cre</i> -mediated excision of exons 10–13 results in loss of detectable <i>Opa1</i> [153]	OPA1 is required for memory T cell formation [61]
MFN2	Mitofusin 2 Mitochondrial membrane fusion	Charcot-Marie-Tooth disease, Motor and sensory neuropathy (608507)	<i>Mfn2^{loxP}</i> floxed mice, tissue-specific deletion of exon 6 via <i>cre</i> -mediated excision [154]	MFN2-mediated mitochondria-ER tethering maintains lymphoid-biased HSCs through calcium-dependent NFAT signaling [155]
			MitoCharc1 transgenic mice w/ <i>Eno2</i> promoter directing R94Q mutated human MFN2; mouse model for Charcot-Marie-Tooth disease type2A (CMT2A) [156]	Activation-induced downregulation of MFN2 is a prerequisite for human T cell proliferation and entry into cell cycle [157]
DRP1	Dynammin-related protein 1 Mitochondrial membrane fusion	Encephalopathy, lethal (603850)	Drp1 KO generated via in frame deletion of exon 2 using Cre-loxP system [158]	Reduced NKT cell activation following disruption of RIPK3/PGAM5/Drp1/NFAT signaling axis [159]
				Inhibition of Drp1 in T cells results in reduced ROS, NFkB associated with impaired proliferation and activation-induced cell death [160]
				Drp1 controls mitochondrial positioning at the immunological synapse and modulates localized TCR signaling [161]
TAZ	Tafazzin 1 Mitochondrial membrane cardiolipin content	Barth syndrome (300394)	TAZKD mice undergo tetracycline inducible shRNA-mediated TAZ knock-down; mouse model of Barth syndrome [162]	Lymphoblasts from Barth syndrome patients exhibit reduced respiratory chain super complex formation [163, 164], increased mitochondrial biogenesis [165], and cell death [164, 166]
				TAZ KD in HL60 human myeloid progenitor cells led to loss of mitochondrial membrane potential and increased cell death [167]
				Neutrophils from Barth syndrome patients undergo PS exposure-independent apoptotic mechanism, are not recognized by macrophages [168]

MOUSE MODELS OF MITOCHONDRIAL DISEASE				
mtDNA REPLICATION, TRANSCRIPTION, TRANSLATION, AND MAINTENANCE				
Human Gene	Name & Function	Human Disease (OMIM)	Mouse model	Experimental links to immune dysfunction
CALCIUM FLUX				
Human Gene	Name & Function	Human Disease (OMIM)	Mouse model	Experimental links to immune dysfunction
<i>MICU1</i>	Mitochondrial calcium uptake 1 Calcium import	Myopathy with extrapyramidal signs (605084)	CRISPR-mediated deletions generated MICU1 KOs via targeting exon 2 or translational initiation codon [53]	Mitochondrially-mediated Ca ²⁺ uptake buffers cytosolic Ca ²⁺ in mast cells [46]
			Cre-loxP system used to target exon 3 of the <i>MICU1</i> gene for removal [52]	