Article Title: Abnormal mitochondrial physiology in the pathogenesis of systemic lupus erythematosus

Authors

Chris Wincup BSc (hons), MBBS, MRCP, Department of Rheumatology, Division of Medicine, University College London, United Kingdom

Anna Radziszewska BSc (hons), MSc, Centre for Adolescent Rheumatology Versus Arthritis at UCL, UCLH, GOSH, London, UK Centre for Rheumatology Research, Division of Medicine, University College London, United Kingdom

Mailing address and email address

CW – Rayne Institute, University College London, 5 University Street, London, WC1E 6JF, United Kingdom

c.wincup@ucl.ac.uk

Twitter @chriswincup

AR – Rayne Institute, University College London, 5 University Street, London, WC1E 6JF, United Kingdom

ania.radziszewska@ucl.ac.uk

Corresponding author – Chris Wincup (c.wincup@ucl.ac.uk)

Disclosure Statement – The authors have nothing to disclose

Key Words

- Systemic lupus erythematosus
- Mitochondria
- Immunometabolism
- Reactive oxygen species
- T-cells
- Autoimmunity
- Mitophagy
- Anti-mitochondria antibodies

Key Points

1. Activation of immune responses requires a significant increase in cellular energy generation in order to initiate and sustain effector cell functions.
2. Aside from their role in energy metabolism, mitochondria are the primary source of endogenous reactive oxygen species and orchestrate apoptosis.
3. Both T-cells and macrophages from patients with SLE have been shown to demonstrate enhanced cellular energy demands.
4. Oxidative stress induced damage to genomic and mitochondrial DNA can promote the formation of autoantibodies directed against nuclear components.
5. Metabolic reprogramming may restore homeostatic immune cell function and thus represents a potentially novel avenue of future drug development in SLE.

Synopsis

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterised by abnormalities within the innate and adaptive immune system. Activation and proliferation of a wide array of immune cells requires significant upregulation in cellular energy metabolism with the mitochondria playing an essential role in the initiation and maintenance of this response.

Herein, we highlight how abnormal mitochondrial function may occur in SLE and focus on how energy metabolism, oxidative stress and impaired mitochondrial repair play a role in the pathogenesis of the disease. We also discuss how this may represent an appealing novel therapeutic target for future drug therapy in SLE.

Introduction

Systemic lupus erythematosus (SLE) is a chronic, systemic autoimmune condition characterised by the formation for autoantibodies directed against nuclear components (1). Over recent years there have been a number of significant advances in the understanding of the underlying pathological mechanisms at play in the development of this highly heterogenous disease (2-4). Abnormalities within both the innate and adaptive immune response have been demonstrated to play a role in the pathogenesis (5). These immune processes require significant changes in cellular activation and proliferation in order to induce antibody and pro-inflammatory cytokine production. However, until recently, the way in which these vast and highly active systemic immune responses are initiated and maintained in terms of cellular bioenergetics has been poorly understood. Here, we report on the latest understanding with regards to the changes in cellular energy metabolism observed within the immune system and how this may result in SLE pathogenesis. Furthermore, we consider how these advances may be utilised in clinical care as a novel target for future therapeutic options in the management of the disease. In particular, we focus on the role of the mitochondria, the intracellular organelle that not only plays an essential role in energy metabolism, but also in a number of other cellular processes that are relevant to the underlying pathogenesis of SLE.

The role of mitochondria in energy metabolism in health

All cellular processes require a careful balance between energy generation and energy consumption. Before considering how this is altered in SLE, it is important to understand energy biogenesis in health. A variety of metabolic substrates are required for energy

production and predominantly include carbohydrates (such as polysaccharides), lipids (including triglycerides) and proteins (that are broken down into amino acids). Conversion of these substrates to energy in the form of adenosine triphosphate (ATP) can occur through either the breakdown of glucose molecules via glycolysis or through oxidative phosphorylation (OXPHOS) within the mitochondria. This is summarised in Figure 1.

Glycolysis is the primary metabolic pathway and foundation of both aerobic and anaerobic respiration. The process centres on the breakdown of glucose (a six-membered ring molecule) through a series of reactions. This is a large molecule that enters the cell from the circulation by facilitated diffusion through cell surface glucose transporters. Oxidation of a single glucose molecule results in the generation of four ATP molecules, however, two ATP molecules are used in this series of reactions, therefore resulting in a net gain of two ATP molecules per glucose. Oxidation of glucose results in the release of electrons that in turn results in the generation of intermediate molecules through the reduction of NAD^+ to $NADH^+$. Another important product of glycolysis is pyruvic acid, which under anaerobic conditions forms lactic acid. Importantly, if there is sufficient oxygen (O_2) then pyruvic acid enters the mitochondria where it is converted to acetyl coenzyme A (acetyl CoA), which enters the Krebs cycle.

When a sufficient supply of glucose is not available, lipids can be converted into fatty acids by the enzyme lipase, which is also able to be converted to acetyl CoA. Within the Krebs cycle, acetyl CoA is converted to citrate and the continued oxidation of the intermediate molecules produced during glycolysis occurs, which in turn results in the production of carbon dioxide. Ultimately this series of chemical reactions generates energy through the oxidation of acetyl CoA and the resultant hydrogen carrying compounds generated from these reactions ($NADH^+$ and $FADH_2$) are then used to generate ATP through OXPHOS.

The most abundant source of ATP generation is through OXPHOS, which takes place on the inner mitochondrial membrane electron transport chain (ETC). $NADH^+$ and $FADH_2$ generated from the Krebs cycle carry high energy electrons that are used to generate a proton gradient across the membrane as it allows for hydrogen ions (H^+) to be actively pumped across the membrane from the mitochondrial matrix to the intermembrane space. The ETC is comprised of five respiratory chain complexes that generate an electric potential within the mitochondrion. This is known as the mitochondrial transmembrane potential, $\Delta\Psi m$, in which the mitochondrial membrane is negatively charged on the outside and positively charged on the inside (6). Finally, H^+ is able to flux back across the membrane through the fifth respiratory chain complex (ATP synthase), which forms ATP via the phosphorylation of adenosine diphosphate (ADP). This is summarized in Figure 2.

Abnormal mitochondrial bioenergetics in SLE

OXPHOS vs Glycolysis

A number of recent studies have shown evidence of abnormal mitochondrial energy biosynthesis in the immune response in lupus. In health, quiescent T-cells have very low cellular energy demands and can use a combination of glucose, lipids and amino acids as substrates for OXPHOS (7, 8). Upon activation of the T-cell receptor, the naïve T-cell undergoes a rapid increase in biosynthesis. In order to meet these energy demands, the resultant T effector cell relies on increased ATP production predominantly through glycolysis in order to fuel the inflammatory effector function (9). Upon conclusion of the immune response, as the cell transitions towards a memory T-cell phenotype, it returns to a more quiescent metabolic

state in which fatty acid oxidation is the primary substrate of OXPHOS energy metabolism (10). These memory T-cells display increased mitochondrial mass and spare respiratory capacity, which suggests that they are primed to respond upon repeat activation in the future (8, 11). In the state of chronic T-cell activation, mitochondrial metabolism has been shown to be the predominant source of cellular ATP (12).

Studies in murine models of SLE have demonstrated that $CD4^+$ T-cells harvested from lupus-prone mice show significantly increased rates of both mitochondrial OXPHOS and glycolysis compared with healthy mice (13). In the same study, $CD4^+$ T-cells from patients with SLE were analysed and this too revealed enhanced ATP energy production through increased rates of glycolysis and OXPHOS when compared with healthy controls. Further evidence supporting enhanced glycolysis in the autoimmune response comes from mouse models in which overexpression of the key glucose transporter, Glut1, was noted on CD4+ T-cells. The authors also noted that the T-cell co-stimulatory molecule CD28 was required to induce maximal glucose uptake by the cell (14) suggesting that this has a vital role in facilitating energy metabolism required during T-cell proliferation and effector function. Further evidence of the role of altered metabolism is provided by the intracellular effects of complement on mitochondria within T-cells (15). Activation of the complement system is a hallmark of SLE with low C3 and C4 levels suggestive of active disease. Previously it has been suggested that complement can have numerous effects on the metabolic state of immune cells. For example, intracellular complement C1q has been shown to drive OXPHOS protein expression in muscle cells (16) and has also been demonstrated to restrict the adaptive immune response to self-antigens through altering $CD8^+$ T-cell metabolism (17). More recently, it has been proposed that this response is predominantly driven by Type I Interferon, another key driver of SLE

pathogenesis (18). This represents an interesting area of potential further research exploring the interaction between abnormal T-cell metabolism and complement activation in SLE.

In addition to T-cell energy metabolism, macrophage metabolism has also been shown to be abnormal in SLE. In the context of inflammation, tissue resident macrophages switch to glycolysis as the primary source of energy production and this is associated with a reduction in OXPHOS derived energy biosynthesis (19). A study by Jing et al found that IgG immune complexes were capable of inducing glycolysis in macrophages (20). Interestingly, the authors also found that *in vivo* inhibition of glycolytic pathways resulted in reduced macrophage IL-1β production and decreased neutrophil recruitment to kidneys in a murine model of lupus nephritis. This suggests that by directly targeting cellular metabolism, it may be possible to reduce immune-mediated inflammation in SLE in the future.

Mitochondria transmembrane potential

There is growing evidence to support that the T-cell mitochondrial transmembrane potential ($\Delta\Psi m$) , is elevated in SLE. A study by Perl et al, reported that T-cells from patients with SLE had higher $\Delta\Psi m$ and were in a state of persistent mitochondrial hyperpolarization, thus suggesting that at a bioenergetic level, T-cell mitochondria are primed for a sudden increase in OXPHOS-dependent ATP production (21). As such, this may drive rapid effector T-cell responses that could be present in active SLE, however the precise balance between glycolysis and OXPHOS in the chronically activated immune response context of SLE is still poorly understood. The authors also reported that there was an increase in cytoplasmic alkalinization, more reactive oxygen species (ROS) generation, and reduced intracellular ATP in T-cells derived from patients with SLE.

In addition to the activation and proliferation of T-cells, mitochondria play an important role in cell death through apoptosis and the clearance of this apoptotic cellular matter has been found to be defective in the pathogenesis of SLE. It has previously been reported that $\Delta\Psi m$ disruption is an essential, irreversible step in the induction of apoptosis (22). Therefore, altered T-cell mitochondrial bioenergetics may not only play a vital role in the effector immune response in SLE but may also have a secondary role in apoptosis that may ultimately result in the exposure of self-antigens to autoantibodies in the disease. The precise reason for this persistent mitochondrial hyperpolarization in SLE is not clear, although it has previously been shown that several of the central pro-inflammatory mediators of SLE can induce hyperpolarization (23).

Abnormalities in the mitochondrial electron transport chain

As previously outlined, OXPHOS occurs at the site of the ETC on the inner mitochondrial membrane. As shown in Figure 2, the ETC is comprised of five individual respiratory chain complexes that allow for the sequential transfer of energy and ultimately generates the transmembrane gradient required to convert ADP to ATP. A number of studies have sought to investigate the function of the ETC and its components as a possible cause of abnormal immune cell bioenergy synthesis in SLE. A study by Leishangthem et al, used spectrophotometry to evaluate the activity of these complexes in peripheral blood mononuclear cells (PBMCs) from patients with SLE and in healthy controls. Complex I and IV activity was noted to be lower in those with SLE compared to healthy controls. In addition, Complex V enzymatic activity was found to be significantly reduced in SLE which may have implications for ATP generation as this complex plays an essential role in the conversion of ADP to ATP

(24). However, the underlying reason for the reduced enzymatic respiratory chain activity in SLE is not understood. Conversely, a subsequent study evaluated ETC activity using oxygraphy to measure mitochondrial O_2 consumption. Complex I activity was measured through the addition of its inhibitor Rotenone. The authors report that Complex I activity was increased in SLE T-cells after 24 hours of *in vitro* stimulation when compared with healthy controls. It was also found that Complex I is the main source of oxidative stress in SLE (25).

Mitochondrial oxidative stress in the pathogenesis of SLE

Oxidative stress refers to the state of impaired equilibrium in the body's ability to neutralise ROS. This often occurs as a result of excessive production of ROS that cannot sufficiently be counterbalanced by scavenging antioxidant reactions. ROS can include superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO•) (26). These are the result of incomplete reduction of oxygen molecules and often may include toxic by-products induced through impaired mitochondrial bioenergetics within the ETC (25, 27, 28). The role of oxidative stress in the pathogenesis of SLE has been well described, however the precise implication of mitochondrial derived ROS in the disease state is not yet fully understood (29). It is possible that a number of environmental factors that have reported to play a role in SLE pathogenesis may also do so through induction of ROS generation (30); such as ultraviolet (UV) light (31), tobacco smoking (32) and silica exposure (33).

Mitochondrial ROS have been reported to have a direct influence on immune cells. T-cells, in particular, are susceptible to oxidative stress which can alter their activation and induce pro-inflammatory cytokine release (6). T-cells derived from patients with SLE have previously shown to display significantly higher amounts of ROS, especially in those with active disease

(34). Furthermore, it has been reported that oxidative stress may induce lupus flares through inhibition of the intracellular ERK signalling pathway within T-cells (35). ROS also play a vital role as an induction signal in the initiation of apoptosis, which has been demonstrated to be markedly abnormal in SLE and ultimately results in increased exposure of self-antigens from cellular debris that in turn results in autoantibody formation. In addition, ROS may be generated as a result of increased mitochondrial permeability, which is a terminal event in cell death (36).

ROS have also been shown to result in oxidative stress through interaction with other metabolites involved in cellular bioenergetics, such as lipids (in which mitochondria play a key role in fatty acid oxidation). This has been demonstrated by Park et al, who investigated how oxidative stress results in changes to oxidation status and susceptibility to oxidation of lipoproteins in patients with SLE. It was found that in spite of similar serum levels of low-density lipoproteins (LDL) between SLE patients and healthy controls, those derived from patients with SLE were significantly more oxidized. Furthermore, *in vitro* studies confirmed that LDL derived from patients with SLE exhibited higher rates of *de novo* oxidation (37). The authors suggest that this may in turn result in vascular inflammation that may prompt premature atherosclerosis, which is a major challenge in the clinical management of SLE.

Another potential role for ROS in the pathogenesis of SLE is through their role in damaging DNA, which is a primary antigenic target of autoantibodies in the disease (38). This has the potential to induce immunogenicity against self-antigens, in particular to nuclear components, which is a hallmark of SLE pathogenesis. Oxidative stress may not only give rise to damage of genomic DNA but also has the potential to target mitochondrial DNA (mtDNA), which is particularly susceptible to the effects of oxidative stress (39). This damage may in turn result

in impaired cellular energy metabolism given that mtDNA encodes the thirteen proteins that give rise to the ETC complexes (40).

Mitochondria are not only capable of inducing an autoimmune inflammatory response as a result of oxidative stress, but they may play a role in the pathogenesis as a result of an abnormal response to impaired repair of damage that they sustain following exposure to ROS.

Impaired mitochondrial repair in SLE

Mitophagy

Within the cell, mitochondria are constantly undergoing a state of fusion and fission. When a mitochondrion is damaged it is removed by a specialist type of autophagy, known as mitophagy (41). This process is essential for maintaining mitochondrial homeostasis and keeping the immune system in check. The persistence of dysfunctional mitochondria can impact on ROS generation which in turn can lead to inflammatory cytokine secretion, and immune cell activation (42). In addition, as outlined above, the presence of mtDNA from fragmented mitochondria can elicit abnormal immune responses (42).

It has been shown that lupus T-cells are resistant to autophagy and that mitophagy is supressed in T-cells of SLE patients and in lupus prone mice (43, 44). In T-cells from lupus patients mitochondrial hyperpolarization leads to increased mammalian target of rapamycin (mTOR) activation which in turn leads to overexpression of HRES-1/RAB4 protein and depletion of Drp1 (a key mediator of mitophagy) (45). Overexpression of RAB4A protein (the mouse equivalent of the human HREs-1/RAB4) in lupus prone mice results in accumulation of

mitochondria, ANA production, and nephritis (44). These disease manifestations, however, can be alleviated by RAB4A blockade indicating that the modulation of mitophagy may serve as a potential therapeutic strategy (44).

Current evidence also suggests that in SLE monocytes IFN-α signalling induces oxidative stress and affects lysosomal alkalinization through mTOR (46). The resultant impairment of mitophagy leads to accumulation of mtDNA which is sensed by stimulator of interferon genes (STING) to promote the differentiation of monocytes into autoreactive dendritic cells (46). Taken together, growing evidence of defects in mitophagy in multiple immune cell lineages in lupus suggests that aberrations in this process may be important in the pathology of the disease and this avenue of research warrants further investigation.

Anti-mitochondrial antibody formation

More recently there has been increased focus on mitochondria as a possible target for auto-antibody formation in SLE. A variety of cell linages including T-cells (47) and neutrophils (48) are able to extrude their mitochondria outside the cell as a consequence of cell death pathway activation. Mitochondria may also be released from cells during tissue damage and inflammation and mitochondrial components, such as mtDNA, can enter the extracellular milieu during cell death (49, 50) . These processes expose mitochondrial antigens, which under normal conditions would be sequestered inside cells, and this may contribute to breaking of tolerance in predisposed individuals.

Antibodies directed at several components of the mitochondrion, including to mtDNA, the inner mitochondrial membrane, and mitochondrial RNA (mtRNA) as well as antibodies to

whole mitochondria have been reported in lupus. Anti-mtDNA antibodies are present in a subset of lupus patients (51), particularly in those with active disease and in lupus nephritis (52, 53), raising the possibility that mtDNA can be a source of antigen for double stranded DNA (ds-DNA) antibodies common in SLE (54).

Antibodies targeting inner mitochondrial components are also found in SLE. For instance, antibodies to cardiolipin (a phospholipid found uniquely on the inner mitochondrial membrane) are detectable in some patients with SLE and antiphospholipid syndrome (APS) (53). The presence of these antibodies is clinically associated with increased risk of thrombotic events and thrombocytopaenia (55). HSP60 which is a chaperonin involved in mitochondrial protein transport is another potential mitochondrial antigen. Antibodies to HSP60 are present in patients with SLE (56) and are associated with vascular events in patients with anti-phospholipid antibodies (57).

A recent study found that antibodies to mitochondrial RNA (AmtRNA) are also present in lupus patients (58). AmtRNA-IgG levels correlated with anti mtDNA antibody titres and were highest in patients with anti-dsDNA antibodies. Although further studies are needed to confirm these findings, the researchers were able to use AmtRNA-IgG titres to specifically discriminate patients from healthy controls. AmtRNA-IgG titres were also found to be negatively associated with plaque formation and nephritis suggesting the potential for using AmtRNA-IgG titres to stratify patients and help predict those at risk of kidney damage (58).

Finally, patients with SLE have also been reported to have higher levels of antibodies to whole mitochondria (53, 59). In a large study of 204 participants with SLE, levels of anti- whole-

mitochondria antibodies were higher in active patients and correlated with levels of anti-dsDNA (59).

Collectively these findings suggest that in SLE the adaptive immune system recognises mitochondrial components. However, it is as yet unclear whether anti mitochondrial antibodies are initiators of the autoimmune response or whether they are the consequence of aberrant immune activation. The suggestion that various clinical disease manifestations of SLE associate with different mitochondrial antibody specificities raises the possibility of using anti mitochondrial antibody titres to predict disease activity and to stratify SLE patients in the future (53).

Targeting mitochondria in the treatment of SLE

Having highlighted the various ways in which mitochondrial physiology is altered in SLE, in is important to consider how restoring bioenergetic homeostasis may in turn improve symptoms of the disease. Furthermore, these new treatment options may potentially convey additional benefits as metabolic reprogramming could restore normal cellular function without resulting in systemic immunosuppression. Novel metabolic therapeutic targets have shown promise in both *in vitro* and animal models of SLE.

Evidence for the use of treatment targeting the mitochondria in animal models of SLE was recently reported by Fortner et al, who demonstrated the efficacy of oral MitoQ, a mitochondrial anti-oxidant, which was found to significantly reduce neutrophil ROS formation in the MRL-*lpr* murine model of SLE (60). The authors also noted that in the mice treated with MitoQ also had a significant reduction in serum Type I interferon (INF), which is known to

play a central role in SLE pathogenesis. This provides interesting evidence to support the role of directly altering mitochondrial function to bring about restoration of immune homeostasis. This has also been considered in a study by Blanco et al, in which the authors investigated the potential benefits of the mitochondrial coenzyme analog, Idebenone, on metabolic and immunological markers of SLE in the MRL-*lpr* murine model. Mice that received Idebenone for eight weeks were found to have substantially improved survival compared with untreated mice. Furthermore, those receiving Idebenone were noted to have less glomerular damage and improved mitochondrial metabolism (with a 30% increase in ATP production observed) (61).

In addition to investigating novel drugs that directly target the mitochondria, a number of recent studies have looked at the potential role of repurposing drugs that are already used in the treatment of a variety of other disorders in clinical practice that act by restoring cellular bioenergetics. Metformin, which is commonly used in the management of diabetes, has been demonstrated to restore metabolic homeostasis in $CD4^+$ T-cells derived from a murine mouse model of SLE. The authors also evaluated the potential benefit of 2-deoxy-D-glucose (2-DG), which acts a competitive inhibitor in the production of glucose-6-phosphate from glucose (a rate limiting step in glycolysis). This was demonstrated to reduce the production of INFγ by $CD4^+$ T-cells *in vitro* thus suggesting that it may be possible to downregulate generation of this key inflammatory mediator of SLE (13). In addition to targeting T-cell glycolysis, inhibition of macrophage glycolytic pathways has been shown to reduce disease severity and attenuated pro-inflammatory cytokine release in animal models of lupus nephritis (20).

Another potential target for metabolic reprogramming in SLE is mTOR, which can be inhibited through the use of Rapamycin (Sirolimus). This macrolide compound that has been shown to reduce the activity of T-cells by reducing their sensitivity to Interleukin-2 (IL-2) as an effect

of mTOR inhibition (62). The efficacy of Rapamycin has previously been investigated in mouse models of lupus. It was found that mice treated with the drug had significantly reduced levels of anti-dsDNA antibodies, lower proteinuria and significantly increase in survival when compared with untreated mice (63). This has also been translated into human studies by Fernandez *et al*, who studied the efficacy of Rapamycin in patients with refractory SLE, in which previous treatments had not been successful in controlling the disease. Nine patients were given a dose of 2 mg a day for between six and forty-eight months, whilst seven patients with refractory SLE receiving standard care were recruited as controls. In those treated with Rapamycin, there was a statistically significant reduction in disease activity as measured by both the British Isles Lupus Assessment Group (BILAG) score and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (64). The authors also investigated how this treatment mechanistically altered T-cell metabolism and noted that although mitochondrial hyperpolarization persisted, T-cell activation was reduced in those taking Rapamycin.

In addition, N-acetylcysteine (NAC), which is more commonly used in the management of paracetamol overdose, has demonstrated to be effective in stabilizing mitochondrial metabolism *in vitro*. A study by Doherty *et al* found that NAC selective inhibits the activity of Complex I on the ETC (25). The authors measured mitochondrial respiration as a marker of ETC activity in peripheral blood lymphocytes from patients with SLE and noted increased O_2 consumption following T-cell stimulation. When these cells were treated with NAC, mitochondrial respiration was significantly reduced, thus suggesting that this drug could have an immunomodulatory effect on immune cell metabolism in SLE. However, further studies and especially larger clinical trials are required to assess whether these drugs could be effective in long-term management of the disease.

Conclusions

In summary, there is increasing evidence that abnormal immunometabolism may play a role in the pathogenesis of SLE. In particular, there is a growing understanding of how these changes occur in both T-cell and macrophage bioenergetics and how this plays a role in pathogenesis. However, given the wide array of effector cells involved in mediating the autoimmune responses of the disease, there is still much to learn (in particular in relation to the role played by alteration to B-cell metabolism). Future studies are required in order to better translate this understanding into clinical care. It is possible that in the future, SLE could be stratified by immunometabolic signature and strategies directed at restoring immune homeostasis through metabolic reprogramming could pave the way for newer, more targeted and better tolerated treatments for this disease.

References

1. Bakshi J, Segura BT, Wincup C, Rahman A. Unmet Needs in the Pathogenesis and Treatment of Systemic Lupus Erythematosus. Clin Rev Allergy Immunol. 2018;55(3):352-67.
2. Bruce IN, O'Keeffe AG, Farewell V, Hanly JG, Manzi S, Su L, et al. Factors associated with damage accrual in patients with systemic lupus erythematosus: results from the Systemic Lupus International Collaborating Clinics (SLICC) Inception Cohort. Ann Rheum Dis. 2015;74(9):1706-13.
3. Carter EE, Barr SG, Clarke AE. The global burden of SLE: prevalence, health disparities and socioeconomic impact. Nat Rev Rheumatol. 2016;12(10):605-20.
4. Murphy G, Isenberg DA. New therapies for systemic lupus erythematosus - past imperfect, future tense. Nat Rev Rheumatol. 2019;15(7):403-12.
5. Tsokos GC, Lo MS, Costa Reis P, Sullivan KE. New insights into the immunopathogenesis of systemic lupus erythematosus. Nat Rev Rheumatol. 2016;12(12):716-30.
6. Perl A, Gergely P, Jr., Nagy G, Koncz A, Banki K. Mitochondrial hyperpolarization: a checkpoint of T-cell life, death and autoimmunity. Trends Immunol. 2004;25(7):360-7.
7. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. Nature reviews Immunology. 2005;5(11):844-52.

8. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. Science. 2013;342(6155):1242454.
9. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, et al. The CD28 signaling pathway regulates glucose metabolism. Immunity. 2002;16(6):769-77.
10. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. Annu Rev Immunol. 2013;31:259-83.
11. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. J Immunol. 2011;186(6):3299-303.
12. Byersdorfer CA, Tkachev V, Opipari AW, Goodell S, Swanson J, Sandquist S, et al. Effector T cells require fatty acid metabolism during murine graft-versus-host disease. Blood. 2013;122(18):3230-7.
13. Yin Y, Choi SC, Xu Z, Perry DJ, Seay H, Croker BP, et al. Normalization of CD4+ T cell metabolism reverses lupus. Science translational medicine. 2015;7(274):274ra18.
14. Jacobs SR, Herman CE, Maciver NJ, Wofford JA, Wieman HL, Hammen JJ, et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. J Immunol. 2008;180(7):4476-86.
15. Rahman J, Singh P, Merle NS, Niyonzima N, Kemper C. Complement's favourite organelle-Mitochondria? Br J Pharmacol. 2020.
16. Feng H, Wang JY, Zheng M, Zhang CL, An YM, Li L, et al. CTRP3 promotes energy production by inducing mitochondrial ROS and up-expression of PGC-1α in vascular smooth muscle cells. Exp Cell Res. 2016;341(2):177-86.
17. Ling GS, Crawford G, Buang N, Bartok I, Tian K, Thielens NM, et al. C1q restrains autoimmunity and viral infection by regulating CD8(+) T cell metabolism. Science. 2018;360(6388):558-63.
18. Buang N, Tapeng L, Gray V, Sardini A, Whilding C, Lightstone L, et al. Type I interferons affect the metabolic fitness of CD8(+) T cells from patients with systemic lupus erythematosus. Nature communications. 2021;12(1):1980.
19. Galván-Peña S, O'Neill LA. Metabolic reprograming in macrophage polarization. Front Immunol. 2014;5:420.
20. Jing C, Castro-Dopico T, Richoz N, Tuong ZK, Ferdinand JR, Lok LSC, et al. Macrophage metabolic reprogramming presents a therapeutic target in lupus nephritis. Proc Natl Acad Sci U S A. 2020;117(26):15160-71.
21. Perl A, Hanczko R, Doherty E. Assessment of mitochondrial dysfunction in lymphocytes of patients with systemic lupus erythematosus. Methods in molecular biology (Clifton, NJ). 2012;900:61-89.
22. Susin SA, Zamzami N, Castedo M, Daugas E, Wang HG, Geley S, et al. The central executioner of apoptosis: multiple connections between protease activation and mitochondria in Fas/APO-1/CD95- and ceramide-induced apoptosis. J Exp Med. 1997;186(1):25-37.
23. Gergely P, Niland B, Gonchoroff N, Pullmann R, Phillips PE, Perl A. Persistent Mitochondrial Hyperpolarization, Increased Reactive Oxygen Intermediate Production, and Cytoplasmic Alkalinization Characterize Altered IL-10 Signaling in Patients with Systemic Lupus Erythematosus. The Journal of Immunology. 2002;169(2):1092-101.
24. Leishangthem BD, Sharma A, Bhatnagar A. Role of altered mitochondria functions in the pathogenesis of systemic lupus erythematosus. Lupus. 2016;25(3):272-81.
25. Doherty E, Oaks Z, Perl A. Increased mitochondrial electron transport chain activity at complex I is regulated by N-acetylcysteine in lymphocytes of patients with systemic lupus erythematosus. Antioxidants & redox signaling. 2014;21(1):56-65.
26. Nagy G, Koncz A, Fernandez D, Perl A. Nitric oxide, mitochondrial hyperpolarization, and T cell activation. Free radical biology & medicine. 2007;42(11):1625-31.

27. Chen SX, Schopfer P. Hydroxyl-radical production in physiological reactions. A novel function of peroxidase. Eur J Biochem. 1999;260(3):726-35.
28. Schopfer P, Plachy C, Frahry G. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. Plant Physiol. 2001;125(4):1591-602.
29. Shah D, Mahajan N, Sah S, Nath SK, Paudyal B. Oxidative stress and its biomarkers in systemic lupus erythematosus. J Biomed Sci. 2014;21(1):23.
30. Somers EC, Richardson BC. Environmental exposures, epigenetic changes and the risk of lupus. Lupus. 2014;23(6):568-76.
31. Golan TD, Dan S, Haim H, Varda G, Sol K. Solar ultraviolet radiation induces enhanced accumulation of oxygen radicals in murine SLE-derived splenocytes in vitro. Lupus. 1994;3(2):103-6.
32. Cui J, Raychaudhuri S, Karlson EW, Speyer C, Malspeis S, Guan H, et al. Interactions Between Genome-Wide Genetic Factors and Smoking Influencing Risk of Systemic Lupus Erythematosus. Arthritis & rheumatology (Hoboken, NJ). 2020;72(11):1863-71.
33. Morotti A, Sollaku I, Catalani S, Franceschini F, Cavazzana I, Fredi M, et al. Systematic review and meta-analysis of epidemiological studies on the association of occupational exposure to free crystalline silica and systemic lupus erythematosus. Rheumatology (Oxford, England). 2021;60(1):81-91.
34. Oates JC, Gilkeson GS. The biology of nitric oxide and other reactive intermediates in systemic lupus erythematosus. Clin Immunol. 2006;121(3):243-50.
35. Li Y, Gorelik G, Strickland FM, Richardson BC. Oxidative stress, T cell DNA methylation, and lupus. Arthritis & rheumatology (Hoboken, NJ). 2014;66(6):1574-82.
36. Jabs T. Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. Biochem Pharmacol. 1999;57(3):231-45.
37. Park JK, Kim JY, Moon JY, Ahn EY, Lee EY, Lee EB, et al. Altered lipoproteins in patients with systemic lupus erythematosus are associated with augmented oxidative stress: a potential role in atherosclerosis. Arthritis research & therapy. 2016;18(1):306.
38. Cooke MS, Mistry N, Wood C, Herbert KE, Lunec J. Immunogenicity of DNA damaged by reactive oxygen species--implications for anti-DNA antibodies in lupus. Free radical biology & medicine. 1997;22(1-2):151-9.
39. Jönsen A, Yu X, Truedsson L, Nived O, Sturfelt G, Ibrahim S, et al. Mitochondrial DNA polymorphisms are associated with susceptibility and phenotype of systemic lupus erythematosus. Lupus. 2009;18(4):309-12.
40. Lemarie A, Grimm S. Mitochondrial respiratory chain complexes: apoptosis sensors mutated in cancer? Oncogene. 2011;30(38):3985-4003.
41. Palikaras K, Lionaki E, Tavernarakis N. Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. Nature Cell Biology. 2018;20(9):1013-22.
42. Xu Y, Shen J, Ran Z. Emerging views of mitophagy in immunity and autoimmune diseases. Autophagy. 2020;16(1):3-17.
43. Alessandri C, Barbati C, Vacirca D, Piscopo P, Confaloni A, Sanchez M, et al. T lymphocytes from patients with systemic lupus erythematosus are resistant to induction of autophagy. Faseb j. 2012;26(11):4722-32.
44. Caza TN, Fernandez DR, Talaber G, Oaks Z, Haas M, Madaio MP, et al. HRES-1/Rab4-mediated depletion of Drp1 impairs mitochondrial homeostasis and represents a target for treatment in SLE. Ann Rheum Dis. 2014;73(10):1888-97.
45. Fernandez DR, Telarico T, Bonilla E, Li Q, Banerjee S, Middleton FA, et al. Activation of Mammalian Target of Rapamycin Controls the Loss of TCRζ in Lupus T Cells through HRES-1/Rab4-Regulated Lysosomal Degradation. The Journal of Immunology. 2009;182(4):2063-73.

46. Gkirtzimanaki K, Kabrani E, Nikoleri D, Polyzos A, Blanas A, Sidiropoulos P, et al. IFNα Impairs Autophagic Degradation of mtDNA Promoting Autoreactivity of SLE Monocytes in a STING-Dependent Fashion. Cell Reports. 2018;25(4):921-33.e5.
47. Maeda A, Fadeel B. Mitochondria released by cells undergoing TNF-α-induced necroptosis act as danger signals. Cell Death Dis. 2014;5(7):e1312-e.
48. Lood C, Blanco LP, Purmalek MM, Carmona-Rivera C, De Ravin SS, Smith CK, et al. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. Nat Med. 2016;22(2):146-53.
49. McDonald B, Pittman K, Menezes GB, Hirota SA, Slaba I, Waterhouse CC, et al. Intravascular danger signals guide neutrophils to sites of sterile inflammation. Science. 2010;330(6002):362-6.
50. Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T, et al. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. Nature. 2012;485(7397):251-5.
51. Caielli S, Athale S, Domic B, Murat E, Chandra M, Banchereau R, et al. Oxidized mitochondrial nucleoids released by neutrophils drive type I interferon production in human lupus. J Exp Med. 2016;213(5):697-713.
52. Wang H, Li T, Chen S, Gu Y, Ye S. Neutrophil Extracellular Trap Mitochondrial DNA and Its Autoantibody in Systemic Lupus Erythematosus and a Proof-of-Concept Trial of Metformin. Arthritis & rheumatology (Hoboken, NJ). 2015;67(12):3190-200.
53. Becker Y, Loignon R-C, Julien A-S, Marcoux G, Allaeys I, Lévesque T, et al. Anti-mitochondrial autoantibodies in systemic lupus erythematosus and their association with disease manifestations. Scientific Reports. 2019;9(1):4530.
54. Reimer G, Rubin RL, Kotzin BL, Tan EM. Anti-native DNA antibodies from autoimmune sera also bind to DNA in mitochondria. J Immunol. 1984;133(5):2532-6.
55. Hudson M, Herr AL, Rauch J, Neville C, Chang E, Ibrahim R, et al. The presence of multiple prothrombotic risk factors is associated with a higher risk of thrombosis in individuals with anticardiolipin antibodies. J Rheumatol. 2003;30(11):2385-91.
56. Dieudé M, Senécal JL, Raymond Y. Induction of endothelial cell apoptosis by heat-shock protein 60-reactive antibodies from anti-endothelial cell autoantibody-positive systemic lupus erythematosus patients. Arthritis Rheum. 2004;50(10):3221-31.
57. Dieudé M, Correa JA, Neville C, Pineau C, Levine JS, Subang R, et al. Association of autoantibodies to heat-shock protein 60 with arterial vascular events in patients with antiphospholipid antibodies. Arthritis Rheum. 2011;63(8):2416-24.
58. Becker Y, Marcoux G, Allaeys I, Julien A-S, Loignon R-C, Benk-Fortin H, et al. Autoantibodies in Systemic Lupus Erythematosus Target Mitochondrial RNA. Frontiers in Immunology. 2019;10(1026).
59. Pisetsky DS, Spencer DM, Mobarrez F, Fuzzi E, Gunnarsson I, Svenungsson E. The binding of SLE autoantibodies to mitochondria. Clinical Immunology. 2020;212:108349.
60. Fortner KA, Blanco LP, Buskiewicz I, Huang N, Gibson PC, Cook DL, et al. Targeting mitochondrial oxidative stress with MitoQ reduces NET formation and kidney disease in lupus-prone MRL-lpr mice. Lupus science & medicine. 2020;7(1).
61. Blanco LP, Pedersen HL, Wang X, Lightfoot YL, Seto N, Carmona-Rivera C, et al. Improved Mitochondrial Metabolism and Reduced Inflammation Following Attenuation of Murine Lupus With Coenzyme Q10 Analog Idebenone. Arthritis & rheumatology (Hoboken, NJ). 2020;72(3):454-64.
62. Ray JP, Staron MM, Shyer JA, Ho PC, Marshall HD, Gray SM, et al. The Interleukin-2-mTORc1 Kinase Axis Defines the Signaling, Differentiation, and Metabolism of T Helper 1 and Follicular B Helper T Cells. Immunity. 2015;43(4):690-702.

63. Warner LM, Adams LM, Sehgal SN. Rapamycin prolongs survival and arrests pathophysiologic changes in murine systemic lupus erythematosus. Arthritis and rheumatism. 1994;37(2):289-97.
64. Fernandez D, Bonilla E, Mirza N, Niland B, Perl A. Rapamycin reduces disease activity and normalizes T cell activation-induced calcium fluxing in patients with systemic lupus erythematosus. Arthritis and rheumatism. 2006;54(9):2983-8.

Figures

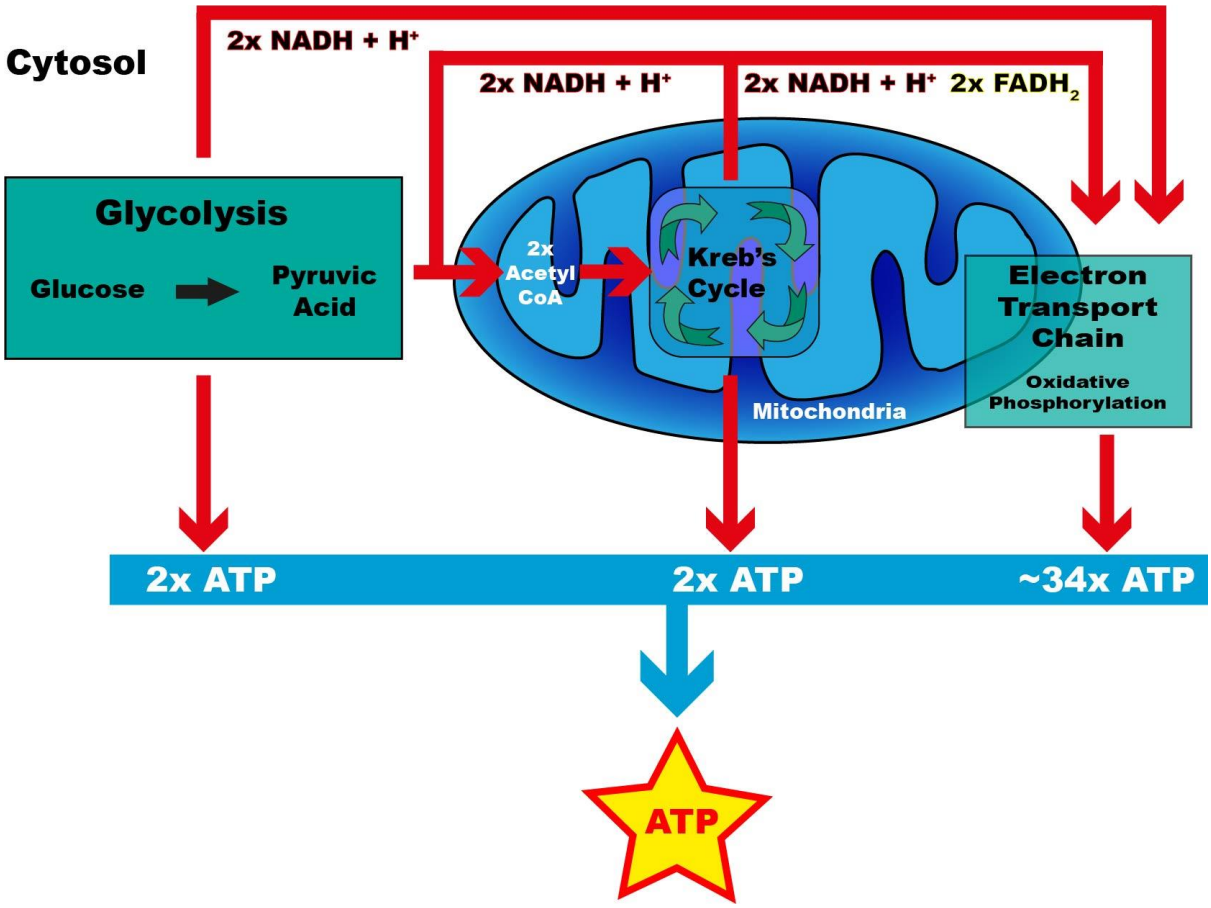

Figure 1: Cellular energy metabolism consists of a series of enzymatic reactions in which glucose is broken down to pyruvic acid, which forms acetyl coenzyme A before entering the Krebs cycle. The metabolic products of this process allows for oxidative phosphorylation on the inner mitochondrial membrane electron transport chain under aerobic conditions

NADH, nicotinamide adenine dinucleotide; H^+, hydrogen; Acetyl CoA, acetyl coenzyme A; $FADH_2$, flavin adenine dinucleotide; ATP, adenosine triphosphate

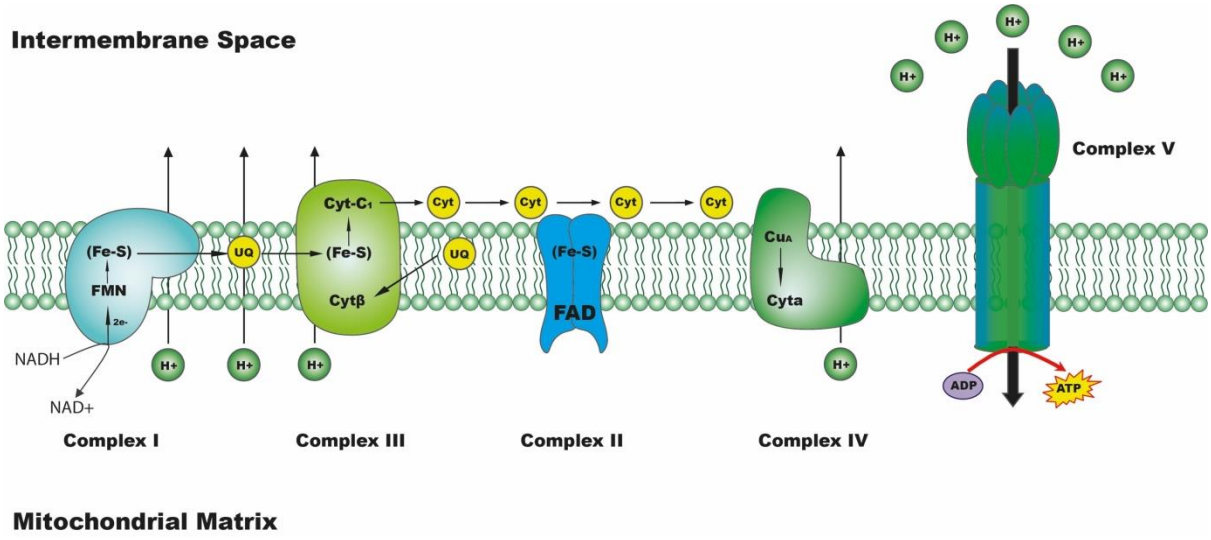

Figure 2: The Electron Transport Chain (ETC) is situated on the inner mitochondrial membrane and is the site of ATP generation via oxidative phosphorylation. The ETC is comprised of five respiratory chain complexes that generate a proton gradient across the membrane, which is essential for the conversion of ADP to ATP

Fe-S, Iron-sulphur cluster; FMN, flavin mononucleotide; e^-, electron; NAD^+, NADH, nicotinamide adenine dinucleotide; H^+, hydrogen; UQ, ubiquinone; Cytβ, cytochrome beta; Cyt, cytochrome; FAD, flavin adenine dinucleotide; ADP, adenosine diphosphate; ATP, adenosine triphosphate