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METABOLIC REGULATION OF ORGANELLE HOMEOSTASIS IN LUPUS T CELLS

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Abstract

Abnormal T-cell signaling and activation is a characteristic feature in systemic lupus erythematosus (SLE). Lupus T cells are shifted towards an over-activated state, important signaling pathways are rewired, and signaling molecules are replaced. Disturbances in metabolic and organelle homeostasis, importantly within the mitochondrial, endosomal, and autophagosomal compartments, underlie the changes in signal transduction. Mitochondrial hyperpolarization, enhanced endosomal recycling, and dysregulated autophagy are hallmarks of pathologic organelle homeostasis in SLE. This review is focused on the metabolic checkpoints of endosomal traffic that control immunological synapse formation and mitophagy and may thus serve as targets for treatment in SLE.

Keywords

Systemic lupus erythematosus; T cell; mitochondria; endocytic recycling; mammalian target of rapamycin; autophagy

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease of complex etiology where immune system deregulation results in widespread inflammation and tissue damage [1]. Pathogenic factors include influences from the environment [2], genetics [3], and epigenetic modifications including DNA hypomethylation [4]. Genetic factors result in a 20–60% concordance of lupus in monozygotic twins, indicating that genetics alone are not solely responsible for development of autoimmunity in SLE [5]. Early environmental exposures to ultraviolet irradiation, chemicals, and infectious agents can permanently alter plasticity of the developing immune system, where overproduction of pro-inflammatory cytokines, including IL-6, results in impaired tolerance induction [2].

SLE is characterized by autoantibody formation against nucleosome components, resulting in anti-nuclear antibody (ANA) production by auto-reactive B cells [1]. Autoantibody production occurs secondary to activation of dendritic cells by necrotic, but not apoptotic, debris [6]. SLE T cells have an increased propensity to undergo necrosis upon stimulation,

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providing substrates for autoantibody generation. Circulating auto-antibodies form immune complexes which deposit in vasculature, activate complement, and incite inflammation, resulting in end-organ damage [7].

While SLE T cells have increased necrosis, activation induced cell death (AICD) is reduced, allowing for persistence of autoreactive lymphocytes [8]. The balance between apoptotic and necrotic cell death in SLE T cells is disturbed due to mitochondrial dysfunction, characterized by increased mass and transmembrane potential ($\uparrow\Delta\psi_m$) [9]. Mitochondrial accumulation may occur as a result of defective autophagic turnover of mitochondria (mitophagy) [10] and increased nitric oxide (NO)-dependent biogenesis [11, 12]. Increased mitochondrial mass and $\uparrow\Delta\psi_m$ allows for sustained T cell activation [13, 14].

Increased T cell activation is central to SLE pathogenesis, as lupus T cells provide help to autoreactive B cells, produce pro-inflammatory cytokines, and stimulate dendritic cell function [15]. Lupus T cells are more sensitive to T cell antigen receptor (TCR) stimulation, with a decreased threshold of activation required to induce intracytoplasmic calcium (Ca^{2+}) fluxing [16]. Pre-clustering of lipid raft domains, which are required for IS formation, and altered activity of protein tyrosine kinases and phosphatases within these raft domains, contribute to increased sensitivity to T cell receptor (TCR) stimulation [17]. Over-expression of endosomal Rab GTPases in SLE T cells [18] contributes to altered recruitment of TCR-associated components to the IS [19] and is required for activation of the mammalian target of rapamycin (mTOR) [20].

mTOR is a key integrator of nutrient signals and regulator of cellular metabolism, which is over-expressed in SLE T cells [18]. mTOR activation controls differentiation of $CD4^+$ and $CD8^+$ T cells through transcriptional regulation [21]. Lupus T cells exhibit altered lineage specification, characterized by depletion of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) [15], and accumulation of uncommitted $CD4^-CD8^-$ double negative (DN) T cells [22, 23], which could result from increased mTOR activity. The lower frequency of Tregs results in failure to maintain immune tolerance [15]. Correcting this deficit may be clinically beneficial in SLE [24]. DN T cells are the major source of IL-17 production, which promotes inflammation through stimulating cytokine and NO production with secretion of IL-1, IL-6, and TNFα [25, 26]. These inflammatory mediators enhance T cell priming, as well as stimulate dendritic cells and macrophages [25]. Blockade of the IL-23/IL-17 axis could be a therapeutic target in SLE [27], through reversal in commitment or depletion of Th17 cells [28, 29].

Mitochondrial dysfunction, endocytic pathway activation, calcium homeostasis, mTOR activation, and autophagy in T cells can serve as biomarkers for SLE and act as potential targets for therapy. New biologic therapies aimed at regulation of T and B cell activation and metabolism show promise in clinical studies and animal models of SLE.

2. Altered T cell receptor signaling machinery in lupus

T cell receptor (TCR) signal transduction is aberrant in SLE. Early T cell receptor associated molecules, which contribute to formation of the immunological synapse, are altered within SLE T cells. CD3ζ and CD4 co-receptor are degraded by lysosomal degradation via the endosomal GTPase HRES-1/Rab4 [18]. A consequence of CD3ζ depletion in lupus $CD4^+$ T cells is re-wiring of the TCR with replacement of CD3ζ for FcεRIγ [30]. FcεRIγ activates tyrosine kinase Syk replacing CD3ζ-ZAP-70 interactions, with Syk being a more potent kinase than ZAP-70, capable of propagating signaling downstream of the TCR under conditions of reduced TCR ligation [31]. Signaling through FcεRIγ-Syk induces stronger and faster Ca^{2+} fluxing in response to T cell activation than CD3ζ-ZAP-70, providing SLE T cells with a lower threshold for activation [32]. Inhibition of Syk has shown to be

therapeutic in lupus-prone mice [33]. CD3ζ depletion also results in reduced CTLA-4 mediated immune suppression, as CTLA-4 requires phosphorylated CD3ζ for its function [34]. Deficiency of CD3ζ results in systemic autoimmunity in mice [35], similar to CTLA-4 deletion [36, 37], with T cell function normalized upon reconstitution of these molecules [38, 39].

CD44 surface expression is increased on T cells, resulting in defective homing and kidney infiltration [40, 41]. Additionally, SLE T cells have deficient production of the homeostatic cytokine IL-2, required during autocrine T cell activation [42]. IL-2 deprivation might contribute to defective function of $CD8^+$ cytotoxic T cells. These changes shape the pathogenic T cell phenotype in SLE.

3. Metabolic dysfunction in lupus T cells

SLE T cells exhibit mitochondrial dysfunction, characterized by increased mass, $\uparrow\Delta\psi_m$, and reduced production of ATP [43]. Mitochondria are essential organelles within all cells, functioning as an energy source and a reservoir for Ca^{2+} [44]. Increased mitochondrial mass and $\uparrow \Delta\psi$m in SLE T cells leads to increased Ca^{2+} stores within mitochondria, resulting in enhanced intracytosolic Ca^{2+} fluxing upon stimulation [45]. Mitochondria integrate signals during apoptosis by regulating the balance between pro- and anti-apoptotic proteins, producing reactive oxygen species, and maintaining ψ_m [46]. Mitochondria are highly dynamic organelles, with organized movement within the cell [44]. They undergo fusion and fission as a response to various stimuli [47] and damaged mitochondria are consumed as a result of organelle autophagy, which is called mitophagy [48].

Mitochondrial function is crucially important in T cells for energy homeostasis. While developing in the thymus, autoreactive T cells undergo apoptosis during negative selection to prevent autoimmunity. The mitochondrial content of thymocytes is regulated by mitophagy, suggesting that cells with improper mitochondrial clearance can contribute to the defective T cell compartment [49]. IL-15 is an important regulator of mitochondrial biogenesis, required of survival and function of $CD8^+$ memory T cells to respond to stimuli [50]. This is mediated through increased expression of carnitine palmitoyl transferase, which increases fatty acid oxidation, oxidative phosphorylation, and ATP production [51]. The circulating, but not the urinary, $CD8^+$ T cell memory compartment is reduced in SLE suggesting that the mitochondrial homeostasis may be affected [52]. In lupus, there is an increased response to IL-15 [50], which contributes to increased mitochondrial biogenesis. IL-15 expression is up-regulated upon T cell activation [53] and is required for survival and function of cytotoxic cells, including $CD8^+$ T cells and natural killer cells [54]. Up-regulation of IL-15 production during autoimmunity can promote accumulation of mitochondria, but may be a protective measure to maintain NK cell function, as NK deficiency promotes systemic autoimmunity and tumor formation [53]. Further studies are required to elucidate the role of cytokines in mitochondrial biogenesis in SLE.

During antigen-specific T cell activation, and following antigen presentation to T cells, redistribution and polarization of the mitochondria occurs towards the site of the immunological synapse (IS) [44, 55]. In T cells, movement of mitochondria is extremely important during IS formation, regulated by the dynamin related protein-1 (Drp-1) which regulates mitochondrial fission [56, 56, 57]. Morphologically, SLE T cells have megamitochondria [58] which could result from disturbed mitophagy.

Persistent mitochondrial hyperpolarization (MHP) predisposes SLE T cells to undergo necrosis in response to stimulation [59, 60], compared to normal T cells which exhibit only transient MHP following activation by CD3/CD28 [60]. Necrotic, but not apoptotic, T cell debris is responsible for the activation of the innate immune system, especially pDCs. pDCs

respond to DNA and RNA remnants of the necrotic cells, produce IFN-α, and infiltrate sites of inflammation [61]. ATP depletion and increased reactive oxygen species generation is also characteristic of lupus T cells and contribute to necrosis [9, 62].

Lupus T cell have a characteristic mitochondrial gene signature. Elevated VDAC (voltage dependent anion channel), SOD2 (superoxide dismutase), and transaldolase expression contribute to increased mitochondrial mass and elevated potential in lupus T cells [18]. The main pathogenic driver of the MHP is NO which is released by monocytes and produced by the activation of inducible nitric oxide synthase (iNOS) [12]. T cells express the endothelial and the neuronal isoforms of nitric oxide synthase [60], which contribute to nitrosative stress in lupus. In patients with SLE and multiple sclerosis (MS), polymorphisms of electron transport chain proteins were observed, which could provide genetic predisposition to mitochondrial dysfunction [63].

Mitochondrial membrane potential is regulated by pyridine nucleotides and reduced glutathione (GSH) levels in lupus T cells. GSH is elevated in whole blood of lupus patients but is depleted within T cells, suggesting that production is not impaired, but GSH deficiency could result from oxidation [64]. Global mitochondrial dysfunction affecting both innate (neutrophils and monocytes) and adaptive immune components (T and B cells), has been identified in patients with anti-phospholipid syndrome, a common comorbidity in SLE patients, providing evidence that mitochondrial dysfunction is not restricted to T cells. Anti-phospholipid antibodies could elicit membrane changes on monocytes via Fc-receptor signaling, which induces alterations in mitochondrial homeostasis and promotes mitochondrial fission [65]. The main differences in mitochondrial homeostasis between a normal and the lupus T cell are depicted schematically in Figure 1.

3.1. Activation of the mammalian target of rapamycin (mTOR) in SLE

mTOR is a serine/threonine protein kinase conserved from fission yeast to humans that integrates environmental and metabolic signals to modulate the innate and adaptive immune responses [21]. Two multi-protein complexes comprise mTOR, complex 1 (mTORC1) and complex 2 (mTORC2). mTORC1 activity is acutely sensitive to suppression by the antibiotic rapamycin through binding to cellular receptor, FK506 binding protein of 12 kDa, while mTORC2 activity is reduced with higher concentrations and prolonged exposure to rapamycin through preventing assembly of mTORC2 components [66].

Inhibition of mTOR by rapamycin results in impaired maturation of dendritic cells [67], reduced cellular proliferation [68], and hyporesponsiveness of $CD4^+$ T cells [69]. These immunosuppressive effects have led to its efficacy and approval by the Food and Drug Administration (FDA) for anti-rejection treatment post-renal transplantation. mTOR activation has been implicated in the pathogenesis of multiple cancers and autoimmune diseases, and its inhibition was found to be therapeutic in cancer [70], epilepsy [71, 72], SLE [45], tuberous sclerosis [73], and autoimmune lymphoproliferative syndrome [74].

mTORC1 is activated in lymphocytes from human lupus patients and in murine lupus models, measured by increased phosphorylation of downstream targets which regulate protein translation, p70 S6 kinase and 4E-BP1 [45, 75]. mTORC1 activity is implicated in disease pathogenesis, as inhibition of mTOR by rapamycin prevents the onset [76, 77]and treats established disease in lupus-prone mice [78] and reduces disease activity in patients with SLE [45]. Rapamycin treatment prevents antinuclear antibody production [75, 76, 79, 80] and suppresses production of pro-inflammatory cytokines produced by $CD4^+$ T cells, including interferon-γ and IL-17A [79, 81], which have been shown to play a key role in lupus pathogenesis [29, 82–85].

One mechanism through which rapamycin exerts therapeutic efficacy in lupus is through shifting lymphocyte cellular metabolism away from glycolysis. Increased mTORC1 activity results in an increased dependence of glycolytic activity and lipid biosynthesis, with reduced oxidative phosphorylation and lipid oxidation [86]. Increased dependence on glycolytic activity supports T_H1, T_H2, and T_H17 metabolism, with these $CD4^+$ T cells expressing high levels of Glut1 receptor [87]. Enhanced mTORC1 activity and reduced lipid oxidation suppresses activation of cAMP-associated protein kinase (AMPK) [88] required for regulatory T cell metabolism, shifting the balance to support effector T over regulatory T cell function [87].

mTORC1 activity directs metabolism in T lymphocytes through control of mitochondrial function. Changes in the mitochondrial transmembrane potential are sensed by mTOR through formation of a complex containing voltage-dependent anion channel and Bcl-xL, components of the outer mitochondrial membrane [89]. mTORC1 activation is associated with increased mitochondrial transmembrane potential, oxygen consumption, and ATP synthetic capacity, as treatment with rapamycin reduces these parameters *in vitro* [90]. Rapamycin may reduce mitochondrial oxidative capacity through transcriptional regulation [91]. SLE T cells have increased mTOR activity [18] and mitochondrial transmembrane potential [9], however, mTOR activation is only one factor regulating mitochondrial function, as treatment with rapamycin *in vivo* in SLE patients has not been found to reduce mitochondrial mass or potential in T cells [45].

Inhibition of mTOR activity by rapamycin promotes T cell anergy [69] and induces peripheral tolerance. mTOR exerts a dominant role in determining antigen responsiveness, reducing effector and promoting Treg lineage commitment in naïve $CD4^+$ T cells [92]. In the absence of mTOR, $CD4^+$ T cells are unable to undergo T_H1, T_H2, or T_H17 lineage commitment in response to polarizing cytokines. mTORC1 promotes T_H1 and T_H17 differentiation through activation of lineage specific transcription factors T-bet and RORγT, respectively [93]. mTORC2 activity promotes T_H2 specification via activation of T_H2 transcription factor GATA-3 [93]. In the absence of mTOR, T cells will undergo Treg specification. Treg commitment occurs through release of inhibition of Foxo1 and Foxo3a transcription factors and increased Smad3 activation, which promotes peripheral generation of Tregs in the presence of TGFβ [92]. mTOR enhances Treg generation, rapamycin promotes tolerance through selective expansion of $CD3^+CD4^+CD25^+Foxp3^+$ T regs, which retain suppressive activity *in vitro* and *in vivo* [94].

Oxidative stress stimulates mTORC1 activity, with increased interaction of Raptor with mTOR and subsequent phosphorylation of S6 kinase in the presence of thiol oxidants [95]. Increased mTOR activity in response to oxidative stress is promoted through inhibition of the tuberous sclerosis 1/2 complex, an upstream negative regulator of mTORC1 [96]. Amelioration of oxidative stress has been shown to reduce mTOR activity *in vitro* [18] and *in vivo* [64] and is therapeutic in human and murine SLE [64, 97, 98]. In a placebo-controlled clinical trial testing the efficacy of antioxidant N-acetyl-cysteine (NAC) in SLE, NAC was found to reduce mTOR activity in T cells and promote expansion of regulatory T cells (Tregs) *in vivo* [64]. This correlated with reduced disease activity by SLEDAI and BILAG indices, reduced fatigue assessment scores, and reduced titers of antinuclear autoantibodies (ANA) [64]. The exact mechanism of mTOR inhibition by NAC remains to be established.

An additional point of regulation for mTOR activation is through altering its intracellular distribution via the endocytic pathway. Early endosomal Rab GTPases, Rab4A and Rab5A, are over-expressed in SLE T cells [18] and regulate recycling and endocytosis of receptors from the plasma membrane, respectively. mTOR colocalizes with HRES-1/Rab4 and Rab5

on early endosomes and with Rab7 of the late endosome [18][20]. Target of rapamycin components have been identified on isolated endosomes, suggesting endocytic trafficking controls localization of mTOR within the cell [99]. mTORC1 function was found to require intact early to late endosomal conversion, as expression of a GTP-locked constitutively active form of Rab5 or knockdown of a Rab7 guanine nucleotide exchange factor, resulted in the inability to activate mTORC1 in response to amino acids [100]. This is due to preventing association of mTOR with Rag GTPases when GDP/GTP cycling is impaired on hybrid early/late endosomes [101]. mTOR is recruited to $Rab7^+$ late endosomes by the Ragulator complex, which associates RagB and RagD, small GTPases which bind raptor, and the amino acid transporter PAT1 to recruit mTOR to localize to endosomal/lysosomal compartments during nutrient activation by amino acids [102–104].

Activity of endosomal Rab GTPases was found to be indispensible for mTOR activation [100]. To sustain activation, mTOR participates in a positive feedback loop with the early endosome. mTOR over-expression in SLE T cells results in increased expression of HRES-1/Rab4, modified by rapamycin treatment [18].

3.2. Enhanced endosome traffic mediates increased T cell activation through reorganization of the immunological synapse

In addition to enhancement of mTOR activity, activation of the endocytic pathway in SLE T cells increases recycling of the T cell antigen receptor (TCR) and CD4 co-receptor in T cells [18], which enhances T cell activation. Increased endocytic activity results in enrichment of TCRs and other components to lipid raft microdomains that make up the T cell-antigen presenting cell interface at the immunological synapse by polarized exocytosis [105]. This process is mediated in part by Rab GTPases and the guanine nucleotide activating proteins that regulate their activity [19]. Recycling of TCRs occurs constitutively [106], allowing T cells to maintain responsiveness upon serial activation by peptide-MHC complexes when an immunological synapse forms [105]. Conversely, chronic stimulation should result in down-regulation of TCR surface expression through blockade of endocytic recycling, while maintaining the same TCR internalization rate [107]. This is a protective measure to limit T cell exhaustion and resulting antigen-induced cell death, which is impaired in SLE T cells, allowing for persistence of autoreactive T cells [8].

Recycling of CD3 and CD4 surface receptors are increased on SLE T lymphocytes, associated with increased expression of HRES-1/Rab4, an early endosomal small GTPase over-expressed in SLE T cells [18]. HRES-1/Rab4 forms direct interactions with CD4 and CD3ζ, and its over-expression results in targeting of CD4 and CD3ζ to lysosomes for degradation [18, 108]. The E3 ubiquitin ligase Cbl down-regulates the TCR upon sustained engagement through targeting CD3ζ [109], which could be mediated by HRES-1/Rab4 through formation of a Rab4-CD2 adaptor protein-Cbl complex [110]. HRES-1/Rab4 could also direct CD3ζ for degradation by microautophagy, a catabolic process that delivers cytosolic cargo into multivesicular bodies formed from endosomes, which transfers cargo to lysosomes by fusion [111].

Activation of early endocytic recycling could also enhance lymphocyte activation in SLE through regulation of antigen processing in B cells. Peptide-MHC class II complexes are loaded into endocytic compartments [112] and early endosomal recycling is required for antigen processing within B cells and presentation to $CD4^+$ T lymphocytes [113]. Rab4 is required for efficient presentation of antigens that are internalized by the B cell receptor (BCR) and is important in processing of receptor-bound antigens [114, 115]. Rab4 transcription is promoted by the MHC class II transactivator, which could allow B cells to enhance their ability to present antigen through coordinately increasing Rab4-dependent recycling. Increased antigen processing by Rab4 could contribute to SLE pathogenesis

through promoting generation of autoantigens through molecular mimicry of endocytosed material, and increased MHC class II processing would enhance presentation of these autoantigens, leading to stimulation of autoreactive $CD4^+$ T cells [116].

Antigen presentation can be promoted by Rab GTPases through increased autophagy [117]. Several Rab GTPases that localize to membrane sources (endoplasmic reticulum, mitochondria) or endosomes (early and late) have been implicated in formation and maturation of autophagosomes [118]. Increased phagocytosis from early endosomes results in a need for increased biogenesis of degradative compartments to eliminate pathogens and foreign substances that are internalized by the cell [119]. Production of pro-inflammatory cytokines, including interferon-γ and IL-6, are up-regulated in SLE [120, 121], which can contribute to increased biogenesis and maturation of phagocytic compartments, through promoting Rab5 transcription [122–124].

3.3. Autophagy is up-regulated within lupus T cells

Autophagy is a well conversed cellular regulatory mechanism in which proteins (microautophagy) or organelles (e.g. mitochondria, previously mentioned as mitophagy) are sequestered and degraded by the autophagosome and autolysosome [125]. The key event of autophagic flux is the assembly of the autophagosome in which several proteins of the Atg family are implicated. Besides Atg proteins, the most notable component is the microtubule associated protein LC3, which is used as marker for identifying autophagosomes [126].

Autophagy regulates the biology of both the innate and adaptive immune systems [127]. Induction of autophagy is essential for the proliferation, homeostatic maintainence, and survival of T lymphocytes. Deficiency of autophagic proteins in knockout mice results in reduced intrathymic development of T cells (Atg6, [128]), impairment of T cell survival and proliferation (Atg5, [129]), defective activation-induced effector cytokine production upon activation (Atg7, [130]), and exaggerated T cell apoptosis upon stimulation (Atg6, [131]). Defective autophagy prevents turnover of damaged endoplasmic reticulum (ER), resulting in its accumulation within effector T cells of knockout mice (Atg3, [132], Atg 7 [133]). Impaired ER homeostasis due to deficient autophagy results in reduced recruitment of stromal interaction molecule-1 (STIM-1) towards Orai1 [133], preventing store-operated Ca^{2+} release activated Ca^{2+} current (CRAC) from the ER, required to sustain T cell activation [134]. Up-regulation of autophagy during T cell activation spares mitochondria [130], which elongate and concentrate towards the immunological synapse to meet the energetic requirements of stimulation [57, 135].

In accordance with these observations, increased autophagy promotes autoimmunity through enhanced survival and reduced apoptosis of autoreactive lymphocytes. Phosphoinositide-3 kinase Vps34, plays an important role in autophagosome formation by producing phosphoinositide 3-phosphate [PI(3)P], which regulates Rab5-directed vesicle traffic. Vps34 expression promotes increased mitochondrial mass and enhanced production of reactive oxygen intermediates within T cells [136]. Lupus T cells exhibit reduced activation-induced cell death [8], increased intracytoplasmic Ca^{2+} fluxing upon stimulation [137], and over-production of effector cytokines [138], all of which could be related to increased autophagy.

Both murine and human lupus T cells have elevated numbers of autophagosomes. Despite increased autophagosome formation, mitochondrial mass remains increased in SLE T cells [58]. HRES-1/Rab4 over-expression, which occurs in SLE T cells, was found to increase microautophagy (with increased lysosomal degradation of proteins CD3ζ, CD4), while inhibiting macroautophagy of mitochondria through degradation of Drp1, a mitochondrial fission initiator required early during mitophagy [139]. Drp1 is depleted in SLE T cells [139], resulting in defective clearance and promoting the formation of megamitochondria.

Increased autophagy can lead to the survival of auto-reactive T cells in lupus, a defect which precedes disease onset [140]. Up-regulation of autophagy in SLE T cells could be mediated by circulating auto-antibodies, as complement-inactivated autoimmune sera from patients with diabetes mellitus has been shown to stimulate autophagy *in vitro* [141]. Polymorphic alleles of autophagy-related genes in SLE patients may also be responsible. In genome-wide association studies in SLE, single nucleotide polymorphisms within the locus of autophagy-related gene ATG5 were identified, resulted in increased transcription, and were linked to lupus susceptibility [142]. Although functional consequences of ATG5 over-expression have not been investigated in SLE, it is thought to be pathogenic through promoting survival and expansion of autoreactive T cells, identified in studies of ATG5 up-regulation during acute demyelination in MS [143].

Modulation of autophagy may improve outcomes in SLE and several medications currently used in SLE management affect the autophagic machinery. Anti-malarial drugs, including chloroquine and hydroxychloroquine, act through raising the pH of endosomes and lysosomes, resulting in an accumulation of ineffective autophagosomes [144]. The P140 phosphopeptide, an inhibitor of Hsc70, prevents formation of autolysosomes [145, 146], and shows promise in a recent phase II clinical trial [147]. Up-regulation of autophagy occurs in SLE lymphocytes despite increased mTORC1 activation, and agents that reduce mTOR activity are effective in SLE although they act as autophagy inducers, including corticosteroids, proteasome inhibitors, and rapamycin. Glucocorticoids reduce mTOR activity and induce autophagy by inhibiting Ca^{2+} signaling, resulting in up-regulation of AMPK, which inhibits mTORC1 activation [148]. Proteasome inhibitors, including Bortezomib, inhibit mTORC1 through blockade of the ubiquitin-proteasome system [149].

4. New therapies target B and T cell signal transduction in SLE

Currently, available therapeutics in SLE are limited and are strong immunosuppressants. Serious side effects, such as infections and impaired wound healing, limit long-term use and efficacy of immunosuppressive regimens. Only four therapies have been approved by the Food and Drug Administration (FDA) for management of SLE [150]. These include 1) glucocorticoids [150] 2) aspirin [150]3) anti-malarial drugs [151]and 4) belimumab [152], an inhibitor of B cell activation. There is an unmet need for safer medications for SLE treatment that can be used during disease flares and for long-term management. There are increasing number of biological targets currently under investigation for SLE, focusing on blockade of major pathways involved in activation and survival of auto-reactive B and T lymphocytes due to metabolic and autophagic disturbances. Some of these targets have been studied in murine models of SLE, but have yet to be investigated in clinical trials. A schematic diagram of these targets is shown in figure 2.

B cell depletion therapies targeting CD20 and CD22 have been utilized to reduce the source of auto-antibody generation and reduce activation of auto-reactive T cells [153–156]. These have achieved limited success due to survival of long-lived plasma cells. However, proteasome inhibitors, including Bortezomib, result in plasma cell depletion and have been shown to be effective in preventing onset of disease in mice [157]. Generation of immune complexes (ICs) by ANA produced by plasma cells results in activation of TLRs and complement activation, which have been effectively blocked by toll-like receptor antagonists, monoclonal antibodies, and complement inhibitors in lupus-prone mice [158–160].

Targets to inhibit B cell activation with reduced B cell depletion are also under investigation, including Atacicept, a fusion protein that targets TACI, which interacts with BAFF during B cell activation in a phase II/III clinical trial [161]. Medications aimed at

tolerizing auto-reactive B or T cells have achieved limited success [162, 163], but additional tolerogenic therapies are under investigation. Additionally, blockade of T cell-B cell co-stimulation with CTLA-4 Ig, has been shown to have modest effects in reducing flares in SLE [164]. Co-stimulatory inhibition by blockade of B7RP-1 is therapeutic in lupus-prone mice and is under investigation in a phase I clinical trial [165]. B7RP-1 inhibition prevents activation of $CD4^+$ T cells, including follicular helper T cells which provide B cell help in splenic germinal center reactions [165].

Blocking co-stimulation prevents T and B cell activation. Exaggerated Ca^{2+} responses result from enhanced T and B cell activation and promote survival and proliferation of autoreactive lymphocytes. Enhanced intracytoplasmic Ca^{2+} fluxing is a target for treatment in SLE, which can be mitigated by mTOR blockade by rapamycin [45], or inhibition of calcium-activated calmodulin kinase [166], PI3Kγ [167], or calcineurin [168].

Blockade of pro-inflammatory cytokines produced by B cells, T cells, or macrophages by monoclonal antibodies has shown promise. Toclizumab, which targets IL-6R, reduces disease activity, ANA, and IL-17 production through reduction in IL-21, which prevents differentiation of pathogenic Th17 cells from naïve $CD4^+$ T cells and DN T cells [169]. IL-17 and IL-21 are over-produced in SLE due to activation of IRF4, and result in increased generation of pathogenic Th17 cells in lupus-prone mice, reversed by Rho kinase 2 inhibitor, Fasudil [170]. Inhibition of IL-10 reduces Th2 differentiation and reduces disease activity [171]. Effects of IL-1 over-production by activated macrophages can be blocked by Anakinra, an IL-1 receptor antagonist [172]. Neutralization of IFN-α produced by pDCs, has shown promise in murine lupus models and a phase I trial [173]. Production of pro-inflammatory cytokines can also be effectively reduced by treatment with rapamycin [45]. Modulation of oxidative stress through use of high-potency anti-oxidant N-acetylcysteine (NAC), has been found to be effective in reducing disease activity in SLE [64]. Descriptions of these therapeutic targets are included in Table 1.

5. Discussion

Numerous disturbances in T cell signaling occur within lupus lymphocytes. These changes are characterized by the altered metabolic flux, mitochondrial homeostasis, early endosome activation, and autophagic activity of T cells. Activation of the mTOR pathway that affects lineage specification, MHP which increases ROS production and predisposes activated cells to necrosis, over-expression of Rab GTPases which enhances recycling and degradation of TCR signaling components, and increased autophagy within T cells contributes to these abnormalities. These can function as potential biomarkers and therapeutic targets to reverse aberrant T cell activation and reduce clinical severity in SLE.

Abbreviations

$\uparrow\Delta\psi_m$	increased mitochondrial transmembrane potential
AICD	activation-induced cell death
AMPK	cAMP-activated protein kinase
ANA	antinuclear autoantibodies
ATG	autophagy-related gene
BCR	B cell receptor
BILAG	British Isles lupus assessment group
$\mathbf{Ca^{2+}}$	calcium

CRAC	calcium release activated calcium current
CTLA-4	cytotoxic T lymphocyte antigen-4
DN T cell	$CD4^{-}CD8^{-}$ double negative T cell
Drp1	dynamin-related protein 1
ECLAM	European consensus lupus activity measurement index
ER	endoplasmic reticulum
FDA	Food and Drug Adminstration
GN	glomerulonephritis
GSH	reduced glutathione
IC	immune complex
IFN	interferon
IL-	interleukin-
iNOS	inducible nitric oxide synthase
IS	immunological synapse
MHP	mitochondrial hyperpolarization
MRL/lpr mouse	murine research laboratory lymphoproliferative mouse
MS	multiple sclerosis
NAC	N-acetylcysteine
NK	natural killer
NO	nitric oxide
NZB/WF1	New Zealand Black × New Zealand White F1 progeny, a mouse model of SLE
mTOR	mammalian target of rapamycin
pDC	plasmacytoid dendritic cell
Rab	Ras-associated in brain
SLE	systemic lupus erythematosus
SLEDAI	systemic lupus erythematosus disease activity score
STAT	signal transducer and activator of transcription
SOCS	suppressor of cytokine signaling
Syk	spleen tyrosine kinase
TCR	T cell antigen receptor
T_H	T helper, $CD4^{+}$ T cell
TLR	toll-like receptor
Treg	regulatory T cell, $CD3^{+}CD4^{+}CD25^{+}Foxp3^{+}$ cell
ZAP-70	CD3 zeta associated protein of 70 kDa

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Highlights

- Organelles, such as endosomes and mitochondria, regulate T cell activation
- Endosomes recycle surface receptors that transmit signals from the T-cell receptor
- Endosome control traffic of proteins and organelles for disposal via autophagy
- Mitochondria control T-cell activation and death
- Organelle dysfunction represents target for treatment in SLE

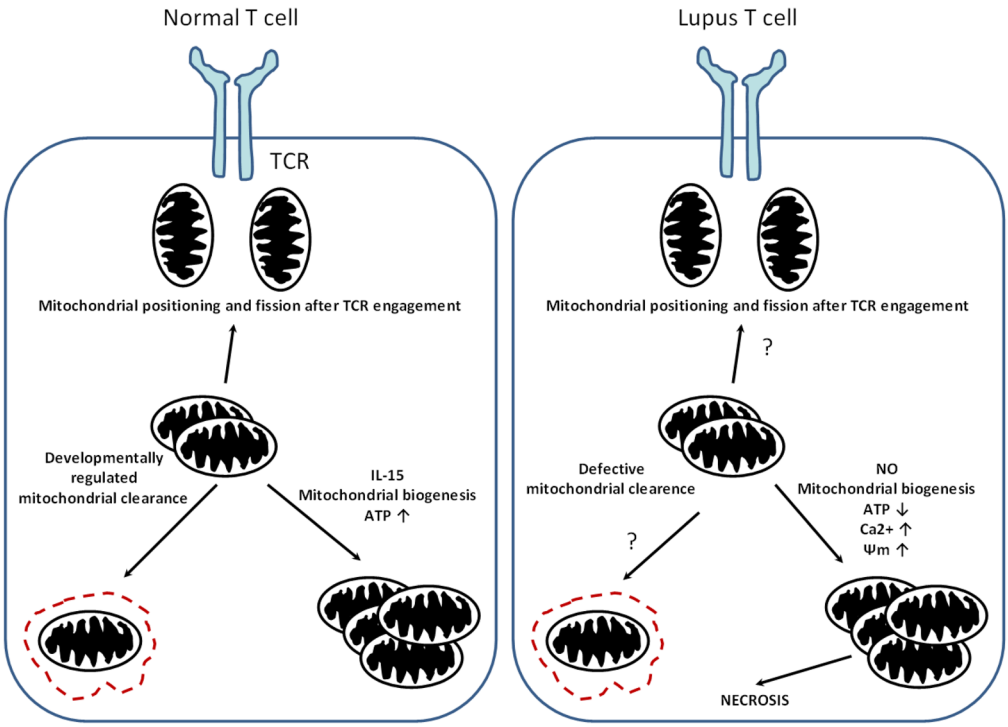

Fig. 1.

Mitochondrial homeostasis in a normal and a lupus T cells. During the formation of the IS, mitochondria are shuttled and redistributed towards the TCR signaling complex. Mitochondrial content is regulated during T cell differentiation allowing damaged mitochondria to be eliminated by autophagy. During T cell memory formation, mitochondrial biogenesis and ATP content are increased via the effect of IL-15. However, in lupus T cell, higher mitochondrial mass and elevated potential is observed, which could be due to higher NO production in these cells. Mitochondria are larger in size and have elevated Ca^{2+}, which alters mitochondrial movement and contributes to defective IS architecture. Removal of mitochondria could be disturbed during T cell differentiation. Contrary to the normal T cell, SLE T cells produce less ATP. The result of mitochondrial dysfunction can be necrotic cell death upon stimulation.

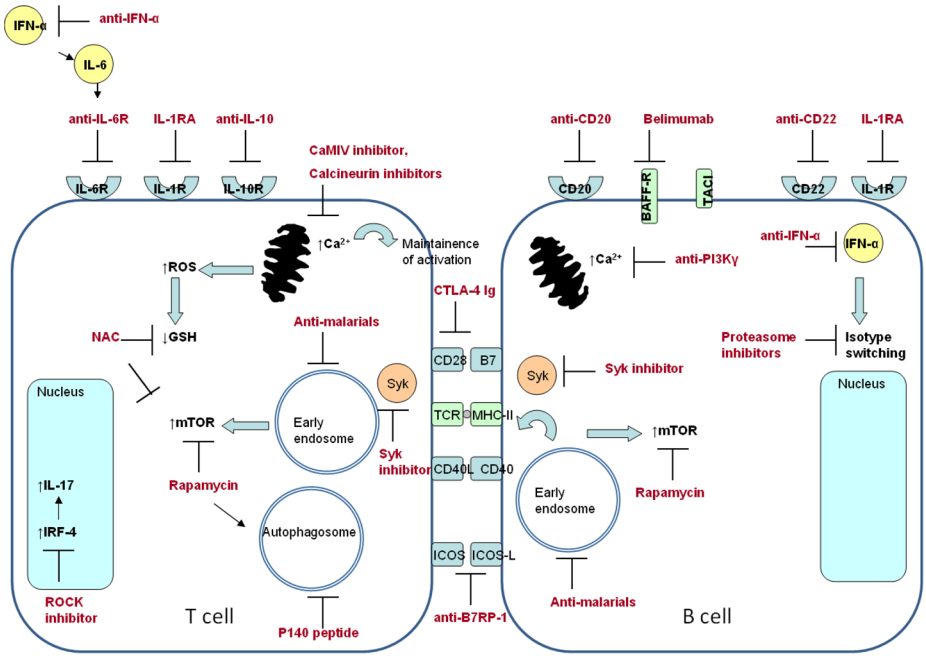

Fig. 2.

Biological targets under investigation for treatment of SLE. Depletion of autoreactive B cells is achieved by treatment with monoclonal antibodies against CD20 and CD22. CD22 also depletes plasma cells, which can also be targeted through blockade of isotype-switching by proteasome inhibition or anti-IFNα antibodies. Activation of lupus B cells can be inhibited by targeting survival (BAFF targeted by Belimumab, APRIL targeted by TACI-Ig) and co-stimulatory signals. Lupus T cell activation is targeted by blockade of cytokine action (IL-6, IL-1, and IL-10), cytokine production (IL-17), and co-stimulation (CD28-B7 interaction by CTLA-4 Ig, ICOS-ICOS ligand interaction by anti-B7RP-1 antibodies). Activation of SLE T and B cells results in a rise of cellular Ca^{2+}, which results from mitochondrial dysfunction, mTOR activation, and endocytic pathway activation. Intracellular Ca^{2+} can be modulated by treatment with calcium calmodulin kinase inhibitors, calcineurin inhibitors, and anti-PI3Kγ. Early endosome and mTOR activation in SLE T and B cells are inhibited with anti-malarials and rapamycin. Autophagy in SLE lymphocytes can be reduced by treatment with P140 peptide and anti-malarial drugs.

Of these targets, antimalarial drugs and Belimumab have been FDA-approved for SLE disease management. The other targets mentioned are under intensive investigation in pre-clinical and clinical studies.

Table 1

Current and prospective therapies for SLE

Molecular target	Treatment	References
FDA approved therapies:		
Homeostatic survival of T cells, B cells, & macrophages	Glucocorticoids Prednisone, Triamcinolone hexacetonide	148, 150
Non-steroidal anti- inflammatory drugs	Aspirin	150
Endosome function, activation of toll-like receptors, antigen processing / presentation	Chloroquine; hydroxychloroquine	151
BLγS/BAFF (B cell cytokine)	Belimumab	152
B cell targeted therapies:		
B cell depletion	Rituximab (anti-CD20) Epratuzumab (anti-CD22) Atacicept (TACI-Ig fusion protein)	153. 154 155, 156 161
Proteasome (↓ plasma cells)	Bortezomib (MRL/lpr; NZB/WF1 mice)	157
Syk	Fostamatinib (MRL/lpr; BAX/BAK mice)	33
T cell targeted therapies:		
Glutathione depletion	N-acetylcysteine	64
Follicular helper T cells	Anti-B7RP-1 Ab (NZB/WF1 mice)	165
Rho kinase (ROCK) Inhibits IRF4 phosphorylation	Fasudil, ROCK2 inhibitor (NZB/WF1 mice)	170
Blockade of B cell – T cell co-stimulation:		
T cell-B cell costimulation	Abatacept (CTLA-4 Ig)	164
Tolerogenic therapies	Edratide (hCDR1 peptide, T cell tolerogen) Abetimus (B cell tolerogen)	162 163
PI3Kγ	AS605240 (MRL/lpr mice)	167
Regulation of intracellular Ca2+:		
Calcineurin	Dipyridamole (MRL/lpr mice)	168
Calcium-activated calmodulin kinase	KN-93, CaMKIV inhibitor (MRL/lpr mice)	166
Cytokine blockade:		
Monoclonal antibodies	Tocilizumab (anti-IL-6R) Anti-IFNα IgG1κ neutralizing antibody Anti-IL-10 monoclonal antibody (B-N10) Anakinra (IL-1 receptor antagonist)	169 173 171 172
mTOR	Rapamycin	45