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T-cell and B-cell signaling biomarkers and treatment targets in lupus

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Abstract

Purpose of review—Systemic lupus erythematosus is characterized by the production of antinuclear autoantibodies and dysfunction of T-cells, B-cells, and dendritic cells. Here, we review newly recognized genetic factors and mechanisms that underlie abnormal intracellular signal processing and intercellular communication within the immune system in systemic lupus erythematosus.

Recent findings—Activation of the mammalian target of rapamycin plays a pivotal role in abnormal activation of T and B-cells in systemic lupus erythematosus. In T-cells, increased production of nitric oxide and mitochondrial hyperpolarization were identified as metabolic checkpoints upstream of mammalian target of rapamycin activation. Mammalian target of rapamycin controls the expression T-cell receptor-associated signaling proteins CD4 and CD3 ζ through increased expression of the endosome recycling regulator HRES-1/Rab4 gene, mediates enhanced Ca²⁺ fluxing and skews the expression of tyrosine kinases both in T and B-cells, and blocks the expression of Foxp3 and the expansion of regulatory T-cells. Mitochondrial hyperpolarization and the resultant ATP depletion predispose T-cells to necrosis, thus promoting the dendritic cell activation, antinuclear autoantibody production, and inflammation.

Summary—Mitochondrial hyperpolarization, increased activity of mammalian target of rapamycin and Syk kinases, enhanced receptor recycling and Ca²⁺ flux have emerged as common T and B-cell biomarkers and targets for treatment in systemic lupus erythematosus.

Keywords

calcium; glutathione; lupus; mitochondria; mTOR; nitric oxide; receptor recycling; Syk

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease in which the pathogenesis is attributed to genetic and environmental factors causing the dysfunction of T

and B-lymphocytes [1–3] and dendritic cells [4,5]. Abnormal T-lymphocyte activation and cell death signaling underlie the pathology of SLE [6] (Table 1) [7–15]. Mitochondria, the organelles that control death signal processing, are dysfunctional in lupus T-cells. They exhibit a persistent elevation of the mitochondrial trans-membrane potential (ψ_m) or mitochondrial hyperpolarization (MHP) [8] and ATP depletion which predispose to cell death by necrosis that is highly proinflammatory [16]. The increased release of necrotic materials from T-cells could drive disease pathogenesis by activating macrophages and dendritic cells and enhancing their capacity to produce nitric oxide and interferon α (IFN- α) in SLE [16]. Indeed, dendritic cells exposed to necrotic, but not apoptotic, cells induce lupus-like disease in Medical Research Laboratories (MRL) mice and accelerate the disease of MRL/lpr mice [17].

Dysregulated signaling through TCR ζ is a critical determinant of abnormal T-cell activation in SLE [18,19]. Activation of the mammalian target of rapamycin (mTOR) contributes to abnormal signaling through the T cell receptor (TCR) by enhancing the expression of the small GTPase HRES-1/Rab4 that targets CD4 [20] and TCR ζ for lysosomal degradation [21*]. Lupus B-cells also exhibit abnormal signaling through the B-cell receptor (BCR) [22*], mTOR activation [23], and enhanced Ca^{2+} flux (Table 2) [8,10,14,15,18,21*,25–27,28*,29–34,35*,36,37*, 38,39*,40*]. Thus, activation of mTOR and enhanced receptor recycling and Ca^{2+} flux have emerged as new common biomarkers of T and B-cell dysfunction in SLE.

T-cell dysfunction in systemic lupus erythematosus

T-cells exert key regulatory and effector functions in the immune system both of which are severely compromised due to defective processing of activation and death signal processing in SLE.

The impact of mitochondrial dysfunction and oxidative stress on T-cell activation

Lupus T-cells exhibit oxidative stress that results from MHP and extrusion of H^+ ions from the mitochondrial matrix [8]. With MHP, the cytochromes within the electron transport chain (ETC) become more reduced which promotes reactive oxygen intermediates (ROI) production [41]. ROI modulates T-cell activation, cytokine production, and proliferation at multiple levels [16]. The antigen-binding $\alpha\beta$ or $\gamma\delta$ TCR is associated with a multimeric receptor module comprising the CD3 $\gamma\delta\epsilon$ and ζ chains (Fig. 1) [42]. The cytoplasmic domain of CD3 ζ chain contains an immunoglobulin receptor family tyro-sine-based activation motif (ITAM) which is crucial for coupling of intracellular tyrosine kinases [43*]. Expression of CD3 ζ is suppressed by ROI [33]. Binding of p56^{lck} to CD4 or CD8 attracts this kinase to the TCR–CD3 complex, leading to phosphorylation of ITAM. Phosphorylation of both tyrosines of each ITAM is required for SH-2-mediated binding by zeta-associated protein-70 (ZAP-70) or the related SYK. ZAP-70 is activated through phosphorylation by p56^{lck} . Activated ZAP-70 and SYK target two key adaptor proteins LAT and SLP-76 [44]. Phosphorylated LAT binds directly to phospholipase C- γ 1 (PLC γ 1) that controls hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) to inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Phosphorylation of inositol lipid second messengers is mediated by phosphatidylinositol 3'-hydroxyl kinase (PI3K). The stimulatory effect of the TCR alone on

PI3K activity is small. Concurrent triggering of the CD28 costimulatory molecule by its ligands CD80 or CD86 is required for optimal PI3K activation. IP3 binds to its receptors in the endoplasmic reticulum, opening Ca^{2+} channels that release Ca^{2+} to the cytosol. Decreased endoplasmic reticulum Ca^{2+} concentration activates the Ca^{2+} release-activated Ca^{2+} channel (CRAC) in the cell membrane. Ca^{2+} depletion in the endoplasmic reticulum initiates the redistribution of STIM1 towards endoplasmic reticulum–plasma membrane junctions and direct binding of Stim1 to Orai1 that opens the CRAC channel. The resultant Ca^{2+} influx activates the phosphatase calcineurin which dephosphorylates the transcription factor NFAT. Dephosphorylated NFAT can translocate to the nucleus in which it promotes transcription of interleukin (IL)-2 in concert with AP-1, NF κ B, and Oct-1. Whereas activities of AP-1 and NF κ B are increased by oxidative stress [45], both thiol insufficiency and H_2O_2 treatment suppress calcineurin-mediated activation of NFAT [46]. Lupus T-cells have decreased amounts of DNA-binding 98 kDa form of the Elf-1 transcription factor that reduces the expression of TCR ζ and augments the expression of Fc ϵ RI γ . Further upstream, the expression of protein phosphatase 2A (PP2A), that dephosphorylates Elf-1 at Thr-231, is enhanced in lupus T-cells. Dephosphorylation results in limited expression and binding of the 98 kDa Elf-1 form to the TCR ζ and Fc ϵ RI γ promoters. Suppression of the expression of the PP2A leads to increased expression of TCR ζ and decreased expression of Fc ϵ RI γ and correction of the early signaling response [28*]. PP2A expression is increased through demethylation of its promoter [35*], a process triggered by oxidative stress [32]. Thus, expression of T-cell signal transduction molecules (TCR ζ and Fc ϵ RI γ) and cytokines, that is IL-2 (with AP-1 and NFAT motif-containing promoter) and IL-4 (with AP-1-less NFAT enhancer), can be selectively regulated by oxidative stress depending on the relative expression level of transcription factors involved (Fig. 1). Expression of Stat3, which is also driven by oxidative stress [47], is increased in both lupus T [38] and B-cells [23]. Stat3-dependent signals play a key role in the differentiation of Th17 cells [48], which appear to mediate nephritis in patients with SLE [39*,40*].

Activation of the mammalian target of rapamycin in lupus T-cells

Rapamycin normalized T-cell mitogen-stimulated spleenocyte proliferation and IL-2 production prevented the typical rise in antidouble-stranded DNA antibody and urinary albumin levels and glomerulonephritis (GN), and prolonged survival of lupus-prone MRL/lpr mice [49]. mTOR is associated with the outer mitochondrial membrane and senses mitochondrial dysfunction and changes in the ψ_m of T-cells [50]. With a focus on mitochondrial dysfunction, we began to utilize rapamycin for treatment of SLE patients resistant or intolerant to conventional medications. Rapamycin markedly improved disease activity [26]. This occurred with the normalization of baseline Ca^{2+} levels in the cytosol and mitochondria and of CD3/CD28-induced Ca^{2+} fluxing while MHP persisted, indicating that increased Ca^{2+} fluxing is downstream or independent of MHP in the pathogenesis of T-cell dysfunction in SLE [26].

The immunosuppressive properties of rapamycin have been attributed to the blocking of TOR complex 1 (TORC1) that is required for transducing T-cell activation initiated by cytokines [51]. TORC2 is required for organization of the actin cytoskeleton and it is rapamycin-insensitive. Whereas mTOR expression was similar in lupus and control T-cells,

phosphorylation levels of two key mTOR substrates S6K1 and 4E-BP1 were dramatically increased in lupus T-cells and such changes were reversed in rapamycin-treated patients [21*]. These findings suggest that mTOR kinase activity is increased in lupus T-cells and it is reversed by rapamycin treatment. Overexpression of the cellular receptor of rapamycin, FKBP12, was identified as a component of the mitochondrial gene expression signature in lupus T-cells. In accordance with the persistence of increased mitochondrial mass and MHP in rapamycin-treated patients [26], elevated expression of FKBP12 was unaffected by rapamycin treatment [21*], suggesting that mTOR activation may be a consequence of genetic factors underlying MHP and increased mitochondrial biogenesis in lupus T-cells [52*]. Such genetic factors may also operate in lupus B-cells [23,53*]. Autophagy mediates the bulk degradation of cytoplasmic contents, proteins and organelles including mitochondria in T-cells [54], in lysosomes; this process is induced by rapamycin through inactivating mTOR [55]. Interestingly, rapamycin treatment *in vivo* did not affect MHP or mitochondrial mass of lupus T-cells [26]. However, rapamycin treatment normalized baseline and T-cell activation-induced Ca^{2+} fluxing. Such outcome may result from modulating the expression of small GTPases Rab5 and HRES-1/Rab4 that control endocytic traffic and degradation of key molecules of T-cell signal transduction including CD4 [20] and TCR/CD3 ζ [21*].

Akt, a kinase that phosphorylates multiple targets in T-cells, may be a key link in the chain of events that activate mTOR [52*]. Upstream, phosphatidylinositol accumulation by phosphoinositide 3-kinases (PI3K) induces the localization of 3-phosphatidylinositide-dependent protein kinase 1 (PDK1) to the plasma membrane that in turn phosphorylates and activates Akt [56]. Activation of class IA-PI3K in T-cells extends CD4+ memory cell survival, triggering an invasive lymphoproliferative disorder and systemic lupus [57]. In turn, the lipid phosphatase PTEN (phosphatase and tensin homolog deleted in chromosome ten) counteracts phosphatidylinositol accumulation by PI3K and mice with PTEN haploinsufficiency also develop systemic autoimmunity [58]. Importantly, the rictor–mTOR complex directly phosphorylates Akt/PKB on Ser473 *in vitro* and facilitates Thr308 phosphorylation by PDK1 [59]. Thus, activation of mTOR may also account for elevated Akt activity and provide a positive feedback loop of T-cell activation in SLE. mTOR controls the expression of Foxp3 and development of regulatory T-cells [60*,61*] which are deficient in patients with SLE [62*,63*]. The effectiveness of rapamycin in murine and human SLE suggests that mTOR is a key mediator of autoimmunity in SLE. Therefore, understanding the mechanisms of persistent MHP that leads to mTOR activation and enhanced Ca^{2+} fluxing may be fundamental to the pathogenesis of lupus.

Activation of endocytic recycling pathway is characterized by partially nitric oxide-inducible and mammalian target of rapamycin-dependent overexpression of HRES-1/Rab4

The overexpression of Rab5A and HRES-1/Rab4, which control internalization and recycling of surface receptors via early endosomes, respectively [64,65], are markers of an activated recycling gene expression signature in lupus T-cells [21*]. In accordance with a dominant impact of HRES-1/Rab4 on the endocytic recycling of CD4 [20], there is an inverse correlation between enhanced HRES-1/Rab4 expression and diminished CD4 expression in negatively isolated CD4 T-cells. Overexpression of HRES-1/Rab4 is also

inversely correlated with TCR ζ protein levels. These changes in gene expression are associated with enhanced constitutive recycling of CD3 ϵ and CD4 in lupus T-cells relative to healthy and RA disease controls. mTOR emerged as a critical checkpoint between the enhanced mitochondrial and receptor recycling gene expression signatures [21 $\bullet$]. Such gatekeeper function of mTOR is consistent with its role in sensing mitochondrial dysfunction and changes of the ψ_m in T-cells [50] and modulating the traffic of GLUT4 [66,67] and transferrin receptor (TFR) [68] which are associated with Rab4-positive endosomes in adipocytes [69] and epithelial cells [65], respectively. Rapamycin treatment *in vivo* not only reduced the expression of HRES-1/Rab4 and Rab5A but also reversed the loss of CD4, Lck, and TCR ζ chain and the overexpression of Fc ϵ RI γ and Syk in lupus T-cells. GST pull-down studies revealed a direct interaction of HRES-1/Rab4 with TFR, CD4, CD2AP, and TCR ζ . Both the knockdown of HRES-1/Rab4 expression by siRNA and the inhibition of lysosomal function increased TCR ζ levels in lupus T-cells. These observations identified HRES-1/Rab4-dependent lysosomal degradation as a novel mechanism contributing to the critical loss of TCR ζ in lupus T-cells [1]. The HRES-1 endogenous retrovirus was earlier mapped to 1q42 [70] and polymorphism of its long terminal repeat (LTR) region has been associated with the development of SLE [71,72]. Although HRES-1/Rab4 overexpression was moderated in rapamycin-treated patients, it remained elevated relative to healthy controls [21 $\bullet$]. Increased expression of HRES-1/Rab4 may also be influenced by polymorphic haplotypes of the LTR [73 $\bullet$] which harbors an enhancer of HRES-1/ Rab4 gene transcription [20].

Checkpoints of Ca²⁺ fluxing

The Ca²⁺ signal is dysregulated in SLE T-cells in a number of ways. SLE T-cells have elevated intracellular and mitochondrial Ca²⁺ at baseline [15], whereas the amount of Ca²⁺ present in the endoplasmic reticulum is normal [25]. Although the T-cell activation-induced Ca²⁺ flux is elevated initially, the plateau phase is reduced relative to activated control T-cells [15]. There are at least three potentially interrelated mechanisms that account for altered Ca²⁺ flux in SLE: abnormal formation of the immunological synapse in T-cells [29,31] and possibly also in B-cells [74,75], increased mitochondrial biogenesis and Ca²⁺ storage in mitochondria [15], and mTOR activation enhances IP3R-mediated Ca²⁺ release from the endoplasmic reticulum [42]. Dysregulation of nitric oxide production may play a role in all three mechanisms since normal T-cells pretreated with nitric oxide donors recapitulate the Ca²⁺ fluxing abnormalities observed in SLE T-cells [15]. Nitric oxide induces the expression of HRES-1/Rab4 that regulates receptor recycling and targets CD4 [20] and TCR ζ for lysosomal degradation [21 $\bullet$] and promotes mitochondrial biogenesis and mitochondrial storage of Ca²⁺ [15] and enhances mTOR activation [21 $\bullet$]. Considering that Ca²⁺ is required for activation of PKC, calcineurin, NFAT, and production of IL-2, the altered Ca²⁺ homeostasis may account for the inappropriate activation of T-cells in SLE [6].

The importance of T-cell synapse formation and Ca²⁺ homeostasis in lupus has been highlighted by the recent discovery that a nonsense mutation of the filamentous actinin-inhibiting Coronin-1A gene inhibits T-cell synapse formation and T-cell activation-induced Ca²⁺ fluxing [76 $\bullet$]. This mutation suppressed the development of lupus on the MRL/lpr background which was associated with functional alterations in T-cells, reduced T-

dependent humoral responses, and no detectable intrinsic B-cell defects. By transfer of T-cells it was shown that suppression of autoimmunity could be accounted for by the presence of the Coronin-1A mutation in T-cells [76*].

B-cell dysfunction in systemic lupus erythematosus

Whereas increased production of antinuclear autoanti-bodies is a hallmark of the diagnosis and pathogenesis of SLE, new information has also emerged on the important regulatory roles that B-cells play in activating T-cells and dendritic cells.

Abnormal signaling through the B-cell receptor in systemic lupus erythematosus

The BCR is a hetero oligomer comprising membrane immunoglobulin (mIg), which confers antigen specificity, and the disulfide-linked CD79 α/β heterodimer which is noncovalently linked to mIg (Fig. 2) [77–83]. Cross-linking of the BCR is rapidly followed by phosphorylation of ITAMs in the intracellular domains of CD79 α/β [84]. The ITAMs on the cytoplasmic domains of CD79 α/β associate noncovalently with Src family protein tyrosine kinases (PTK) Lyn, Fyn, Lck, and Blk via their SH2 domains [85]. A prerequisite for this binding is the phosphorylation of CD79 α/β by Lyn. Phosphorylation of two precisely spaced tyrosine residues within the ITAM allows recruitment and activation of Syk, a gatekeeper of downstream signaling through PKC, MAPK, or phospholipase $C\gamma 2$ (PLC $\gamma 2$). BTK contributes to BCR-stimulated calcium signaling by phosphorylating and activating PLC $\gamma 2$ (Fig. 2) [77]. PLC $\gamma 2$ cleaves the membrane phosphoinositide, phosphatidyl inositol 4,5-bisphosphate (PIP $_2$) generating DAG and inositol 1,4,5-trisphosphate (IP $_3$). DAG activates protein kinase C β (PKC β), whereas IP $_3$ binds to specific receptors on the endoplasmic reticulum inducing the Ca $^{2+}$ release from intracellular stores [24]. Both *Lyn* and *Lyn*-deficient mice develop mild lupus-like disease. *Lyn* deficiency was associated with high levels of autoantibody production and glomerulonephritis [86]. B-cells from young *Lyn* $^{-/-}$ mice are hyper-responsive to anti-IgM-induced proliferation, suggesting involvement of Lyn in negative regulation of BCR signaling. Tyrosine phosphorylation of Fc γ RIIB and CD22 coreceptors, which are important for feedback suppression of BCR-induced signaling, is severely impaired in *Lyn* $^{-/-}$ B-cells. Hypophosphorylation on tyrosine residues of these molecules resulted in failure of recruiting the tyrosine phosphatase SHP-1 and inositol phosphatase SHIP, SH2-containing potent inhibitors of BCR-induced B-cell activation. Consequently, *Lyn* $^{-/-}$ B-cells exhibit defects in suppressing BCR-induced Ca $^{2+}$ influx and proliferation [87]. Interestingly, mice expressing a constitutively activated form of Lyn due to a single point mutation (Y508F) that negatively regulates Lyn activity, also display heightened Ca $^{2+}$ flux in response to BCR stimulation, develop circulating auto-antibodies and lethal glomerulonephritis [88]. Thus, both overexpression and underexpression of Lyn results in lupus-like autoimmunity, suggesting that signaling molecules such as Lyn are tightly regulated [85].

Fc γ RIIB is an important downstream target of Lyn and a negative regulator of signaling through the BCR [85]. Inhibition through Fc γ RIIB is mediated primarily by the activation of SHIP which hydrolyzes PI(3,4,5)P $_3$, an essential element of BCR signaling. SHIP protein levels are lower in SLE B-cells, suggesting that SHIP is less available to connect with Fc γ RIIB and inhibit BCR signaling [89]. B-cells of lupus-prone mice exhibit diminished

SHIP and stress-activated protein kinase (SAPK) activity [90]. An I232T variant of Fc γ RIIB, that has been genetically linked to lupus, was found less effectively distributed to detergent-insoluble lipid rafts at baseline and after BCR ligation [91]. Membrane fractionation revealed that Fc γ RIIB readily segregated into the lipid rafts, whereas the Fc γ RIIBI232T isoform was excluded [92]. Although the mechanism by which this transmembrane domain polymorphism prevents partitioning into the lipid raft fraction is unclear, this study suggests that defective signaling through Fc γ RIIB enhances the B-cell reactivity in SLE (Table 3) [23,24,74,75,86,89,93,94*,95].

The potential importance of B-cell synapse formation has been revealed by the observation that a decrease of Lyn is accompanied by concomitant increase of CD45 in lipid raft microdomains [74]. CD45 is a protein tyrosine phosphatase, involved in regulating B-lymphocyte selection and modulating signal thresholds. CD45 couples BCR signaling to both cell proliferation and differentiation. Knock-in mice with a single E613R point mutation in the inhibitory wedge of CD45 develop a lymphoproliferative syndrome, with polyclonal T and B-lymphocyte activation, anti-DNA antibody production and severe autoimmune nephritis [96]. CD45 can dephosphorylate both the activating pY-396 and negative regulatory pY-507 tyrosines in Lyn. CD45 dephosphorylates the fraction of Lyn that is localized in lipid rafts. Thus, the Lyn that is in proximity to relevant substrates would be inactive, static, and unable to downregulate BCR signaling. Such uninhibited B-cell activation appears to drive autoimmunity in mice with the CD45 E613R wedge mutation and Fas deficiency [97]. The CD45 E613R wedge mutation also promotes autoimmunity in concert with the deficiency of the PEST domain-enriched tyrosine phosphatase (Pep $-/-$) [98]. Here, the Pep deficiency serves as a surrogate for lupus-linked inactivating R620W mutation in the analogous human gene PTPN22 [99]. Whereas the CD45 E613R wedge mutation appears to drive B-cell autoreactivity, Pep deficiency promotes T-cell autoreactivity [98].

Activation of mTOR was recently demonstrated in B-cells isolated from genetically distinct mouse models of lupus and rapamycin derivative ameliorated clinical disease [23]. Surprisingly, mTOR blockade was associated with the crippling of several other signaling axes, suggesting that activation of this pathway plays a central role in human [21*,26] and murine lupus [23,49].

Enhanced Ca²⁺ fluxing is linked to altered composition of lipid rafts in lupus B-cells

Following BCR stimulation, the activation of phospholipase C- γ 1 and phospholipase C- γ 2 enzymes leads to the hydrolysis of phosphatidylinositol 4,5 bisphosphate to inositol 1,4,5 trisphosphate (IP₃) and DAGl. IP₃ binds to its receptor on the endoplasmic reticulum and releases Ca²⁺ to the cytosol by opening Ca²⁺ channels, followed by capacitative Ca²⁺ entry through the plasma membrane. Lupus B-cells have higher intracytoplasmic Ca²⁺ responses than controls when stimulated with anti-sIgM or anti-IgD mAb [24]. The magnitude of anti-sIgM-mediated protein tyrosine phosphorylation and Ca²⁺ release from intracellular stores are increased in SLE patients' B-cells [24]. Fc γ RIIB-dependent inhibition of sIgM-mediated Ca²⁺ flux is deficient in lupus B-cells irrespective of disease activity [89]. The accelerated Ca²⁺ flux has been associated with an altered composition of lipid rafts in membrane

domains of B-cells from patients with SLE [74]. B-lymphocytes from SLE patients, but not those from healthy controls, expressed a low molecular weight isoform of CD45 in lipid raft signaling microdomains. SLE patients had increased numbers of B-lymphocytes (20–60%) with CD45 in the lipid rafts. Importantly, there was an inverse relationship between CD45 in the lipid rafts and Lyn levels in these microdomains. This inverse correlation was not significantly associated with disease activity, nor was it influenced by medication use [74,75,89]. Thus, activation of mTOR, altered lipid raft composition, and enhanced Ca^{2+} flux represent common metabolic pathways underlying T and B-cell dysfunction in patients with SLE (Tables 2 and 3).

Targeting biomarkers of T-cell and B-cell dysfunction for treatment of systemic lupus erythematosus

As common signaling pathways have recently emerged that mediate both T and B-lymphocyte signaling defects, key regulatory nodes of those pathways may represent optimal pharmaceutical targets in SLE.

Depletion of intracellular glutathione

Reduced glutathione (GSH) is profoundly depleted in lymphocytes of SLE patients [8]. Low GSH in T-cells overexpressing transaldolase predispose to MHP [100]. GSH depletion is a robust trigger of MHP via S-nitrosylation of complex I upon exposure to nitric oxide [101]. Thus, the effect of nitric oxide on MHP is tightly related to GSH levels. Diminished production of GSH in the face of MHP and increased ROI production are suggestive of a metabolic defect in de-novo GSH synthesis or maintenance of its reduced state due to deficiency of NADPH [102]. Recent studies showed diminished GSH/GSSG ratios in the kidneys of 8-month-old versus 4-month-old (NZB $\times$ NZW) F1 mice; treatment with N-acetylcysteine (NAC), a precursor of GSH and stimulator of its de-novo biosynthesis, prevented the decline of GSH/GSSG ratios, reduced autoantibody production and development of glomerulonephritis and prolonged the survival of (NZB $\times$ NZW) F1 mice [103]. Oral NAC has been used to treat oxidative stress in patients with idiopathic pulmonary fibrosis (IPF) [104]. In a 1-year study of IPF patients treated with prednisone and azathioprine, addition of NAC (3×600 mg/day) improved vital capacity and reduced myelotoxicity in comparison to placebo. Therefore, prospective clinical studies appear justified to assess whether NAC treatment can reverse GSH depletion, correct T-cell signaling defects and provide clinical benefit to patients with lupus.

Inhibiting nitric oxide production

Nitric oxide induces MHP and mitochondrial biogenesis, increases Ca^{2+} in the cytosol and mitochondria of normal T-cells, and recapitulates the enhanced CD3/CD28-induced Ca^{2+} fluxing of lupus T-cells [15]. As recently reported, eNOS is recruited to the site of T-cell receptor engagement, locally increasing nitric oxide at the immunological synapse in a Ca^{2+} and PI3K-dependent manner, resulting in reduced IL-2 production [105] which is characteristic of SLE [6]. Nitric oxide contributes to the development of glomerulonephritis in the MRL/*lpr* lupus mouse model [106]. Inactivation of iNOS does not block the development of lupus [107], suggesting a role for eNOS and nNOS isoforms

expressed in T-cells. However, given the widespread expression of these isoforms in vascular smooth muscle and brain, it will be necessary to develop T-cell-specific approaches for inhibiting NOS to avoid potentially deleterious side effects.

Rapamycin

With a focus on mitochondrial dysfunction, we began to utilize rapamycin for treatment of SLE patients resistant or intolerant to conventional medications. We observed normalization of baseline Ca^{2+} levels in the cytosol and mitochondria and of CD3/CD28-induced Ca^{2+} fluxing as well as persistence of MHP [26], indicating that increased Ca^{2+} fluxing is downstream or independent of MHP in the pathogenesis of T-cell dysfunction in SLE. The effectiveness of rapamycin in murine [49] and human SLE suggests that mTOR is a key mediator of autoimmunity in SLE. Rapamycin can selectively expand CD4⁺/CD25⁺/Foxp3⁺ regulatory T-cells [108,109] which appear to be deficient in patients with SLE [62^{*}]. Therefore, understanding the mechanism of persistent MHP that leads to mTOR activation and enhanced Ca^{2+} fluxing may be fundamental to the pathogenesis of SLE.

Syk inhibition

TCR ζ depletion and its functional replacement by Fc ϵ RI γ is a hallmark of altered TCR signaling in lupus T-cells [27]. The abnormal expression the spleen tyrosine kinase Syk and its preferentially binding to Fc ϵ RI γ greatly contribute to enhanced Ca^{2+} fluxing after TCR cross-linking [110^{*}]. Lupus B-cells also have high basal levels of phosphorylated Syk and ERK1/2 in response to BCR cross-linking that promotes differentiation into plasma cells *in vitro* [94^{*}]. R788, an orally bioavailable Syk inhibitor, was recently shown to prevent the development of renal disease and to treat established nephritis in NZB/W mice [111^{*}]. R788 minimally affected auto-antibody titers, whereas it dose-dependently reduced the numbers of CD4⁺ activated T-cells, suggesting that T-cells might be the effective targets of Syk inhibition [111^{*}].

Targeting the interaction of T and B-cells

CD40 ligand: CD40 binding to CD40 ligand (CD154) is one of the most robust costimulatory signals for T-cells. CD154 a member of the TNF family is inducibly expressed on the surface of CD4 T-cells, whereas CD40 is constitutively expressed on B-lymphocytes. CD154–CD40 interactions are essential in the formation of germinal centers and the differentiation of memory and plasma cells. CD154-expressing T-cells engage CD40-expressing B-cells inducing them to express activation markers (CD154, CD69) and differentiation markers (CD38, CD5, CD27). CD154 have been found to be hyperexpressed on T-lymphocytes from patients with active SLE [112]. CD40–CD154 and B7/CD28 costimulation blockade effectively treated lupus in mice [113]. Anti-CD154 treatment also appeared initially well tolerated and potentially effective in patients with SLE [114]; however, it has been discontinued due to thrombotic events [115]. CTLA4Ig: Costimulatory blockade with CTLA4Ig is an effective treatment of nephritis in lupus-prone mice [116^{*}]. Clinical trials with CTLA4Ig (abatacept), which is FDA-approved to treat RA, are under way in patients with lupus nephritis.

Conclusion

With the arrival of the postgenomic era and approaches of systems biology in the last couple of years, a series of novel genetically influenced common molecular pathways have been identified that underlie both T and B-cell dysfunction in lupus. These pathways include altered formation of the immunological synapse, increased activity of the signaling network controlled by the kinases mTOR and Syk, and enhanced Ca^{2+} fluxing upon activation of the TCR or BCR. Increased production of nitric oxide and IFN- α as well as cytokines promoting Th2 and Th17 activities represent key messengers of intercellular communication and targets for therapeutical intervention in SLE.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 559–560).

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Figure 1. Schematic outline of mTOR activation and its role in the recycling of surface receptors such as CD4 and TFR and signaling and adaptor proteins such as TCR ζ and CD2AP
mTOR senses the ψ_m and regulates IP3R-mediated Ca²⁺ release [42]. Activation of mTOR controls the expression of TCR ζ and Lck, mediates their lysosomal degradation by activating HRES-1/Rab4 which in turn results in the substitution of TCR ζ by Fc ϵ RI γ and the recruitment of Syk. The intracellular rapamycin receptor FKBP12 directly binds the RyR. The small GTPase HRES-1/Rab4 regulates endocytic recycling of surface receptors such as CD4 and adaptors such as TCR ζ and their traffic to lysosomes. Ca²⁺ controls the activation of calcineurin, a phosphatase that dephosphorylates the transcription factor NFAT and allows it to translocate into the nucleus. Ca²⁺ release from the endoplasmic reticulum also initiates opening of the CRAC channel through activation of Stim1 and Orai1. mTOR, mammalian target of rapamycin; TFR, transferrin receptor.

Figure 2. Schematic diagram of B-cell activation

In resting B-cells, the BCR is excluded from lipid rafts. The rafts concentrate GPI-linked proteins and myristylated proteins, such as LYN and phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG). After antigen engagement, the BCR relocates within rafts. The phosphoinositide 3-kinases (PI3Ks) are recruited to the BCR through BCR and SYK-dependent phosphorylation of CD19 and BCAP, which contains a tyrosine-X-X-methionine activation motif. BCAP and Vav contribute to the activation of PI3K. BTK contributes to BCR-stimulated calcium signaling by phosphorylating and activating PLC γ 2 [77]. The subsequent recruitment of PKC β leads to the phosphorylation of S180 of the TEC-homology domain and inactivation of BTK. Fc γ RIIB, an inhibitory receptor for the Fc of immunoglobulin G, contains one immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic domain. When Fc γ RIIB is cross-linked along with the BCR, the SH2-domain-containing inositol 5-phosphatase (SHIP) is recruited, leading to the abrogation of BCR signaling by the hydrolysis of phosphatidylinositol-3,4,5-triphosphate (PIP3). B-cell activation is also downregulated by LYN that phosphorylates a tyrosine of PAG which then recruits c-SRC tyrosine kinase (Csk) to the rafts. Csk phosphorylates and decreases the enzymatic activity of LYN. The accumulation of PIP3 activates the Akt/mTOR axis [78] and stimulation of the IP3 receptor and the RyR on the endoplasmic reticulum. The subsequent release of Ca²⁺ from the endoplasmic reticulum turns on the CRAC channel, which also operates in B-cells [79]. Elevated Ca²⁺ levels activate NFAT [80,81] and NFAT-dependent B-cell stimulators such as Blys [82]. Rab4 regulates IgG internalization-dependent antigen processing [83]. BCAP, B-cell PI3K adaptor protein; BCR, B-cell receptor; GPI, glycosylphosphatidylinositol; IgH, immunoglobulin

heavy chain; IgL, immunoglobulin light chain; P, phosphate; PIP2, phosphatidylinositol-4,5-bisphosphate or phosphatidylinositol-3,4-bisphosphate.

Table 1

Biomarkers of abnormal T-cell death in patients with SLE

Signal	Effect	Reference
Spontaneous apoptosis ↑	Compartmentalized autoantigen release, disease activity ↑	[7,8]
AICD ↓	Persistence of autoreactive cells	[9,10]
FasL ↑	Spontaneous apoptosis ↑	[11]
ψ_m ↑	ROI ↑, ATP ↓	[8]
GSH ↓	ROI ↑, spontaneous apoptosis ↑	[8,12]
ATP ↓	AICD ↓, predisposes for necrosis ↑	[8,13]
NO ↑	ψ_m ↑, mitochondrial biogenesis ↑	[14,15]
ROI ↑	Spontaneous apoptosis ↑	[8,10]
IL-10 ↑	Spontaneous apoptosis ↓, ROI ↓	[10,11]
IL-12 ↓	Spontaneous apoptosis ↓, ROI ↓	[10]

↑, increase; ↓, decrease. SLE, systemic lupus erythematosus.

Table 2

Biomarkers of abnormal T-cell activation in patients with SLE

Signal	Effect	Reference
CD3-induced Ca^{2+} flux $\uparrow$	Altered T-cell activation	[25]
Baseline $[\text{Ca}^{2+}]_c$ $\uparrow$	Ca^{2+} influx $\uparrow$	[15,26]
Baseline $[\text{Ca}^{2+}]_m$ $\uparrow$	Ca^{2+} influx $\uparrow$	[15,26]
TCR/CD3 ζ $\downarrow$	Ca^{2+} influx $\uparrow$, IL-2 production $\downarrow$	[18]
Fc ϵ RI γ $\uparrow$	Ca^{2+} influx $\uparrow$	[27]
PP2A $\uparrow$	Elf-1 $\downarrow$	[28*]
Lck $\downarrow$	Ca^{2+} influx $\uparrow$	[27,29]
Syk $\uparrow$	Ca^{2+} influx $\uparrow$	[29,30]
Lipid raft formation $\uparrow$	TCR ζ $\downarrow$; Fc ϵ RI γ $\uparrow$; Syk $\uparrow$	[29–31]
Receptor recycling $\uparrow$	CD4 $\downarrow$, TCR ζ $\downarrow$	[21*]
Rab5 $\uparrow$, HRES-1/Rab4 $\uparrow$	Receptor recycling $\uparrow$	[21*]
mTOR $\uparrow$	Ca^{2+} influx $\uparrow$	[26]
mTOR $\uparrow$	Rab5 $\uparrow$, HRES-1/Rab4 $\uparrow$	[21*]
ψ_m $\uparrow$	ROI $\uparrow$, ATP $\downarrow$	[8]
NO $\uparrow$	ψ_m $\uparrow$, mTOR $\uparrow$; Ca^{2+} flux $\uparrow$	[14,15,21*]
ROI $\uparrow$	IL-10 $\uparrow$	[8,10]
ROI $\uparrow$	DNMT1 $\downarrow$	[32]
ROI $\uparrow$	TCR ζ $\downarrow$	[33,34]
DNMT1 $\downarrow$	PP2A $\uparrow$; Elf-1 $\downarrow$; TCR ζ $\downarrow$; Fc ϵ RI γ $\uparrow$	[35*]
PKC δ $\downarrow$	ERK $\downarrow$, DNMT1 $\downarrow$	[36]
IBP $\downarrow$	Rho GTPase $\downarrow$, ERK $\downarrow$	[37*]
Stat3 $\uparrow$	Chemokine-induced T-cell migration $\downarrow$	[38]
CD4-/CD8-/Th17+ $\uparrow$	Nephritis	[39*,40*]

$\uparrow$, increase; $\downarrow$, decrease. mTOR, mammalian target of rapamycin; SLE, systemic lupus erythematosus.

Table 3

Biomarkers of abnormal B-cell activation in patients with SLE

Signal	Effect	Reference
Anti-IgM	Ca ²⁺ flux ↑	[24]
Altered B-cell synapse	Sustained BCR signaling	[74,75]
Lyn ↓	Sustained BCR signaling	[75,86]
CD45 ↑	Sustained BCR signaling	[74]
SAPK ↓; SHIP ↓	Sustained BCR signaling	[90]
FcγRIIB ↓	Sustained BCR signaling	[89,93]
CD19 ^{high} ↑	Syk ↑, ERK ↑	[94]
Blys ↑	B-cell activation ↑	[95]
Akt/mTOR/PKCα/STAT3 ↑	B-cell activation ↑	[23]

↑, increase; ↓, decrease. BCR, B-cell receptor; SLE, systemic lupus erythematosus.