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## Pathogenesis of Human Systemic Lupus Erythematosus: A Cellular Perspective

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### Abstract

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease affecting multiple organs. A complex interaction of genetics, environment, and hormones leads to immune dysregulation and breakdown of tolerance to self-antigens, resulting in autoantibody production, inflammation, and destruction of end-organs. Emerging evidence on the role of these factors has increased our knowledge of this complex disease, guiding therapeutic strategies and identifying putative biomarkers. Recent findings include the characterization of genetic/epigenetic factors linked to SLE, as well as cellular effectors. Novel observations have provided an improved understanding of the contribution of tissue-specific factors and associated damage, T and B lymphocytes, as well as innate immune cell subsets and their corresponding abnormalities. The intricate web of involved factors and pathways dictates the adoption of tailored therapeutic approaches to conquer this disease.

### SLE, a Devastating Disease for Young Women

SLE afflicts mostly women [1] in which the autoimmune response is directed against practically all organs, leading to protean clinical manifestations including arthritis, skin disease, blood cell abnormalities, and kidney damage (Boxes 1 and 2) [1]. Autoantibodies such as those against double-stranded (ds) DNA are a hallmark of lupus, and, together with other cellular and soluble mediators of inflammation, contribute to end-organ damage. With an estimated 1.5 million people suffering from SLE in the USA alone, and an approximate annual cost of more than 13 billion dollars, SLE represents a major diagnostic and therapeutic challenge [1]. The disease is significantly more prevalent among African, Asian, Hispanic, and native-American populations, and these also experience the highest mortality [1]. Currently, the diagnosis, which is frequently difficult and delayed, is based on established criteria by the American College of Rheumatology [2] and the treatment is still limited to the use of general immunosuppressive drugs.

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In recent years, important strides have been made in improving our understanding of the etiopathogenesis of SLE. Several new loci and genetic variants have been identified that are associated with disease in multiple patient cohorts [3]. The abnormal phenotype, subsets, and function of T and B lymphocytes, as well as aberrations in cellular metabolism, have been identified as important factors in the underlying pathophysiology [4–7]. In addition, evidence that aberrant levels of cytokines and soluble mediators of the adaptive immune system appear before autoantibody accrual preceding disease diagnosis highlights their importance in predicting disease onset [8–10]. Recent work has also shed light on various innate immune cell players with novel roles in disease pathogenesis. Despite these major advances, there is a paucity of specific targeted therapies for SLE. In this review we focus on cellular and inflammatory defects that characterize dysregulated immunity in SLE, and examine the underlying molecular pathways and their potential to inform the design of novel putative therapeutic targets and candidate biomarkers. Subtopics include the role of genetic factors in SLE, signaling and gene regulation defects in T cells, mitochondrial hyperpolarization and metabolism, **CD4<sup>+</sup> T helper (Th) cell** (see Glossary) subsets [**T follicular helper (Tfh) cells** and regulatory T cells (Tregs)] and cytokines, CD8<sup>+</sup> T cells, B cell subsets and signaling, innate cells [neutrophils, monocytes, dendritic cells (DCs), platelets], and factors involved in tissue injury.

## Genetics and SLE

The role of various genetic factors in SLE pathogenesis is evident from the high heritability (43.9%) and the relative risk (5.87%) in first-degree relatives of patients with SLE [11]. Although the disease can develop from a single gene deficiency, such as of **complement component 1q** (C1q) subcomponent A (*CIQA*), *CIQB*, *CIQC*, three-prime repair exonuclease 1 (*TREX1*), or deoxyribonuclease 1-like 3 (*DNASE1L3*), in most cases disease results from a combination of multiple gene variant effects [12] (Figure 1).

Several human loci have been linked to impaired immune system functions in SLE. *TREX1*, *DNASE1*, autophagy related 5 (*ATG5*), and *RAD51B* code for proteins functionally related to mechanisms of apoptosis, DNA degradation, and clearance of cellular debris; these are processes which are related to the release of self-proteins and nucleic acids, and are a common source of autoantigens in SLE [3]. **Interferon regulatory factor 5** (*IRF5*), *IRF7*, **signal transducer and activator of transcription 4** (*STAT4*), **Toll-like receptor 7** (*TLR7*), *TLR8*, and *TLR9* are involved in nucleic acid sensing and type I interferon (IFN) production by antigen-presenting cells including DCs, and these are events associated with SLE pathogenesis because they generate a proinflammatory environment [3,13]. Other SLE-associated loci code for proteins involved in T and B cell function, as well as in T cell signaling, and include non-receptor type **protein tyrosine phosphatase 22** (*PTPN22*), *TNFSF4* (tumor necrosis factor superfamily member 4), protein phosphatase 2 catalytic subunit  $\alpha$  (*PPP2CA*), pyruvate dehydrogenase complex component X/cluster of differentiation 44 (*PDHX/CD44*), E74-like ETS transcription factor 1 (*ELF1*), B cell scaffold protein with ankyrin repeats 1 (*BANK1*), B lymphocyte kinase (*BLK*), LCK/YES novel tyrosine kinase (*LYN*) [3], and **cluster of differentiation 3 $\zeta$**  (*CD3Z*) [14], which presumably decrease the activation threshold of CD4<sup>+</sup> T and B cells upon autoantigen encounter [15]. Of note, *IRF5* and *STAT4* have been reported to increase the risk of SLE in

an additive manner, implicating both innate and adaptive immunity in the development of SLE pathogenesis [16].

Most **single-nucleotide polymorphisms** (SNPs) associated with SLE are found within non-coding regulatory regions and can thus lead to altered gene transcription and aberrant gene expression [17]. Transcriptomics refers to the analysis of gene expression at a global level, and microarrays have helped to explore aberrantly regulated genes in peripheral blood mononuclear cells (PBMCs) taken from SLE patients relative to healthy controls [18,19]. In these studies, IFN signaling was identified as a key molecular pathway. Two recent reports, one in PBMCs [20] and another in total T cells enriched from PBMCs derived from SLE patients [21], examined the transcriptome from these cells and analyzed the molecular pathways altered in disease. These studies have confirmed the well-known type I IFN signature. In addition to pathways associated with protein synthesis, cell cycle, and mitochondrial dysfunction, increased molecular signatures of both innate and adaptive immunity were revealed [20]; these included transcriptome modules linked to neutrophils, natural killer (NK) cells, and T and B cells (function), confirming the involvement of both systems in SLE pathogenesis [20]. Moreover, the study on total T cells demonstrated gene signatures associated with antibodies to dsDNA, complement activation, and nephritis, suggesting that T cells can contribute to SLE-associated inflammatory processes [21]. In addition, both studies demonstrated unbiased clustering of transcriptomes, both from PBMCs or T cells, indicating that this type of clustering might enable stratification of patients into subgroups [20,21]. Although the number of patients in both studies was not large, an analysis of these groups over time might enable the development of personalized therapies for SLE patients [22]. Furthermore, the emergence of next-generation sequencing technologies such as RNA sequencing has provided higher resolution in gene expression measurements together with identification of alternative splicing events, noncoding RNAs, and novel loci in SLE [23]. Technological advances including deep sequencing have offered novel high-throughput platforms that will help to decipher the molecular pathways contributing to SLE pathogenesis.

Of note, the increased rate of whole-genome sequencing that has been conducted in patients presenting with complex autoimmune manifestations in multiplex families suggests that the number of patients with monogenic lupus may be increasing, taking into consideration that SLE represents a final common manifestation that results from several overlapping genetic alterations [12]. It is expected that advances in genome editing might allow us to better understand how each polymorphism contributes to disease. Eventually (and hopefully) we should be able to link variants to biochemical/cellular processes as well as to specific disease manifestations. In addition, epigenetic alterations – including changes in DNA methylation, histone modifications, and specific roles of non-coding RNAs – have also been recently associated with SLE pathogenesis and will undoubtedly represent a fruitful area of future research (Box 3).

## T Lymphocyte Abnormalities and SLE Pathogenesis

Multiple subsets of T cells (CD4<sup>+</sup>, CD8<sup>+</sup>, double-negative) from SLE patients are aberrantly activated, mediate inflammatory responses, provide help to B cells, and are unable to

produce enough amounts of the crucial cytokine **interleukin 2** (IL-2) [4]. As described below, both biochemical and molecular defects in T cells coupled to aberrations in gene regulation can lead to an abnormal T cell phenotype in SLE [1,4,5] (Figure 2).

### T Cell Activation and Signal Transduction

SLE human T cells exhibit a rewiring of their T cell receptor (TCR) wherein the expression of the CD3 $\zeta$  chain is decreased, and is frequently replaced by the homologous **Fc $\gamma$  receptor** (Fc $\gamma$ R) chain, which recruits the downstream signaling Syk kinase rather than the CD3 $\zeta$  partner Zap70 (Figure 2). Specifically, mice deficient in CD3 $\zeta$  display inflammation in multiple tissues [24], whereas replenishment of CD3 $\zeta$  can restore IL-2 production from T cells and can resolve the spontaneously aggregated **lipid rafts** in T cells [25]. Indeed, aggregated lipid rafts characterize T cells from the lupus-prone **MRL/lpr mouse model**, and their pharmacologic dissolution with methyl- $\beta$ -cyclodextrin can prevent and resolve disease [25]. Similarly, inhibition of Syk in MRL/lpr lupus-prone mice can prevent and reverse disease symptoms, offering potential approaches to be considered for clinical use [26]. Understanding the biochemical pathways underlying T cell abnormalities in lupus has identified important novel therapeutic targets, such as Syk [26].

### T cell Mitochondrial Hyperpolarization and Altered Metabolism

SLE T cells display abnormal persistent mitochondrial hyperpolarization, depletion of the main intracellular antioxidant glutathione, and reduced ATP synthesis, leading to spontaneous apoptosis and decreased activation-induced cell death [7]. **Mammalian/mechanistic target of rapamycin** (mTOR) is a serine/threonine kinase sensor of mitochondrial membrane potential. T cells from SLE patients exhibit mitochondrial dysfunction, characterized by elevated mitochondrial transmembrane potential [27]. Rapamycin, which blocks mTOR activation, can normalize T cell signaling in SLE T cells, and its use in SLE patients has shown improvement in disease activity [28,29].

Aerobic glycolysis and mitochondrial oxidative phosphorylation are also elevated in T cells from lupus-prone MRL/lpr mice and from patients with SLE [7,30]. In SLE T cells, increased mitochondrial metabolism can contribute to aberrant T cell function [7]. Along these lines, administering 2-deoxy-D-glucose (an aerobic glycolysis inhibitor) and metformin (a mitochondrial metabolism inhibitor) has been shown to suppress autoimmunity and nephritis in **B6.Sle1.Sle2.Sle3** and in **New Zealand black  $\times$  New Zealand white F1** (NZB/W F1) lupus-prone mice [31].

In a double-blind placebo-controlled trial of SLE patients, *N*-acetylcysteine (NAC, an antioxidant), reversed glutathione depletion in blood and improved disease activity by blocking mTOR and expanding Tregs [32]. Furthermore, **quantitative metabolomics analyses** were conducted on total peripheral blood lymphocytes of SLE patients to assess the mechanism of impact of NAC in the context of the trials, and revealed that NAC treatment significantly reduced levels of the metabolite **kynurenine**, and this was the top predictor of NAC effect. Therefore, the accumulation of kynurenine in SLE patients and its stimulation of mTOR might constitute an important metabolic checkpoint in lupus pathogenesis [33]. Knowledge generated from immune cell metabolomics studies should

enrich our understanding of the contribution of cellular metabolism defects in SLE and guide potential approaches to treatment.

### Alternative Splicing in T Cell Gene Regulation

Aberrations in alternative splicing involve several T cell related genes contributing to altered gene expression in SLE. For instance, alternative splicing of genes was first identified in human SLE T cells for the *CD3Z* gene which showed unstable splice variants in lupus [34]. Moreover, alternative splicing of *CD44* leading to the production of CD44v3 and CD44v6 isoforms has been reported in T cells from SLE patients relative to controls [35]. In addition, cAMP response element modulator *CREM* genes have been shown to result in repressive transcriptional isoforms (CREM $\alpha$  and ICER) in SLE patients and mice, and ICER/CREM-deficient B6.*Ipr* mice exhibit recovery from autoimmune symptoms [36]. The **signaling lymphocytic activation molecule** family (*SLAMF*) genes encode cell-surface proteins on T cells and are important in T cell activation. A short spliced isoform of *SLAMF6* (*Ly108HI*) has been found to mitigate the T cell-dependent autoimmunity in B6.*Sle1b* mice [37]. A serine/arginine-rich splicing factor 1 (SRSF1) has also been identified in connection with CD3 $\zeta$  alternative splicing [38], and promotes IL-2 production in human T cells [39]. These findings are relevant because *SRSF1* mRNA and protein levels have been shown to be decreased in SLE T cells relative to healthy T cells and, furthermore, increasing *SRSF1* expression in T cells from SLE patients can rescue IL-2 production [39]. Consequently, aberrant alternative splicing appears to be a common pathogenic mechanism of SLE which is also shared by other autoimmune diseases such as multiple sclerosis [40]. Therefore, a better understanding of the mechanisms of gene regulation in SLE might lead to the potential identification of novel therapeutic targets.

### CD4<sup>+</sup> Helper T Cell Subsets, and Cytokines in SLE

CD4<sup>+</sup> helper T cells control or modify immune responses through cytokine secretion. SLE CD4<sup>+</sup> T cells display aberrant cytokine production, a profound defect in IL-2 production, and their effector and regulatory capacities are compromised; in type 17 T helper (Th17) cell subsets, increased production of **interleukin 17** (IL-17) can increase the inflammatory response [4]. Indeed, using an inducible recombinant adeno-associated virus vector, replenishment of IL-2 in lupus-prone mice (MRL/*Ipr*) has been found to correct immunoregulatory CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg function, and decrease the number of CD4<sup>-</sup>CD8<sup>-</sup> double-negative T cells as well as the number of CD3<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup> T cells producing IL-17; this reduced cell infiltration (and hence inflammation and tissue damage) into several organs, including skin, lung, and kidney [41]. In addition, the subcutaneous administration of low doses of IL-2 (1 million or 1.5 million IU of human IL-2) in patients with SLE has been claimed to be beneficial in case reports [42,43], highlighting the importance of IL-2 deregulation in SLE pathogenesis and the potential of therapeutically targeting this defect.

Along these lines, IL-17 is produced by both CD4<sup>+</sup> and expanded double-negative (CD3<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup>) T cells subsets which are both capable of infiltrating the kidneys of mice and patients, promoting inflammation and recruiting other immune cells such as neutrophils, and thus contributing to lupus disease [44]. Targeting this cytokine pathway should also be

considered because its inhibition by expression of a soluble form of neutralizing IL-17 receptor (IL-17R) using an adenovirus, or by inhibition of IL-23 or STAT-3, has shown disease amelioration in BXD2 and MRL/*lpr* mouse models of lupus [45,46].

The reduced production of IL-2 and the increment in Th17 differentiation and IL-17 production associated with lupus appears to be better understood at this point. For example, **calcium/calmodulin-dependent protein kinase 4** (CaMKIV) – whose activity is increased in SLE T cells – has been reported to promote the activation of transcription factor CREM $\alpha$  [47], which binds to **DNA methyltransferase** DNMT3 [48]; DNMT3 is then recruited to the *IL2* locus in CD4<sup>+</sup> T cells, increasing CpG methylation and reducing *IL2* expression [48]. Moreover, forced expression of CREM $\alpha$  results in reduced CpG methylation and increased transcription of the *IL17A* locus [48]; this suggests that CREM $\alpha$  might mediate epigenetic remodeling of these loci in SLE T cells, and this appears to favor an effector memory subset [48]. Another molecular mechanism of increased production of IL-17 in lupus involves signaling via **Rho-associated coiled-coil domain protein kinase** (ROCK); ROCK activity is promoted by **protein phosphatase 2A** (PP2A), which causes increased binding of IRF4 to the *Il17* promoter in mouse CD4<sup>+</sup> T cells [49,50]. In addition, ROCK is also involved in the phosphorylation of the ezrin, radixin, and moesin (ERM) cytoskeletal complex which leads to increased adhesiveness of hyaluronic acid (CD44) in human SLE T cells [51].

Moreover, Tfh cells, a dynamic subset of CD4<sup>+</sup> T cells (CXCR5<sup>+</sup> **programmed cell death 1** (PD1)<sup>+</sup>OX40<sup>+</sup>ICOS<sup>+</sup>) expressing the transcription factor BCL6, are essential for B cell maturation and antibody production [52]. They secrete **interleukin 21** (IL-21) which drives B cell immunoglobulin production, **isotype switching**, and **somatic hypermutation** [53]. Studies in the lupus-prone mouse model BXSB-Yaa have shown that blockade of IL-21 with IL21R-Fc, or **inducible T cell costimulator** (ICOS) deficiency, can reduce disease progression [54]. Furthermore, genetic deletion of IL-21R in T cells or B cells has resulted in attenuated kidney disease in a P  $\rightarrow$  F1 chronic graft-versus-host disease (cGVHD) mouse model, implicating the IL21/IL21R axis in promoting lupus-like disease; the mechanism appeared to involve both CD4<sup>+</sup> Tfh<sup>-</sup> and B cell-intrinsic mechanisms because deficiency of IL-21R also caused impaired Tfh expansion and reduced **germinal center B cell** differentiation [55]. In addition, a newly described death receptor 6 (DR6) on Tfh cells has been shown to interact with syndecan 1 on autoreactive germinal center B cells in mice [56]; blockade of this axis via monoclonal antibodies has been reported to delay disease progression in NZB/W F1 lupus-prone mice [56]. In addition, **extrafollicular helper T cells** (eTfh) represent a CD4<sup>+</sup> T cell subpopulation analogous to Tfh that can promote immunoglobulin production by B cells in extrafollicular compartments [57]. Remarkably, eTfh cell numbers are increased in the peripheral blood of SLE patients, correlating with **plasmablast** B cell numbers as well as with **anti-dsDNA auto-antibody** titers, implicating this T cell subset in SLE disease pathogenesis [58,59]. Of note, a subpopulation of  $\gamma\delta$  **T cells**, termed  $\gamma\delta 2$  cells, accumulate in the kidneys of SLE patients, express CD40L, and secrete IL-21, which aids the formation of extrafollicular germinal centers in the kidney [60]. However, the contribution of  $\gamma\delta$  T cells in SLE pathogenesis is not known; nevertheless, because IL-21 secretion (from various T cell subsets) appears to play a role in

SLE pathogenesis, it is possible that IL-21 blockade may be helpful to SLE patients, but this remains to be tested.

Treg numbers and/or function exhibit variable reductions in SLE patients [61]. Thus, it is currently not clear what the precise contribution of Tregs to SLE pathogenesis is. The plasticity and stability of Tregs is indeed complex, and may harbor important therapeutic implications [62]. For instance, several approaches have been taken to increase Treg numbers/function [63]. First, low-dose IL-2 concentrations (1 million IU of recombinant human IL-2 subcutaneous administration) seem to increase Treg numbers in SLE patients [42]. Given that the pharmacologic window between high-and low-dose IL-2 administration is small [64], approaches to limit IL-2 delivery to Tregs deserves maximum attention. Second, increased expression and activity of particular molecules have been found to compromise the function of Tregs in mice and humans, including CaMKIV [65], Notch1 [66], leptin [67], and mTORC1 [68,69]. Indeed, inhibition of mTOR has been shown to ameliorate disease activity in SLE patients [32]. Consequently, it is hypothesized that the specific delivery of 'empowering' molecules such as CaMKIV inhibitor(s) to Tregs for SLE treatment might be envisaged, and this deserves further consideration [65].

### CD8<sup>+</sup> T Cells and Cytotoxic Responses in SLE

Although SLE studies in CD8<sup>+</sup> T cells are not as extensive as those for CD4<sup>+</sup> T cells, several reports demonstrate the deficient cytotoxic capacity of CD8<sup>+</sup> T cells in SLE (reviewed in [70]). For instance, reduced numbers of SLAMF4-expressing memory CD8<sup>+</sup> T cells and decreased cytotoxic activity *in vitro* have been reported in samples from SLE patients [71]. In addition, increased expression of PD-1 in CD8<sup>+</sup> T cells from SLE patients, as well as conversion of CD8<sup>+</sup> T cells into double-negative T cells, have been related to dampened cytotoxic function *in vitro*, and to reduced proliferative responses to viral peptides [72]. Indeed, SLAMF4 and its transducer SAP are key molecules involved in cytotoxic responses against Epstein–Barr virus [73]. In addition, lack of CD28 expression in human SLE CD8<sup>+</sup> T cells may be involved in diminished cytotoxicity because these cells might not be able establish immunological synapses with antigen-presenting cells; however, an increased number of CD28-deficient CD8<sup>+</sup> T cells has been associated with increased lupus activity, given that these display a proinflammatory phenotype [74]. Furthermore, engagement of SLAMF7 in CD8<sup>+</sup> T cells from SLE patients has been shown to restore defects in the antigen-specific cytotoxicity of these patient CD8<sup>+</sup> T cells *in vitro* [75]. The defect in cytotoxicity has clinical relevance because it can limit defense against infectious agents, and it is known that bacterial and viral infections represent the most important cause of death in SLE patients [76].

Overall, studies of T cells in human and murine lupus have demonstrated the central functional role of these cells in SLE disease pathogenesis and, furthermore, the elucidation of various molecular aberrations in these cells has revealed several putative targetable molecules, some of which are currently ongoing clinical trials for SLE (Table 1), while others remain to be tested.

**B Lymphocytes and SLE Pathogenesis**—SLE patients and lupus-prone mice show multiple B cell abnormalities including B cell **lymphopenia** and B cell hyperactivity (such as increased immunoglobulin production); therefore, modulating B cell function has been traditionally viewed as an attractive therapeutic approach to treating SLE [77].

**Regulatory Nodes and B Cell Subpopulations**—Patients with active SLE present profound naive B cell (CD19<sup>+</sup>CD27<sup>-</sup>) lymphopenia and increased numbers of **transitional B cells** (CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>hi</sup>), **switched memory B cells** (CD19<sup>+</sup>CD27<sup>+</sup>IgD<sup>-</sup>), **double-negative** (CD19<sup>+</sup>CD27<sup>-</sup>IgD<sup>-</sup>) B cells and plasmablasts/ **plasma cells** (CD27<sup>hi</sup>CD38<sup>+</sup>CD19<sup>dim</sup>sIg<sup>low</sup>CD20<sup>-</sup>CD138<sup>+</sup>), correlating with disease activity [78,79]. Moreover, a fraction of human CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>hi</sup> B cells secrete IL-10 and suppress Th1 and Th17 cell differentiation, and may be limited in SLE patients [80]. The **9G4<sup>+</sup> antibody** represents a major part of the anti-apoptotic cell repertoire in SLE patient sera, and correlates with disease activity [81]. Furthermore, the selection of 9G4<sup>+</sup> B cells by apoptotic cell antigens may represent an important step in SLE progression [82]. SLE patients exhibit increased frequencies of self-reactive B cells, both in recently emigrating and mature naïve B cell subsets, demonstrating a breach in early B cell **tolerance** pathways [83]. In addition, DNA-reactive B cells are more likely to mature, participate in germinal center reactions, and undergo plasma cell differentiation in lupus patients [84].

**B Cell Signaling in SLE**—B cell intrinsic risk alleles including *BANK1*, *BLK*, *CSK*, *FCGR2B*, and *PTPN22* have been linked to increased susceptibility to SLE [84]. Each of these genes, except for *PTPN22*, leads to hyper-responsiveness to B cell receptor (BCR) engagement and enhanced B cell activation [84]. The *PTPN22* risk allele results in altered *PTPN22* signaling, with decreased phosphorylation of proteins in the BCR pathway, and it has been shown to diminish tolerance in human immature B cells [84]. In normal B cells the kinase CSK physically interacts with the intracellular phosphatase Lyp (encoded by *PTPN22*) which can augment inhibitory phosphorylation of downstream Src kinases, such as Lyn [85]. In SLE, a risk allele has also been associated with increased *CSK* expression [85].

In addition, pronounced Syk and Btk phosphorylation – mediating crosstalk between BCR and TLR, as well as between BCR and JAK/STAT pathways – has been observed in B cells of patients with active SLE compared to those of healthy individuals, potentially contributing to their hyperactive state (reviewed in [86]). Another study documented that the formation of antinuclear antibodies in MRL/*Ipr* mice depended on TLR signaling adaptor MyD88 in total B cells; B cell-specific MyD88 deficiency ameliorated nephritis in MRL/*Ipr* mice, clearing antibody-independent interstitial T cell infiltrates, and indirectly suggested that nucleic acid-specific B cells might potentially activate nephrotoxic T cells [87]. Further work in these studies implicated the BCR, TLR, and JAK/STAT signaling pathways in B cells in lupus pathogenesis, and could lead to potential therapeutic targets.

B cells, despite being antibody producers and antigen-presenting cells, are also important mediators of organ inflammation. However, B cell-depleting biologics such as Rituximab (anti-CD20 monoclonal antibody) have failed so far to deliver acceptable clinical effects [88,89] (Table 1); evidently, lupus disease does not depend on B cells alone. Consequently, further research is warranted, and future studies should determine whether (and which)



patients might benefit from B cell-depleting biologics, and whether targeting B cell signaling molecules might preferentially limit their hyperactivity.

### Innate Immune Cells in SLE

As previously mentioned, increasing evidence links the profound defects in innate immunity with SLE disease initiation and progression, as well as with tissue damage [90,91]. Defective phenotypes and functions of neutrophils, monocytes, macrophages, and DCs [92–94] have been identified in SLE patients [95]. These defects play vital roles in the pathogenesis of SLE, including ineffective apoptotic debris clearance [96], self-antigen presentation [92], and inflammatory cytokine production [19,94,97] (Figure 3).

### Neutrophils

Altered functional properties of neutrophils have been observed in SLE, including diminished phagocytic and lysosomal activity, upregulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), increased cellular aggregation, and intravascular activation *in vivo* [98]. In addition, the presence of tissue infiltrating neutrophils is a hallmark of **diffuse proliferative lupus nephritis** [99]. A novel mechanism of neutrophil cell death was recently reported, consisting in the extrusion of **neutrophil extracellular traps** (NETs), a network of chromatin fibers primarily composed of DNA [100]. Indeed, studies have linked NET formation to the source of dsDNA autoantigen in lupus [101]. Moreover, pharmacologic inhibition with peptidylarginine deiminase disrupts NET formation and protects against kidney, skin, and vascular disease in lupus-prone MRL/*Ipr* mice [102]. However, lupus-prone MRL/*Ipr* mice that lack functional NADPH-oxidase (which is required for NET formation) [100] develop a worsening phenotype [103]. Consequently, these observations have raised controversy on the contribution of NETosis to the pathogenesis of SLE. Nevertheless, mitochondrial reactive oxygen species (ROS) production can support NET formation without activation of functional NADPH-oxidase in low-density granulocytes from patients with SLE, thus suggesting that the development of mitochondrial ROS inhibitors may offer a novel putative strategy to perturb SLE progression [104].

### Dendritic Cells

It has long been hypothesized that prolonged self-antigen presentation and enhanced inflammatory cytokine production by DCs are important in the development of autoimmune diseases, and that defects in tolerogenic DC functions are crucial in breaking self-tolerance [105]. *In vivo*, DCs are the major phagocytes involved in the rapid clearance of apoptotic debris, via an immunologically silent process, which, coupled to the production of anti-inflammatory cytokines, is an effective means of self-tolerance [105]. Receptors such as MFG-E8, Tim4, Scarf1, or soluble bridging molecules, as in the case of the **complement cascade**, for example C1q, have been implicated in the clearance of apoptotic cells [106]. It has been proposed that deficiency of these molecules could initiate or promote autoimmunity, in some cases resembling lupus-like disease [107–111], which has been demonstrated by numerous studies in mice. Moreover, alterations affecting DC development and maturation might also promote autoimmunity; for example, mice harboring a deficiency

of the transcription factor Blimp-1 in DCs have been reported to develop spontaneous autoimmune disease resembling features of lupus, including increased anti-dsDNA antibodies, splenomegaly, and glomerulonephritis [112,113].

The current consensus is that two principal subsets of DCs have been identified in mice: classical DCs (cDCs) and plasmacytoid DCs (pDCs) [114]. Equivalent DC subsets exist in humans. Different DCs differ in their development, location, transcriptional regulation, phenotypic features, and immunological functions [92]. pDCs produce large amounts of type I IFN, and this IFN- $\alpha/\beta$  'signature' is often observed in SLE patients [18]. Therefore, pDCs have been hypothesized to contribute to the pathogenesis of SLE [115,116]. Indeed, type I IFNs are proinflammatory cytokines which are cytotoxic for a variety of cells, and thereby can provide a potential source for autoantigens by inducing cell apoptosis [117]. Type I IFNs can directly act on T cells to enhance immune responses *in vivo* by modulating T cell activation, proliferation, differentiation, and survival [118–120]. They can also modulate different aspects of B cell function, including antigen recognition, antigen presentation, cell migration, cytokine production, survival, and class-switch recombination [121–124]. Therefore, it is not surprising that type I IFN targeted therapies to treat lupus are under development.

Various studies on pDCs have characterized the expansion and aggregation of pDCs in the splenic perifollicular region of lupus-prone (BXD2, B6.*Sle1.Sle2.Sle3*) mice as well as in SLE patients [125,126]. Massive pDC infiltrates have also been observed in the renal and skin lesions of SLE patients, suggesting that they contribute to local tissue damage [127]. In addition, transient (7–14 days) ablation of pDCs, mediated by the blood DC antigen 2–diphtheria toxin receptor (**BDCA2–DTR**) transgene in lupus-prone BXSB mice, has been reported to reduce autoantibody production and ameliorate kidney pathology [128,129]. Of note, platelets in human and murine lupus activate pDCs to produce IFN- $\alpha$  and an ensuing lupus-like pathology [130]. Consequently, depleting platelets or administering the P2Y<sub>12</sub> receptor antagonist (clopidogrel) improved disease symptoms and survival in NZB/W F1 and MRL/*lpr* lupus-prone mice [130]. We believe that targeting platelets might have, at least, an adjuvant clinical value for patients with SLE.

Collectively, strategies targeting DCs to limit their self-reactivity and promote their tolerogenic functions are being considered as potential therapeutic tools to re-establish tolerance [92]. Nevertheless, a great deal of research is still necessary to further understand the molecular underpinning and precise contribution of DCs to SLE pathogenesis to inform potential treatment avenues.

### Monocytes/Macrophages

Monocytes and macrophages are both potent phagocytes necessary for the clearance of apoptotic debris. Defects in this process lead to breakdown of immune tolerance by providing autoantigens for adaptive immunity against self [131]. In support of this concept, a reduction in the numbers or function (impaired uptake of apoptotic cells) of **phagocytic tingible body macrophages**, concomitant with an accumulation of apoptotic cells near germinal centers, has been reported in SLE patients, as well as in several murine strains such as MFG-E8<sup>-/-</sup> and Mer<sup>-/-</sup> mice, which display features of lupus-like disease [132,133].

Furthermore, splenic examination of several lupus-prone mice, including BXD2 and B6.*Sle1.Sle2.Sle3* mice, and of patients with SLE, has revealed gradual loss of phagocytic function and survival of a distinct cell population of **marginal zone macrophages** (MZMs) harboring a unique capacity to clear apoptotic cells and induce tolerogenic signals, including the production of TGF- $\beta$  and IL-10 [125,134]; these findings suggest that agents that can specifically restore MZM barriers might be therapeutically considered as candidates to prevent the onset and progression of lupus [125,134]. Recently, a form of noncanonical **autophagy** was described, known as microtubule-associated protein 1A/1B-**light chain 3 (LC3)-associated phagocytosis** (LAP), in which phagosomes recruit elements of autophagy to facilitate the phagocytosis of dying cell debris [135]. Of relevance, knockout of LAP-specific genes, including *Rubico*, autophagy protein 5 (*Atg5*), and *Atg7* in mice has been shown to lead to a lupus-like syndrome that includes the presence of autoantibodies and kidney damage [136]. These findings suggest that pharmacologic approaches aiming to 'bypass' LAP to control inflammation and autoimmunity might be promising.

Although pathogenic roles of different components and cell subsets of the innate immune system are being increasingly recognized, further studies will evidently be necessary to gain increased knowledge of their role in SLE and to aid in the design of novel putative therapeutic approaches.

## Tissue Injury in SLE

Renal impairment is the most common and severe clinical manifestation of tissue damage seen in patients with SLE, and is characterized by **immune complex** deposition, inflammation, and scarring of glomeruli and interstitium; these are followed by skin injury, which also involves autoantibody production together with immune complex formation and deposition [137]. A source of autoantigens and endogenous molecules that promote inflammation is generated by apoptosis, a form of programmed cell death; nucleosomes containing nucleic acids with other endogenous ligands are incorporated in apoptotic blebs, a major source of such damaging molecules. These blebs promote activation of B cells and DCs, breaking tolerance and inducing autoimmunity by priming autoreactive T cells leading to the production of IFN- $\alpha$  and autoantibodies, thus activating TLR and other domain receptors [138]. Although a wide spectrum of autoantibodies and multiorgan tissue damage can be present in SLE, only a few autoantibodies have been found in mice and humans to specifically contribute to disease-related injury, including anti-blood cell antibodies causing **cytopenia**, anti-dsDNA antibodies causing nephritis, and **anti-phospholipid antibodies** causing, among various pathological mechanisms, fetal resorption [79]. Moreover, emerging evidence suggests a crucial role of various cytokines in tissue pathology in addition to antibody-antigen immune complex build-up in organs. These cytokines, including IL-6, BlyS, IL-17, type I IFNs, TNF- $\alpha$ , and IL-18, can induce immune dysregulation followed by local inflammation and tissue damage. It has also been proposed that tissue-resident cells might express receptors for inflammatory cytokines, and respond to these, accentuating local injury; for example, **mesangial cells** produce IL-6, whereas **podocytes** express CD86, which may provide costimulation to lymphocytes [140]. Moreover, confocal microscopy has identified the presence of organized lymphoid aggregates, termed **tertiary lymphoid organs**, in non-lymphoid organs such as the kidneys of patients with lupus nephritis [141].

Furthermore, the use of congenic mice has shown that distinct chromosomal regions determine the development of autoimmunity and chronic kidney damage [142]. The recognition that tissue injury and autoimmunity should be considered as independent processes dictates that we should strive to better understand the nature of organ-specific factors that might enable inflammation in the presence of an autoimmune response.

## Biologics and Biomarkers in the Treatment and Diagnosis of SLE

From a clinical perspective, most trials with biologics have failed to show efficacy in SLE patients. These failures might be due to the clinical heterogeneity of the disease, a multiplicity of pathogenic mechanisms, lack of reliable biomarkers, or improper design of clinical trials. Overall, biologics can be grouped based on the pathological process or cell type they target. Table 1 lists clinical trials for SLE which are ongoing or have concluded (reviewed in [144–146]). The failure of clinical trials and the advancing recognition that distinct molecular and cellular pathways may operate in individual patients dictates the need to consider personalized/precision approaches for SLE.

In terms of diagnostics, an important aspect of SLE management is the prediction of the occurrence of flares, response to therapy, and disease prognosis. Lupus nephritis remains a dreaded complication and a major cause of morbidity and mortality [147,148]. Traditional diagnostics of SLE have included measuring anti-dsDNA, complement, creatinine, or proteinuria, but are not suitable because they do not distinguish renal function from renal damage, a crucial factor [147]. Moreover, the pathogenic events initiating damage may long precede abnormal renal function. Several molecules measured either in the urine or serum have been identified by targeted selection of candidates, or by non-targeted high-throughput proteomics approaches, and are emerging as potential biomarkers for renal disease in SLE. These include various cytokines, chemokines, adhesion molecules, growth factors, miRNAs, and other molecules. Among the most promising are neutrophil gelatinase-B-associated lipocalin (NGAL)/lipocalin 2, MCP-1, TWEAK, IP-10, CXCL-16, IL-6, IL-17, VCAM, TGF- $\beta$ 1 (mRNA), and L-prostaglandin D synthase (PGDS) [147]. While these serum/urinary biomarkers show promise, further work will be necessary to robustly validate their utility in the clinic.

Early and accurate diagnosis of SLE is also vital in the prevention of significant morbidity and mortality. Recent work has shown that serum biomarkers such as type II IFN cytokines appear years before SLE diagnosis, followed by anti-dsDNA autoantibodies and then by type I IFN activity immediately preceding clinical diagnosis, bringing forth the notion that they might be exploited in the potential prevention of SLE [8–10]. These studies support the concept of a gradual chronological disease progression, from a preclinical asymptomatic phase, to incomplete lupus erythematosus, and finally, to complete lupus erythematosus [149]. Clinical and serologic parameters distinguish patients with incomplete lupus from SLE patients, and identification of these parameters is crucial for early management of disease [150–152]. Consequently, tailoring management based on these early identifications has the potential to significantly improve patient outcomes and reduce healthcare costs.

## Concluding Remarks

SLE has been one of the most challenging diseases to understand and treat. It is obvious from the multiple failures of clinical trials that the disease does not depend on a single pathogenic process. Each biologic administered to patients does what it is designed to do. For example, an IFN blocker neutralizes the action of IFN, but fails when tested in clinical trials. This painful experience suggests that many pathways lead to what is clinically defined as SLE. It is true that several pathways are shared by a substantial percentage of patients, indicating that, in each patient, a particular pathway may be leading or dominant, whereas the remaining pathways might be ‘called-on’ to action. The patients who develop SLE as a result of a single genetic defect provide an example of this line of thought. For example, C1q- and C4-deficient individuals, through clearly distinct cellular pathways, share several immunologic and clinical manifestations [153]. In addition, little attention if any is paid to unknown complexities of targeted pathways. For example, does an anti-CD20 antibody deplete B cells in SLE patients from all compartments the same way it does in healthy individuals, or do low doses of administered IL-2 generate or activate STAT5 in SLE T cells compared to normal T cells? Animal studies are used to address specific mechanisms which underwrite autoimmunity and related pathology. Nevertheless, some information derived from preclinical studies is inappropriate for the design of clinical trials in SLE patients. The complexity of the cellular and biochemical events which have been unveiled in SLE patients and documented mechanistically *in vitro* or *in vivo* in animals strengthens the position that much more needs to be learned before venturing into treating the disease, despite the apparent popular urge to proceed (see Outstanding Questions and Box 4). We argue that there is an urgent need for personalized medicine in patients with SLE, which implies methods of stratification. In addition, for each patient, we need to define the origin and processes that have led to disease manifestation and design appropriate treatments. These approaches may be long-winded and expensive, but ultimately may prove to be more targeted, successful, and cost-effective than current practices.

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## Abbreviations

### **9G4<sup>+</sup> antibody**

a major species of anti-apoptotic cell antibodies in SLE serum; associated with disease activity.

### **Anti-dsDNA autoantibodies**

antibodies reacting with double-stranded (ds)DNA; these are highly diagnostic of SLE and are implicated in the pathogenesis of lupus nephritis.

### **Anti-phospholipid antibodies**

antibodies against phospholipids that are found in all living cells and cell membranes.

### **Autophagy**

an intracellular degradation process in which a cell digests itself.

### **B6.*Sle1.Sle2.Sle3* mice**

homozygous mice for three NZB/W-derived lupus susceptibility gene loci (*Sle1*, *Sle2*, *Sle3*) on the C57BL/6J background; animals develop systemic autoimmunity with fatal glomerulonephritis; model for SLE.

### **BDCA2–DTR mice**

mice harboring a blood dendritic cell antigen 2 (BDCA2)–diphtheria toxin receptor (DTR) fusion transgene; this enables efficient plasmacytoid dendritic cell (pDC) depletion *in vivo* after a single dose of diphtheria toxin.

### **Calcium/calmodulin-dependent protein kinase IV (CAMKIV)**

serine/threonine kinase implicated in T cell transcriptional regulation.

### **CD4<sup>+</sup>T helper (Th) cells**

by releasing cytokines, Th cells can help to suppress or regulate immune responses; they are essential for B cell antibody class-switching, the activation and growth of cytotoxic T cells, and for maximizing the bactericidal activity of phagocytes.

### **Cluster of differentiation 3 $\zeta$ (CD3 $\zeta$ )**

a subunit of CD3 complex of the T cell receptor; helps to activate cytotoxic CD8<sup>+</sup> and CD4<sup>+</sup> helper T cells and is downregulated in many chronic inflammatory diseases.

### **Complement component 1q (C1q)**

the first subcomponent of the C1 complex in the classical pathway of complement activation.

### **Complement cascade**

a series of small proteins and protein fragments (>30) that lead to the stimulation of phagocytes to clear foreign and damaged material, proxy inflammation to attract additional phagocytes, and activate the cell-killing membrane attack complex. The complement system includes serum and serosal proteins, as well as cell membrane receptors.

**Cytopenia**

a reduction in the number of cells (blood); often observed in SLE patients.

**Diffuse proliferative lupus nephritis**

class IV disease, the most severe and most common subtype of lupus nephritis. More than 50% of glomeruli are involved. Lesions can be segmental or global, and active or chronic, with endocapillary or extracapillary proliferative lesions.

**DNA methyltransferases (DNMTs)**

catalyze the addition of a methyl group to DNA; they might contribute to aberrant epigenetic regulation in SLE.

**Double-negative B cells**

IgD<sup>-</sup>CD27<sup>-</sup> cells; these are age-related or autoimmune disease-associated.

**Extrafollicular helper T cells (eTfh)**

antigen-experienced CD4<sup>+</sup> T cells found outside B cell follicles of secondary lymphoid organs such as lymph nodes, spleens, and Peyer's patches; eTfh cells are identified by constitutive expression of CXCR4.

**Fc- $\gamma$  receptor (Fc $\gamma$ R)**

a crucial component of Fc receptors expressed on many inflammatory cells. Fc receptors are essential for the initiation or maintenance of immune responses.

**Genome-wide association studies (GWAS)**

examination of a genome-wide set of genetic variants, in different individuals within a population, that are potentially associated with a given trait.

**Germinal center B cells**

mature B cells reside in germinal centers within lymphoid organs, central factories for the generation of affinity-matured B cells specialized in producing refined antibodies.

**Histone deacetylases (HDACs)**

enzymes that catalyze the removal of acetyl groups from histone proteins (epigenetic regulation).

**Immune complexes**

formed by the binding of an antibody to a soluble antigen. Their deposition in tissues (e.g., kidneys) causes damage and is a prominent feature of several autoimmune diseases.

**Inducible T cell costimulator (ICOS)**

a CD28 superfamily costimulatory molecule that is expressed on activated T cells.

**Interferon regulatory factors (IRFs)**

proteins which regulate the transcription of interferons (IFNs) and are crucial for innate immunity. Dysregulation of IRF signaling might contribute to autoimmune diseases.

**Interleukin 2 (IL-2)**



interleukin produced by T cells, has essential roles in tolerance and immunity.

**Interleukin 17 (IL-17)**

produced by T helper cells; acts as a potent mediator of delayed-type immune reactions and inflammation.

**Interleukin 21 (IL-21)**

produced by activated T cells to regulate immune responses; strongly linked to inflammation and autoimmunity.

**Isotype switching**

mechanism by which B cell production of immunoglobulin is changed from one type to another.

**Kynurenine**

metabolite of the amino acid L-tryptophan.

**Light chain 3 (LC3)-associated phagocytosis (LAP)**

an autophagy-related process in which phagosomes become decorated with LC3 and are subsequently trafficked to the lysosome for degradation.

**Lipid rafts**

subdomains of the plasma membrane containing high concentrations of cholesterol and glycosphingolipids. Signaling in these domains differs in T cells from patients with SLE and rheumatoid arthritis.

**Lymphopenia**

low levels of lymphocytes in the blood.

**Mammalian/mechanistic target of rapamycin (mTOR)**

ubiquitous atypical serine/threonine kinase important for cellular processes including survival, growth, and proliferation. Aberrant mTOR signaling is involved in many diseases (including autoimmune).

**Marginal zone macrophages (MZMs)**

a small specialized macrophage subset residing in the marginal zone of the spleen; MZMs play a central role in the clearance of apoptotic cells to minimize the immunogenicity of autoantigens.

**Mesangial cells**

constitute the glomerular mesangium and, with the mesangial matrix, form the vascular pole of the glomerulus. Their primary function is to remove trapped residues and aggregated protein from the basement membrane.

**MRL/lpr mouse model**

mice homozygous for the lymphoproliferation spontaneous mutation (*Fas/lpr*) show systemic autoimmunity, massive lymphadenopathy associated with proliferation of aberrant T cells, arthritis, and immune complex induced glomerulonephrosis; model for SLE.

**Neutrophil extracellular traps**

networks of extracellular fibers that are primarily composed of DNA from neutrophils, and may provide a source of autoantigens in autoimmune diseases.

**New Zealand black × New Zealand white F1 mice (NZB/W F1)**

these mice develop an autoimmune disease resembling human SLE: high levels of antinuclear antibodies, hemolytic anemia, proteinuria, and progressive immune complex glomerulonephritis (more pronounced in females).

**Phagocytic tingible body macrophages**

a subtype predominantly found in germinal centers that contain many phagocytized, apoptotic cells.

**Plasmablasts**

immature B cells that divide rapidly and secrete antibodies (but less than plasma cells).

**Plasma cells**

effector B cells that secrete large volumes of antibodies.

**Podocytes**

cells in the renal Bowman's capsule that wrap around the capillaries of the glomerulus. Their primary function is to filter blood and retain large molecules (e.g., albumin).

**Programmed cell death 1 (PD1)**

inhibitory T cell-surface receptor implicated in mechanisms of self-tolerance. It is an immune checkpoint against autoimmunity.

**Protein phosphatase 2A (PP2A)**

ubiquitously expressed; regulates cellular function by dephosphorylating molecules such as Akt, p53, c-Myc and  $\beta$ -catenin. Deregulation of PP2A in T cells has been implicated in autoimmunity.

**Protein tyrosine phosphatase 22 (PTPN22)**

lymphoid-specific intracellular phosphatase that acts as negative regulator of T cell receptor signaling. Mutations in its coding gene may be associated with autoimmune disorders including SLE.

**Quantitative metabolomics analysis**

quantitative study of chemical processes involving metabolites.

**Rho-associated coiled-coil domain protein kinases (ROCKs)**

proteins involved in cytoskeletal reorganization and control of cell adhesion and migration, apoptosis, proliferation, and differentiation.

**Signal transducers and activators of transcription (STATs)**

a family of signaling intermediates in the Janus kinase (JAK)/STAT signaling pathway which initiate gene transcription. Gain-of-function or loss-of-function mutations of different STATs can lead to immunodeficiency, infections, or multiorgan autoimmunity.

**Signaling lymphocytic activation molecules (SLAMs)**

a group of type I transmembrane receptors. SLAM members appear to play crucial roles in multiple autoimmune diseases.

**Single-nucleotide polymorphisms (SNPs)**

variations in a single nucleotide occurring at a specific position in the genome, where each variation is present to some appreciable degree within a population.

**Somatic hypermutation**

cellular mechanism by which the immune system adapts to new foreign elements. It involves a programmed process of mutation affecting the variable regions of immunoglobulin genes.

**Switched memory B cells**

a B cell subtype that is formed within germinal centers following antigen encounter (switched immunoglobulin receptor).

 **$\gamma\delta$  T cells**

T cells that display the distinctive  $\gamma\delta$  T cell receptor (TCR) on their surface (most T cells bear  $\alpha\beta$  chain TCRs); mostly expressed in mucosal sites; considered as 'first line of defense' or 'bridge between innate and adaptive immunity'.

**Tertiary lymphoid organs**

functional ectopic lymphoid structures formed inside non-lymphoid tissues, presumed to have immune functions.

**T follicular helper (Tfh) cells**

a CD4<sup>+</sup> T cell subset that provides IL-21-mediated help to B cells (Tfh cells express BCL6).

**Tolerance**

the ability of the immune system to recognize and tolerate the body's own proteins and organs, avoiding attacks against 'self'.

**Toll-like receptors (TLRs)**

play key roles in innate immunity; sense danger/damage signals, recognizing structurally conserved molecular patterns derived from microbes (e.g., pattern-recognition receptors). Endogenous TLR ligands, particularly nucleic acids, may contribute to the modulation of aberrant adaptive immunity (e.g., SLE).

**Transitional B cells**

immature B cells residing in peripheral lymphoid tissues; are capable of differentiating into mature B cells.

### Box 1. A Brief Introduction to SLE

SLE is a chronic debilitating disease which mainly afflicts women, especially those of African-American, Asian, or Hispanic descent [1]. Disease follows an unpredictable course of relapses (flares) and remissions, is difficult to diagnose, and there is currently no cure. Immunosuppressive steroids are the mainstay of therapy, which render patients susceptible to opportunistic infections. Because of a combination of factors, including genetic defects, hormones, environmental exposures such as UV light, medications, or infectious agents, the immune system fails to recognize ‘self’ and begins to attack cells and destroy organs such as joints, skin, brain, and kidneys, leading to SLE. Symptoms of the disease include skin rashes (e.g., facial ‘butterfly’), oral ulcers, arthritis (joint pains and swelling), and neurological manifestations (psychosis, seizures). Damage to blood cells is reflected by hemolytic anemia (destruction of red blood cells), leukopenia (low levels of white blood cells), and thrombocytopenia (low platelet numbers). Further complications in vital organs such as the kidneys can lead to renal failure, further increasing morbidity and mortality.

The disease is extremely heterogeneous such that different patients present with different combinations of clinical manifestations. Autoantibodies circulate in the body, deposit into tissues, and contribute to tissue damage, while cell-mediated mechanisms involve the production of inflammatory cytokines, loss of regulatory function, and infiltration into organs, leading to pathology. Current therapeutic strategies with steroids and cytotoxic drugs are aimed to minimize or halt disease progression and organ damage. However, there is a need for better therapeutic approaches that specifically target pathogenic mechanisms while preserving the protective functions of the immune system. In parallel, the identification of biomarkers to predict onset, progression, and worsening of disease are imperative to better manage this complex disease.

### Box 2. Female Gender Bias in SLE

The impressive preponderance of women suffering from SLE (9:1 female to male incidence) demonstrates the importance of the female gender in the pathogenesis of SLE [179,180]. This gender bias implicates both sex chromosomes and sex hormones in disease. Indeed, in a pristane-induced lupus model in mice, the XX sex chromosome complement was shown to lead to increased susceptibility to autoimmune disease relative to XY mice [181]. Female hormones (estrogen in particular) have been implicated in rheumatic autoimmune disease in animal studies, either by removal of ovaries from female mice or by supplementation of male mice with estrogen [182].

The molecular mechanisms of how hormones regulate the immune system are still largely unclear; however, a few studies have shed light on these aspects. Estrogen can increase expression of the Bcl-2 anti-apoptotic molecule to promote the survival of autoreactive B cells in mice [183] and increase the expression of the B helper molecule CD40 ligand in human SLE CD4<sup>+</sup> T cells [184]. Estrogen can increase the expression of the transcription repressor cAMP response element modulator alpha (CREM $\alpha$ ) and suppress IL-2 production in human T cells [185]. Estrogen can also bind to estrogen receptors (ER) in immune cells, controlling gene expression, and ERs have also been implicated in autoimmune disease. For example, genetic ablation of ER $\alpha$  has been reported to lead to reduced kidney pathology in NZM2410 lupus-prone mice [186]. In addition, engagement of ER $\alpha$  has been shown to promote the appearance of self-reactive B cells and autoimmunity in NZB/W F1 lupus-prone mice [187].

### Box 3. Epigenetic Modifications in SLE

DNA methylation, histone modification, and non-coding RNAs are major epigenetic mechanisms of gene regulation, and alterations have been reported in SLE.

#### DNA Demethylation

In SLE patients DNA has been reported to be spontaneously hypomethylated in CD4<sup>+</sup> T and B cells, leading to higher expression of autoimmune-associated genes normally suppressed by DNA methylation [188]. Methylation-sensitive genes such as *IL6*, *IL10*, *IL13*, *CD6*, *CD70*, *CD40L*, and *CD11A* have been found to be overexpressed in SLE T cells [188]. Moreover, increased levels and activity of the serine/threonine protein phosphatase 2A (PP2A) in SLE T cells has been linked to downregulation of DNA methyltransferases (DNMT1 and DNMT3a), leading to DNA hypomethylation [189]. Recently, genome-wide DNA methylation patterns in naive CD4<sup>+</sup> T cells from SLE patients were examined, and several IFN-regulated gene loci, including *IRF7*, were found to be hypomethylated, and therefore potentially over-expressed, indicating that aberrant DNA hypomethylation may be a key process underlying SLE pathogenesis, and therapeutic targeting of this process may be beneficial [190, 191].

#### Histone Deacetylation

Global site-specific histone H3 and H4 hypoacetylation has been described in splenocytes from MRL/*lpr* lupus-prone mice [192] and in CD4<sup>+</sup> T cells from SLE patients [193]. An example of a regulatory mechanism in this process involves the transcription factor CREM $\alpha$  which recruits **histone deacetylase 1** (HDAC1) to the *IL2* promoter, leading to *IL2* silencing through histone H3K18 deacetylation in human SLE T cells [47]. CREM $\alpha$  can also recruit DNMT1 and DNMT3a, leading to DNA methylation of the *IL-2* promoter [48]. Conversely, CREM $\alpha$  fails to recruit HDAC1 and DNMT3a to *IL17A* promoter in CD4<sup>+</sup> T cells from SLE patients, resulting in increased expression of IL-17 [194]. Moreover, the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) has been found to ameliorate disease severity in NZB/W F1 lupus-prone mice, suggesting a potential pathogenic role of histone deacetylation in SLE pathogenesis, and highlighting the therapeutic potential of HDAC inhibitors, but further and robust investigation is warranted [195,196].

#### miRNAs

Altered miRNA expression has been reported in SLE. Downregulation of miR-155 and increased PP2A mRNA expression was found in PBMCs from SLE patients [197]. Expression of another miR, miR-146a, whose targets are IFN $\alpha$  and IFN $\beta$ , in peripheral blood leukocytes from SLE patients was shown to inversely correlate with SLE disease activity [198]. Overexpression of miR-21, miR-148a, miR-126, and miR-29b in SLE CD4<sup>+</sup> T cells may potentially indicate their contribution to DNA hypomethylation by directly or indirectly suppressing DNMT1 [199].

Recently, systematic integration of fine-mapped genetic and epigenetic information derived from primary immune cells including resting and stimulated CD4<sup>+</sup> T cells, Tregs, CD8<sup>+</sup> T cells, B cells, and monocytes was performed to identify causal variants in

autoimmune-associated loci and explore their functions [200]. Comparing SNP locations with a map of *cis*-regulatory elements based on H3 lysine 27 acetylation (H3K27ac) for 56 cell types, predictions were made regarding the SNPs that might contribute to autoimmune diseases such as SLE [200]. Using such genome-wide information might facilitate the identification of genes involved in disease pathogenesis and facilitate therapeutic target discovery.

#### Box 4. Clinician's Corner

SLE primarily affects women with significant morbidity and mortality. Although the American College of Rheumatology criteria are used to diagnose the disease, it is abundantly clear that SLE is not a single disease/pathology.

The increasing number of monogenic cases and the identification of a multitude of pathways leading to disease imply that SLE is a syndrome and should be approached as such. The failure of dozens of clinical trials targeting different pathways highlights this issue.

Classification of patients in the context of the defined involvement of biochemical and cellular pathways is mandatory, and lupus deserves personalized medical approaches to treatment. Until then, physicians should make every effort to avoid approaching every SLE patient as having one same disease, and instead should aim to apply individualized treatment approaches to the extent that it is possible.



### Trends

Recent work has identified patterns of altered gene expression denoting molecular pathways operating in groups of SLE patients.

Studies have identified local, organ-specific factors enabling or ameliorating SLE tissue damage, thereby dissociating autoimmunity and end-organ damage.

Novel subsets of adaptive immune effectors, and the contributions of innate immune cells including platelets and neutrophils, are being increasingly recognized in lupus pathogenesis.

Studies have revealed metabolic cellular aberrations, which underwrite cell and organ injury, as important contributors to lupus disease.

### Outstanding Questions

How can we determine the biologic and functional effects of the **genome-wide association study** (GWAS)-identified predisposing genes and variants associated with SLE using novel gene-editing techniques in primary cells and animals?

How can we determine how signaling defects in SLE T cells are linked to their aberrant function, and contribute to autoimmunity and tissue damage?

How can we elucidate the relationship between aberrant transcription factor expression, chromatin remodeling defects, and skewed differentiation of effector cells in SLE?

How can we dissect the underlying mechanisms of B cell biology to understand the failure of B cell targeted therapies so as to develop better therapeutic strategies?

How can we better identify and distinguish the innate immune cell subsets and mechanistic pathways that might contribute to the initiation and progression of SLE?

How can we better elucidate the tissue-specific molecules and mechanisms that enable, initiate, maintain, or amplify immune-mediated organ damage? What is the exact contribution of NETosis to SLE pathogenesis?

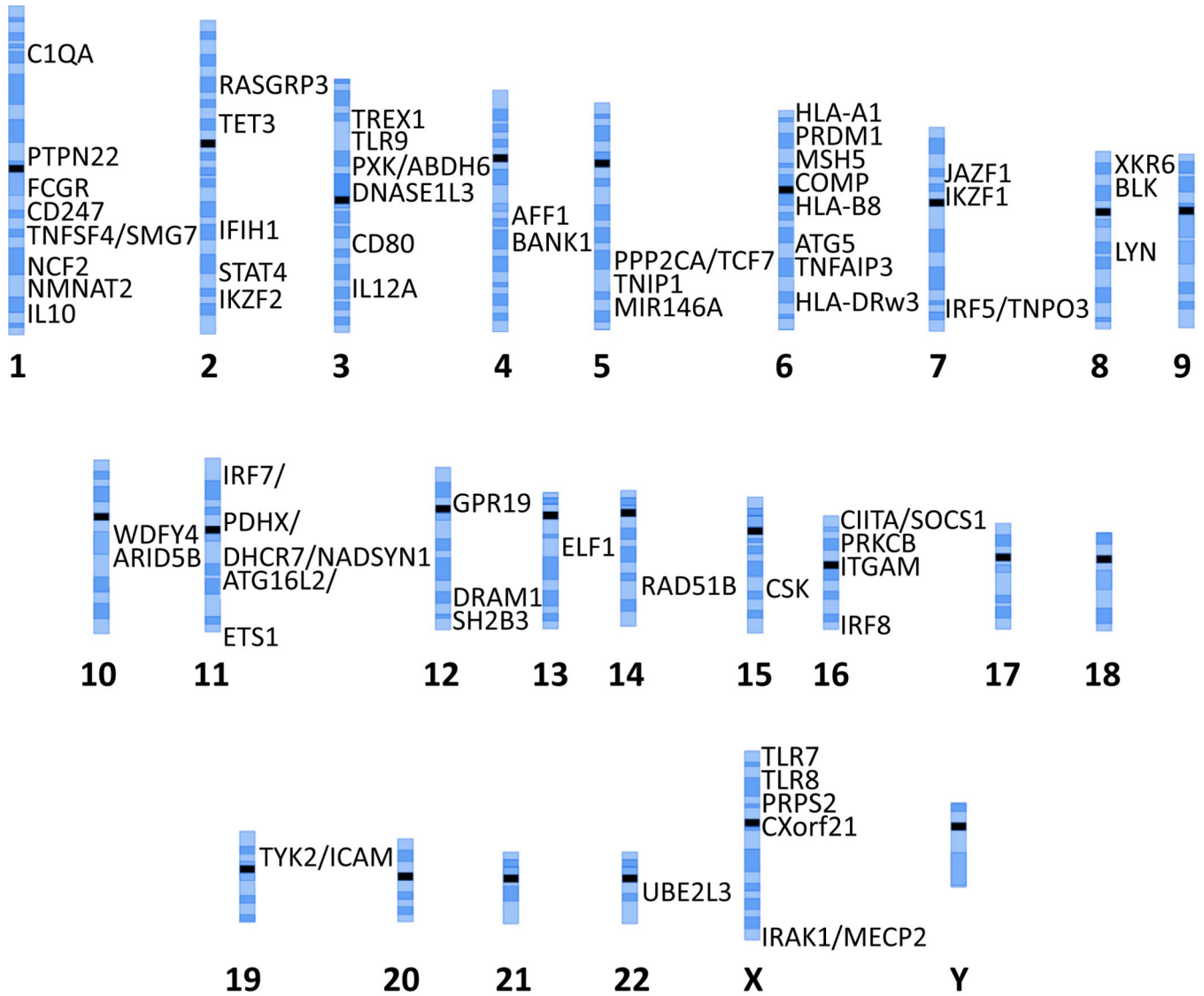
We must develop strategies to identify driving molecular/cellular pathways in individual patients such that each patient can be treated suitably (precision medicine).

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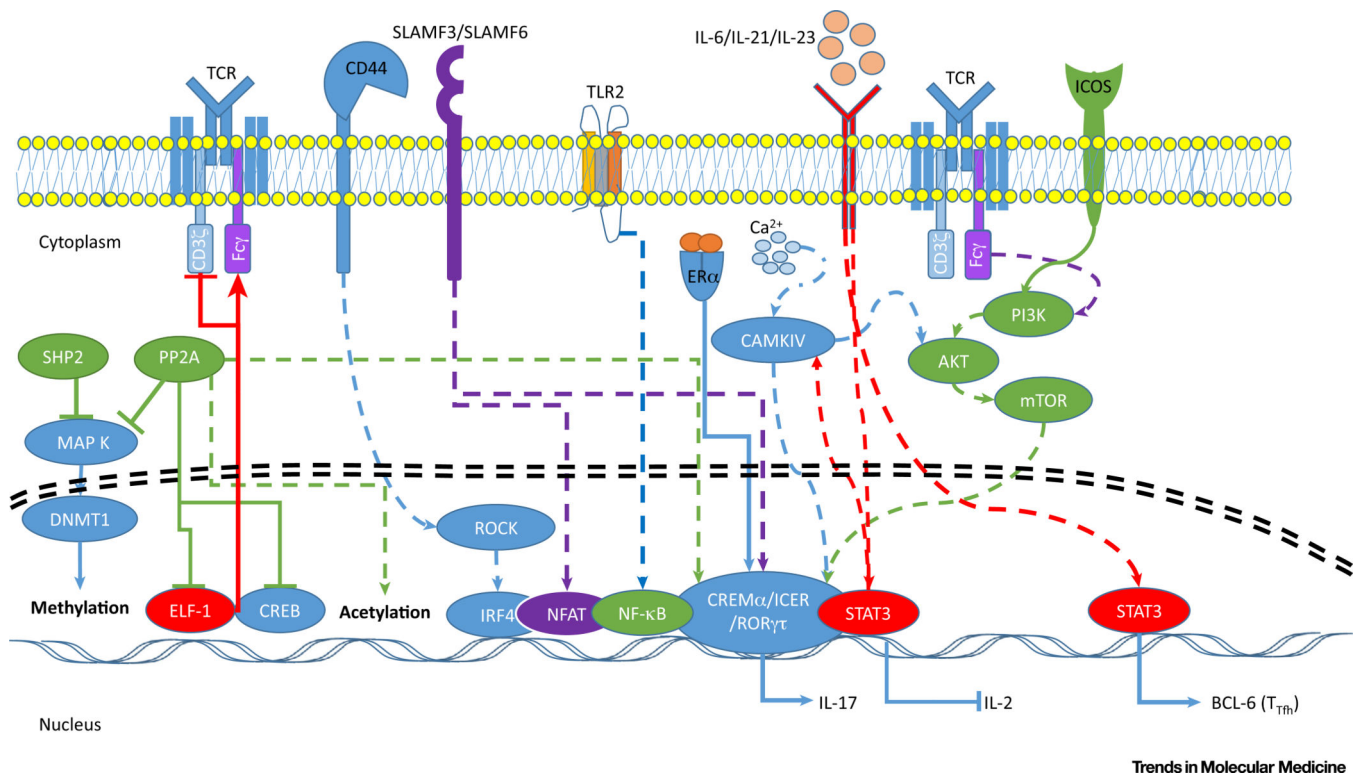
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Trends in Molecular Medicine

**Figure 1.** Genetics of Systemic Lupus Erythematosus (SLE). The schematic depicts a panel of chromosomes showing genes associated with SLE. The approximate positions of SLE-associated loci and genes in the human genome are shown. These genes were selected because they have been validated in two or more studies. FCGR includes *FCGR2A*, *FCGR3B*, and *FCGR3A*; COMP includes *C2*, *C4A*, and *C4B*.



Trends in Molecular Medicine

**Figure 2.**

T Cell Signaling and Gene Regulation Defects in Systemic Lupus Erythematosus (SLE). In SLE patients, increased protein phosphatase 2A (PP2A) levels and activity in T cells suppresses Ets-like-factor-1 (ELF-1), a transcriptional enhancer of the CD3 $\zeta$  chain and a repressor of the Fc $\epsilon$ RI $\gamma$  (Fc $\gamma$ ) chain. TCR/CD3 complex rewiring, via replacement of CD3 $\zeta$  with Fc $\gamma$ , results in increased calcium responses, enhancing the activity of calcium/calmodulin-dependent protein kinase IV (CaMKIV), which in turn increases the binding of cAMP response element modulator (CREM $\alpha$ ) and inducible cAMP early repressor (ICER). CREM $\alpha$  is also activated by ER $\alpha$ , and is recruited to the *IL17A* and *IL2* promoters, enhancing and repressing their transcription, respectively [185,194]. It is known that CaMKIV activity is enhanced by the presence of costimulatory molecules such as ICOS in preformed lipid rafts containing the TCR, which can lead to activation of the PI3K/mTOR pathway; enhanced activity of protein phosphatase 2A (PP2A) and SHP2 suppresses the MAPK–DNMT1 pathway and dephosphorylates cAMP-responsive element-binding protein 1 (CREB), resulting in suppression of *IL2* transcription [189]. PP2A also acts through Rho-associated protein kinase (ROCK) to enhance binding of the *IL17* transcription enhancer interferon regulatory factor 4 (IRF4); signaling through CD44 also activates ROCK, which promotes cell migration and binding of IRF4 to the *IL17* promoter [49,51]. TLRs and SLAMF signaling can activate the transcription factors NF- $\kappa$ B and NFAT, and this contributes to the transcription of proinflammatory cytokines [201]. In addition, proinflammatory cytokines IL-6, IL-21, and IL-23 cause downstream activation of STAT3 transcriptional targets, including *IL17* and *BCL6*, contributing to inflammation and supporting antibody production from B cells. Abbreviations: [46,52]. ER, estrogen receptor; ICOS, inducible T cell costimulator; SHP2, Src homology 2 domain containing

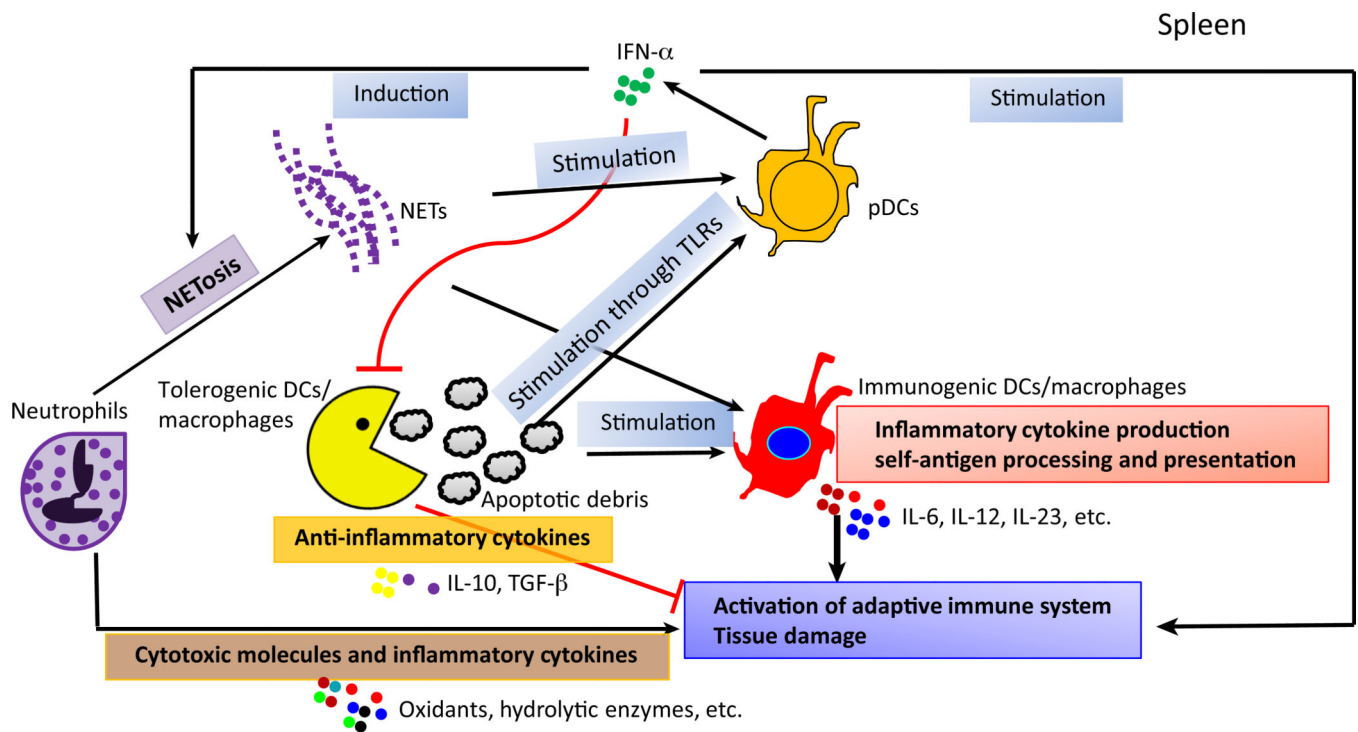
phosphotyrosine phosphatase 2; TCR, T cell receptor; Tfh, T follicular helper cells; TLR, Toll-like receptor.

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**Figure 3.**

Role of Innate Cells (Neutrophils, Macrophages and Dendritic Cells) in Systemic Lupus Erythematosus (SLE) Pathogenesis. Altered functional properties in neutrophils have been observed in SLE, including decreased clearance of apoptotic material and increased synthesis and release of various proteins including oxidants, hydrolytic enzymes, and inflammatory cytokines which contribute to tissue damage. Myeloid dendritic cells (DCs) and plasmacytoid dendritic cells (pDCs) represent two major DC subsets derived from different developmental pathways from their precursors. pDCs are a specialized type of interferon (IFN)-producing cells capable of producing massive amounts of type-I IFNs upon stimulation. Type I IFNs can induce the formation of neutrophil extracellular traps (NETs), which are a source of self-stimuli and reciprocally enhance production of type I IFNs [104]. Based on their function, myeloid DCs can be further divided into tolerogenic and immunogenic DCs. In SLE patients, immunogenic DCs/macrophages acquire activated phenotypes with increased production of inflammatory cytokines or enhanced self-antigen processing and presentation. Tolerogenic DC/macrophages are responsible for the rapid removal of apoptotic cells, and, coupled with their ability to produce anti-inflammatory cytokines, efficiently suppress autoimmunity. In SLE patients, a significant reduction in both the number and function of these cells is observed [105]. Abbreviations: BDCA2–DTR, blood dendritic cell antigen 2–diphtheria toxin receptor transgene; CAMKIV, calcium/calmodulin-dependent protein kinase type IV; DNMT, DNA methyl transferase; HDAC, histone deacetylase; ICOS, inducible T cell costimulatory; IRF, interferon regulatory factor; PD-1, programmed death receptor; LAP, microtubule-associated protein 1A/1B-light chain 3 (LC3)-associated phagocytosis; mTOR, mechanistic target of rapamycin; NET, neutrophil extracellular trap; PP2A, protein phosphatase 2A; PTPN22, protein tyrosine phosphatase,

non-receptor type 22; ROCK, Rho-associated protein kinase; SLAM, signaling lymphocyte activation molecule family of receptors; STAT, signal transducer and activator of transcription; Tfh, T follicular helper cell; TLR, Toll-like receptor.

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**Table 1**Biologics for the Treatment of SLE: Overview of Clinical Trials<sup>a</sup>

Target	Treatment	Mode of action	Clinical trials
BLYS/APRIL	Belimumab (anti-BLYS)	Blocks BLYS	Approved by the FDA for active autoantibody-positive SLE receiving standard therapy [154, 155]
	Atacept (TACI Ig)	Blocks BLYS and APRIL	Phase II/III on moderate-to-severe SLE patients showed low Ig levels and infection-related deaths at the higher dose, and did not show an effect at the lower dose. Recruiting patients for Phase IIb study, completion date: April 2018 [156, 157]
	Blisibimod (peptibody with BAFF binding domains)	Blocks soluble and membrane-bound BLYS	Phase II trials with moderate-severe SLE failed primary end-points [158]
	Tabalumab (anti-BLYS)	Blocks soluble and membrane-bound BLYS	Phase III trials did not meet expected results for efficacy and safety [159]
dsDNA	Abetimus sodium (dsDNA epitopes)	Reduces autoantibody production by inducing selective B cell anergy or apoptosis.	Phase III studies showed no efficacy in SLE patients with lupus nephritis [160]
	Edratide (CDR1 of a human anti-dsDNA mAb)	Decreases gene expression of inflammatory cytokines and pro-apoptotic markers, and increases gene expression of suppressive/regulatory markers	Phase II trial failed to meet primary end-points
CD20	Rituximab (anti-CD20)	Depletes B cells through complement-dependent cytotoxicity, antibody-dependent cytotoxicity, and activation of apoptosis	Failed Phase III studies in SLE patients with moderate to severe activity and lupus nephritis [88]
	Ocrelizumab (anti-CD20)		Failed Phase III studies with non-renal SLE patients or lupus nephritis [89]
CD22	Epratuzumab (anti-CD22 agonist antibody)	CD22 phosphorylation and decreased BCR signaling; less vigorous B cell depletion	Failed Phase III trials in SLE patients with moderate to severe activity [161]
CTLA4	Abatacept (CTLA4-Ig)	Competes with CD28 for binding to CD80 or CD86 and inhibits T lymphocyte activation	Phase IIb trial in SLE patients with non-renal disease failed to meet primary end-points  Two Phase II/III studies failed to meet primary end-points in lupus nephritis patients [162]. A Phase III trial is in process for lupus nephritis
IFN- $\alpha$	Sifalimumab (anti-IFN- $\alpha$ )	Prevents signaling through the type 1 IFN receptor	Phase IIb trial showed significant improvement in mucocutaneous and articular



Target	Treatment	Mode of action	Clinical trials
			involvement in moderate-to-severe SLE [163]
	Anifrolumab (anti-IFNAR1)	Prevents type 1 IFN signaling	Phase IIb trial with SLE or lupus nephritis met primary endpoints [164]
			Phase III trial to evaluate the efficacy and safety in adult SLE recruiting patients. Completion: September 2018
	Rontalizumab (anti-IFN- $\alpha$ )	Neutralizes all human IFN- $\alpha$ subtypes	Phase II trial in moderate-to-severe SLE (no renal or neuropsychiatric) showed no improvement in disease scores [165]
	IFN-K (inactivated IFN- $\alpha$ conjugated to KLH)	Induces anti-IFN $\alpha$ antibodies and neutralization of IFN- $\alpha$ in sera	Phase I/II studies showed decreased expression of IFN-induced genes [166]
IFN- $\gamma$	AMG811 (anti IFN- $\gamma$ )	Blocks type 2 IFN	Completed Phase I testing; was found to suppress IFN-regulated genes including IP-10 [167]
CD40L	IDEC-131 (anti-CD40L)	Blocks CD40L	Phase II trial showed no efficacy [168]
	BG9588 (anti-CD40L)		Phase II trial showed thromboembolic events through platelet activation via the IgG (Fc $\gamma$ RIIIa) receptor [169]
	CDP7657 (PEGylated monovalent Fab' anti-CD40L fragment lacking the Fc domain)		Improves renal disease in lupus-prone mice. Phase I study with tolerability in healthy individuals and in SLE patients [170]
ICOSL	AMG 557 (anti-ICOSL)	Blocks ICOSL	Phase I trial showed successful inhibition of ICOSL [171]
IL-6	Tocilizumab (anti-IL6R)	Inhibits IL-6 signaling	Phase I trial in moderately active SLE showed improvement in disease scores but some patients developed neutropenia and infections [172]
	Sirukumab (anti-IL-6)		Phase I trial in cutaneous lupus erythematosus, stable active SLE, and lupus nephritis did not show efficacy and patients had serious adverse effects [173]
Immunomodulation	Laquinimod (quinoline-3-carboxamide derivative)	Functional changes in APCs, downregulation of Th1 and Th17 cells, increase in Tregs	Phase II trial in lupus nephritis showed better results than standard therapy [174]
	Paquinimod (quinoline-3-carboxamide derivative)		Phase Ib in SLE showed good tolerability. Comparable effectiveness to MMF [175]

Target	Treatment	Mode of action	Clinical trials
Immunomodulation	Lupuzor (phosphorylated analog of a U1 snRNP epitope)	Recognized by IgG and T cells; tolerogenic effects and improvement in renal disease	Phase II trial in moderately active SLE showed reductions in anti-dsDNA, CRP levels, and in SLEDAI scores, and good tolerability [176]
FC $\gamma$ receptors	SM101 (soluble non-glycosylated Fc $\gamma$ RIIb)	Competes with cell-surface FC $\gamma$ receptors for binding to the Fc portion of immune complexes	Phase IIa trial in serologically active SLE reached primary end-points [177]
TWEAK/Fn14	BIIB023 (anti-TWEAK)	Inhibits TWEAK/Fn14 signaling resulting in decreased proinflammatory activity, vascular activation, angiogenesis, mesangial cell proliferation, cell death, and renal fibrosis	Phase I trial in RA patients showed favorable safety and tolerability [178]. Two clinical trials for lupus nephritis are currently ongoing

<sup>a</sup>Abbreviations: APC, antigen-presenting cell; APRIL, a proliferation inducing ligand; BCMA, B cell maturation antigen; BCR, B cell receptor; BLyS/BAFF, B lymphocyte stimulator/B cell activating factor; CTLA-4, cytotoxic T lymphocyte-associated protein 4; ICOSL, inducible costimulator-ligand; IFN, Interferon; KLH, keyhole limpet hemocyanin; MMF, mycophenolate mofetil; SLEDAI, SLE disease activity index; snRNP, small nuclear ribonucleoprotein; TACI, transmembrane activator, calcium modulator, and cyclophilin ligand interactor; TWEAK, TNF-related weak inducer of apoptosis.