ANNUAL REVIEWS

*Annual Review of Immunology*

# Systemic Lupus Erythematosus Pathogenesis: Interferon and Beyond

Simone Caielli, Zurong Wan, and Virginia Pascual

Drukier Institute for Children's Health and Department of Pediatrics, Weill Cornell Medical Center, New York, NY, USA; email: sic2011@med.cornell.edu, zuw4001@med.cornell.edu, vip2021@med.cornell.edu

Annu. Rev. Immunol. 2023. 41:533–60

First published as a Review in Advance on February 28, 2023

The *Annual Review of Immunology* is online at immunol.annualreviews.org

https://doi.org/10.1146/annurev-immunol-101921-042422





## Keywords

lupus, cell death, mitochondria, autoantibodies, extrafollicular

## Abstract

Autoreactive B cells and interferons are central players in systemic lupus erythematosus (SLE) pathogenesis. The partial success of drugs targeting these pathways, however, supports heterogeneity in upstream mechanisms contributing to disease pathogenesis. In this review, we focus on recent insights from genetic and immune monitoring studies of patients that are refining our understanding of these basic mechanisms. Among them, novel mutations in genes affecting intrinsic B cell activation or clearance of interferogenic nucleic acids have been described. Mitochondria have emerged as relevant inducers and/or amplifiers of SLE pathogenesis through a variety of mechanisms that include disruption of organelle integrity or compartmentalization, defective metabolism, and failure of quality control measures. These result in extra- or intracellular release of interferogenic nucleic acids as well as in innate and/or adaptive immune cell activation. A variety of classic and novel SLE autoantibody specificities have been found to recapitulate genetic alterations associated with monogenic lupus or to trigger interferogenic amplification loops. Finally, atypical B cells and novel extrafollicular T helper cell subsets have been proposed to contribute to the generation of SLE autoantibodies. Overall, these novel insights provide opportunities to deepen the immunophenotypic surveillance of patients and open the door to patient stratification and personalized, rational approaches to therapy.

## INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototype autoimmune disease that predominantly affects females and involves a wide array of organs/systems. The disease follows a waxing and waning course, with exacerbations leading to cumulative organ damage over time. Heterogeneity in both inflammatory pathways and clinical manifestations, even within the same organ, contributes to diagnosis delays and complicates both the development of objective disease activity measures and the design of successful clinical trials (1). In fact, while the therapeutic armamentarium to treat rheumatic diseases has expanded considerably in the past 20 years, only 3 drugs, belimumab, anifrolumab, and voclosporin, have been approved by the US Food and Drug Administration (FDA) to treat SLE or lupus nephritis in more than six decades (2–4).

SLE pathogenesis is complex, but two disease-defining and pervasive immune features are the breakdown of tolerance to nucleic acids and the activation of the interferon system. Thus, antibodies against nucleic acids, especially those against naked and nucleosome-associated double-stranded DNA (dsDNA) and RNA/RNA-binding proteins, together with an interferon-stimulated gene (ISG) signature in blood and affected tissues, are hallmarks of the disease. Around the turn of the century, the finding that nucleic acids bound to SLE autoantibodies induce interferon production upon being internalized by plasmacytoid dendritic cells (pDCs) provided a pathogenic link that had been missed in murine lupus models (5). Eventually, this link brought up the fundamental role of endosomal Toll-like receptor (TLR) sensing of nucleic acids in the disease (6–8). Around this time, the pleiotropic effects of interferon on dendritic cell (DC) and B cell differentiation were elucidated (9, 10), and high-throughput transcriptional studies of pediatric and adult SLE peripheral blood mononuclear cells (PBMCs) provided an unequivocal picture of SLE as an interferon-mediated disease (11).

During the 20 years that followed these observations, monoclonal antibodies targeting either B cells or different components of the interferon pathway were tested in clinical trials (12). While most of these approaches progressed to phase 3 studies, only two drugs were eventually granted FDA approval: belimumab, a monoclonal antibody against the interferon-induced B cell proliferation and differentiation factor BAFF, and anifrolumab, a monoclonal antibody blocking the type I interferon receptor 1 subunit (IFNAR1). Even though these are remarkable therapeutic advances, end points for disease improvement and/or flare reduction were not reached in up to 40–50% of SLE patients included in the trials (13, 14). These results underscore the heterogeneity in upstream pathways and/or proinflammatory amplification loops contributing to the lupus phenotype. In fact, neither anti–nucleic acid antibodies nor increased interferon activity are exclusive features of lupus. Thus, as reviewed below, only a few out of the ever-growing spectrum of Mendelian diseases characterized by increased type I interferon activity overlap with SLE (15).

## SLE: AN INTERFERONOPATHY WITH MANY TWISTS

Chronic autoimmune and/or inflammatory diseases result from interplay between genes and the environment. The SLE genetic background is complex, as highlighted by mouse and human studies. Thus, >30 rare mutations in humans and >60 in mice have been associated with a lupus phenotype (16). Even though causal mutations are not identified using either limited-panel or whole-genome sequencing in ~90% of patients with early-onset disease (4, 17), the functional characterization of rare, familial or de novo, monogenic lupus cases has provided fundamental information on basic mechanisms upstream of the SLE phenotype(s) (18–27). Genome-wide association studies (GWAS), on the other hand, identified >100 common alleles that contribute to SLE risk (28, 29). Recently, a synergistic effect between heterozygous, ultrarare coding variants in

genes associated with monogenic lupus and common risk alleles has been described, suggesting a larger impact of rare variants than previously suspected (4).

In addition to genetic studies, high-throughput immune monitoring has informed on cells, transcriptional pathways, and proinflammatory mediators that contribute to SLE pathogenesis. Bulk and single-cell transcriptional profiles confirmed the increased expression of ISGs in patients' blood and tissues such as skin and kidney (30–38). However, many questions remain about the inducers, sensors, and cellular sources of interferon production in SLE. The contribution of the different interferon families to disease is also difficult to assess, as quantification of type I and III cytokines requires ultra-sensitive assays, and transcriptional programs used to monitor patients overlap significantly among members of all three families. While type I interferon has been validated as a target with the approval of the first drug blocking IFNAR1 (39), the role(s) of type II and type III interferon remain unresolved. IFN-γ-producing and/or -responsive cells are expanded in patients (40–42), and increased levels of IFN-γ/IFN-γ-inducible proteins precede clinical disease onset and/or flares (43, 44). However, blocking IFN-γ or upstream cytokines such as IL-12/IL-23 failed to reach the expected end points in clinical trials (45, 46). Finally, type III interferons, which are secreted by discrete immune cell subsets and tissue-resident cells (47), have not yet been targeted therapeutically. Importantly, B cell subpopulations associated with SLE disease activity express high levels of their receptor (40), supporting a potential pathogenic role.

## A Complex Link Between Cell Death and Interferon in SLE

Double-stranded DNA (dsDNA) was one of the first autoantigens identified in SLE. Attempts to understand its source and the conformation responsible for breaking immune tolerance led to nucleosomes, the basic units of genomic dsDNA coiled around histone cores that are normally compartmentalized and shielded within nuclei (48). A major mechanism that exposes nucleosomes to the immune system is cell death. Programmed cell death in the form of apoptosis is immunologically silent, while necrosis is regarded as proinflammatory. However, additional modalities of cell death trigger inflammation (49, 50). Programmed necroses, including necroptosis, pyroptosis, NETosis, and ferroptosis, lead to the release of inflammatory molecules in the form of danger-associated molecular patterns (DAMPs). These different cell death programs have been considered independent of each other, but their plasticity and interconnection are increasingly recognized (50). Defects in apoptosis, and increased rates of ferroptosis and NETosis, have been associated with SLE pathogenesis in mice and humans (51–53). Apoptosis can be triggered by extrinsic death receptor pathways or intrinsic mitochondrial pathways, and it plays a physiological role during development and aging while helping maintain tissue homeostasis (54). In the steady state, apoptotic cells release find-me signals and expose eat-me signals on their surface, leading to their recognition and clearance by phagocytes. In addition, late apoptotic and necrotic cells are recognized by complement initiators such as C1q, mannose-binding lectin, ficolins, and properdin, which trigger complement activation and opsonization (55).

During apoptosis, chromatin DNA is digested intracellularly by DNA fragmentation factor subunit beta (DFFB). Nucleosomes are then either released as cell-free monomers or polymers or incorporated into membrane-coated vesicles or microparticles (56), which are all eventually found in plasma (57). Microparticles expose nucleosomes on their surface, where they become accessible to DNase1L3, which together with DNase1 is responsible for the bulk of DNase activity in the circulation (58). DNase1L3 is unique in its capacity to digest membrane- and/or protein-associated DNA, including intact chromatin (18, 59, 60) as well as long dsDNA fragments within soluble polynucleosomes (61, 62). Following extracellular nuclease digestion, microparticles and apoptotic bodies are cleared by phagocytes within lysosomal compartments, where any remaining dsDNA is degraded by DNase2 (63).

Specific cell types, such as granulocytes, have the unique capacity to expose genomic dsDNA extracellularly as they die by NETosis. Neutrophil extracellular traps (NETs) are large, fibrillary structures composed of cytosolic and granular proteins on a scaffold of decondensed chromatin (64). NETosis is a physiological phenomenon intended to kill extracellular microbes without overtly activating the immune system. This is accomplished in part by the prompt digestion of NETotic dsDNA by the ubiquitous extracellular DNase1 (52).

Inside the cell, genomic dsDNA is not restricted to the nucleus but is also present within micronuclei, which arise as the result of genotoxic stress, as well as in the cytosol as the result, for example, of reverse transcription of retroelements. There, DNA and DNA/RNA hybrids are digested by the 3′ exonuclease TREX1 (65), which restricts DNA sensing by the cytosolic cGAS-STING axis (66).

Alterations in any of the above-reviewed processes lead to disease, but the strongest link to breaking tolerance to dsDNA and eliciting an SLE phenotype in humans remains the deficient extracellular degradation of microparticle-associated nucleosomal DNA (18). Thus, defective apoptosis due to mutations in *FAS/FASL* gives rise to lymphoproliferation and lupus-like disease in mice (67). In humans, *FAS/FASL* mutations also induce lymphoproliferation and autoimmune manifestations as part of the so-called autoimmune lymphoproliferative syndrome (ALPS). Some ALPS patients fulfill criteria for SLE diagnosis, but for the most part they can be distinguished based on clinical and immunological features (68). Accumulation of cell debris, including genomic dsDNA, was thought to underlie the strong association between deficiencies of early components of the complement cascade, especially C1q, and SLE. As discussed later, this concept is being revisited (25).

Defective degradation of extracellular dsDNA within microparticles, as seen in patients with homozygous null mutations in *DNASE1L3*, causes early-onset familial SLE characterized by prominent anti-dsDNA antibody response and renal involvement (69–71). In some cases, the disease initially manifests as hypocomplementemic urticarial vasculitis syndrome (HUVS), but it almost invariably progresses to severe SLE (71, 72). A coding polymorphism in *DNASE1L3* that gives rise to a hypofunctional protein (73) is also associated with autoimmunity, including SLE (74). DNase1L3 deficiency provides ligands sensed by the endosomal TLR9/MyD88 axis. Importantly, studies using *DNASE1L3* knockout mice confirm that this enzyme maintains tolerance to self-DNA in a nonredundant manner (18).

Extracellular dsDNA release during NETosis has been extensively proposed to contribute to SLE pathogenesis (75). In vitro, however, depolymerized nucleosomes within NETs are weakly interferogenic when internalized by pDCs, and they are efficiently degraded by the DNase1 activity of healthy human serum (64, 76). As reviewed elsewhere, the contribution of loss-of-function (LOF) mutations in *DNASE1* to human SLE is controversial (77). Remarkably, however, sera from approximately one-third of SLE patients fail to degrade dsDNA within NETs because of autoantibodies that limit DNase1 accessibility. Patients whose sera fail to degrade NETs tend to suffer from a higher incidence of lupus nephritis. As DNase1 is locally produced in the kidney, decreased accessibility of this enzyme to in situ–generated, autoantibody-protected NETs could contribute to this phenotype (52).

An additional form of neutrophil death recently linked to SLE is ferroptosis. Ferroptosis is driven by iron-dependent reactive oxygen species (ROS) production and cell membrane lipid peroxidation and has been implicated in SLE neutropenia. As reported for NETosis, IgG and IFN-α from SLE sera induce ferroptosis and lead to the release of neutrophil DAMPs. In a lupus mouse model, suppression of ferroptosis reverted disease progression (78).

Downstream of cell death, remnants containing nucleic acids are digested within the lysosomal compartment of phagocytes. Interestingly, the phenotype of mice and humans carrying LOF

mutations in the lysosomal endonuclease *DNASE2* is distinct from SLE. In fact, this enzyme plays a central role in the clearance of nucleic acids generated during enucleation of erythroid progenitors. Consequently, its deficiency causes lethal anemia due to arrested erythropoiesis and activation of type I interferon signaling through the cGAS/STING pathway (79). Blocking interferon signaling in DNase2-deficient mice rescues lethality but gives rise to a deforming, noninflammatory arthropathy. Importantly, humans carrying biallelic LOF mutations in *DNASE2* display a similar phenotype, which is distinct from SLE (80).

Finally, cytosolic DNA and DNA/RNA hybrid accumulation as the result of TREX1 deficiency causes encephalopathy within the spectrum of diseases associated with Aicardi-Goutières syndrome (AGS) (81). About 50% of TREX1-deficient patients develop lupus manifestations; however, they are mainly restricted to the skin (82). Mutations in additional AGS-associated genes have been rarely reported in SLE patients (15, 83), although heterozygous variants might contribute to the disease more than previously thought, as earlier discussed.

Overall, of the growing number of Mendelian or de novo genotypes (>40) associated with increased production of interferon and assembled under the term monogenic interferonopathies, only a minority overlap with SLE (15, 83). Thus, in addition to those involving *DNASE1L3* and *TREX1*, LOF mutations in *ACP5*, which encodes a tartrate-resistant acid phosphatase (TRAP) responsible for the phosphorylation of osteopontin in pDCs, cause SLE together with a syndromic skeletal dysplasia (23, 24). In summary, the remarkable number of molecular pathways and wide range of phenotypic manifestations associated with monogenic interferonopathies, which have been thoroughly reviewed recently elsewhere (15), underscore the complex pathogenic role of interferon and its triggers in human inflammatory and autoimmune diseases.

## THE MULTIFACETED ROLE OF MITOCHONDRIA IN SLE

Loss of tolerance to nucleic acids in SLE has been traditionally linked to those of nuclear origin, but mitochondrial nucleic acids are emerging as both antigenic targets and triggers of interferon in this disease. Mitochondria are most recognized for their function in oxidative phosphorylation (OXPHOS), metabolism, and apoptosis. These organelles, which originate from bacteria, contain their own nucleic acids enclosed within double membranes. In recent years, mitochondrial components have been identified as a major source of DAMPs. In particular, the circular mitochondrial genome with hypomethylated CpG motifs resembling bacterial DNA activates a plethora of pattern recognition receptors, including TLR9, cGAS, and inflammasomes (NLRP3 and AIM2), when released into either the cytoplasm or the extracellular space, leading to type I interferon and/or IL-1β production (84).

Importantly, antibodies directed against an array of mitochondrial components, including mitochondrial DNA (mtDNA), inner mitochondrial membrane (IMM) or outer mitochondrial membrane proteins and lipids, matrix components, and mitochondrial RNA (mtRNA), have been reported in SLE patients (85). A classic SLE specificity is cardiolipin, a phospholipid found uniquely on the IMM. Anti-cardiolipin antibodies are detectable in patients with SLE and those with primary antiphospholipid syndrome and are associated with thrombotic events and thrombocytopenia (86). HSP60, a chaperonin involved in mitochondrial protein transport, is also a mitochondrial antigen in SLE (87). Similarly, autoantibodies against the IMM protein mitofusin 1 (MFN1) seem to predict SLE disease activity and are associated with the presence of antiphospholipid and anti-dsDNA antibodies (88). Finally, antibodies to whole mitochondria have been described in active SLE patients. These antibodies do not overlap with the classic "antimitochondrial antibodies" (AMAs) detected in patients with primary biliary cirrhosis that target the M2 (2-oxo-acid dehydrogenase protein complex) antigen (89).

## Mitochondria Are a Source of Extracellular Interferogenic DNA in SLE

Along with genomic dsDNA, extracellular mtDNA is a constitutive component of NETs (90, 91). Importantly, NETs released from SLE neutrophils are interferogenic due to an enrichment in oxidized mtDNA (Ox mtDNA). Upon internalization by myeloid cells or pDCs, these NETs induce type I interferon production through cGAS or TLR9 sensing, respectively (92, 93). Notably, low-density granulocytes, a distinct proinflammatory neutrophil subset expanded in SLE (94), spontaneously extrude NETs enriched in Ox mtDNA (92) (**Figures 1** and **2**).

Neutrophils and eosinophils also extrude mtDNA while alive as part of a process referred to as vital NETosis (90, 91). This process is immunologically silent in the steady state, due to the

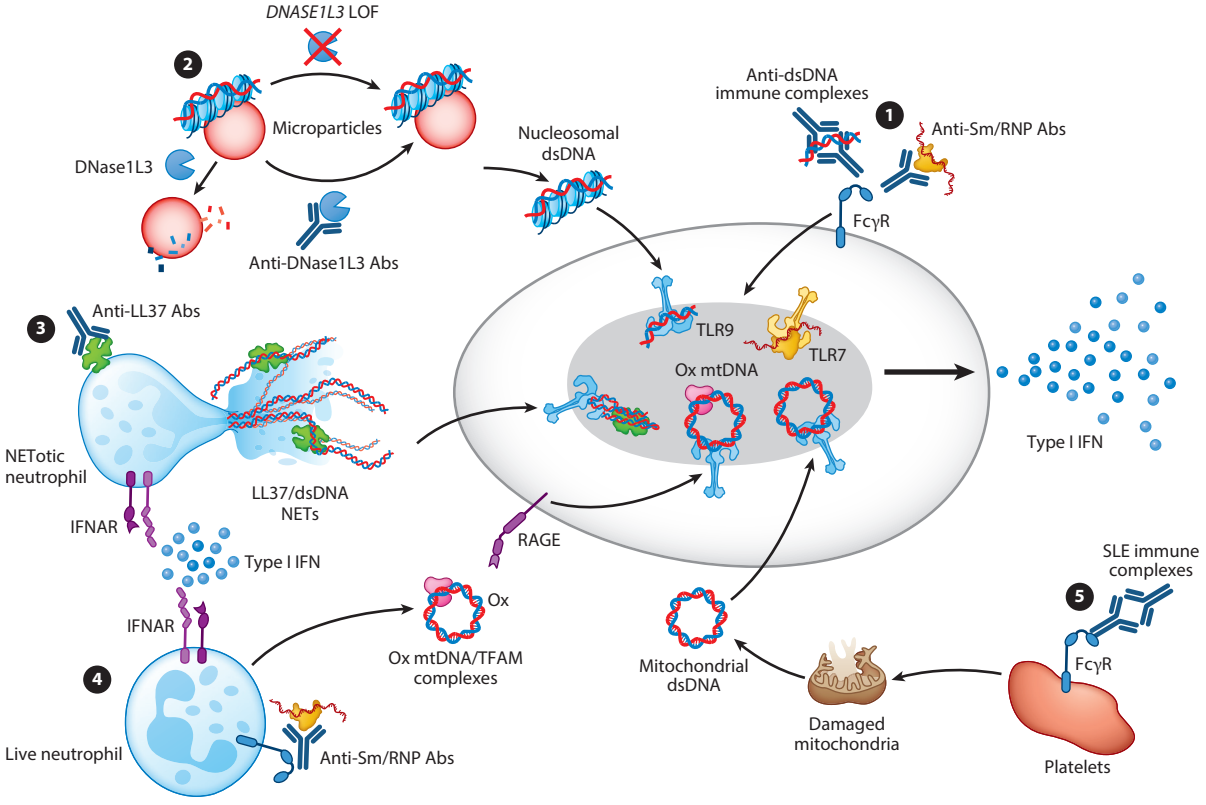


**Figure 1**

Sources of pDC-activating nucleic acids in SLE. (❶) FcγR-mediated internalization of SLE autoantibodies in pDCs delivers dsDNA and single-stranded RNA into endosomal compartments, where they activate TLR7 and TLR9, respectively. (❷) Extracellular nucleosomal dsDNA is associated with microparticles from apoptotic cells. Under physiological conditions, DNase1L3 digests microparticle-associated dsDNA. Loss-of-function *DNASE1L3* mutations, or anti-DNase1L3 antibodies from SLE patients, lead to nucleosomal dsDNA accumulation and TLR9-dependent pDC activation. (❸) Type I interferon primes neutrophils to translocate LL37 to the cell surface. Upon binding anti-LL37 antibodies, LL37-dsDNA complexes are extruded within NETs. LL37 facilitates their internalization in pDCs and induces TLR9 activation. (❹) SLE anti-Sm/RNP antibodies induce the extrusion of oxidized mtDNA/TFAM complexes from type I interferon–primed neutrophils in the absence of cell death. TFAM-RAGE interaction favors their internalization in pDCs and triggers TLR9 activation. (❺) SLE immune complexes activate platelets in an FcγR-dependent manner and induce the extrusion of entire mitochondria/mitochondrial DAMPs, including mitochondrial dsDNA. Abbreviations: DAMP, damage-associated molecular pattern; dsDNA, double-stranded DNA; FcγR, Fcγ receptor; IFN, interferon; LOF, loss of function; Ox mtDNA, oxidized mitochondrial DNA; NET, neutrophil extracellular trap; pDC, plasmacytoid dendric cell; SLE, systemic lupus erythematosus; TFAM, transcription factor A, mitochondrial; TLR7, Toll-like receptor 7.

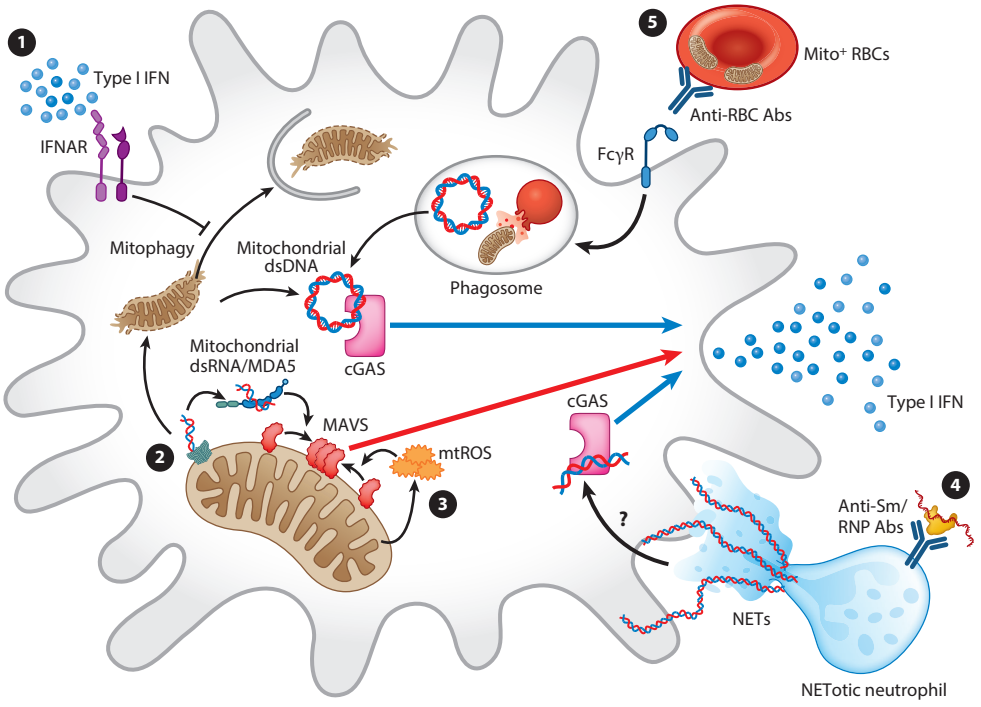


**Figure 2**

Sources of myeloid cell–activating nucleic acids in systemic lupus erythematosus. (❶) Type I interferon impairs monocyte mitophagy, leading to cytosolic accumulation of mitochondrial dsDNA, cGAS activation, and induction of a monocyte-derived inflammatory dendritic cell phenotype. (❷) Upon leaking from the mitochondrial matrix, mitochondrial dsRNA engages the RLR/MAVS pathway, leading to type I interferon production. (❸) mtROS can also trigger MAVS oligomerization in an RLR-independent manner. (❹) Anti-Sm/RNP antibodies induce NETs enriched in mitochondrial DNA. These NETs are internalized in myeloid cells, through a mechanism yet to be determined, and trigger cGAS activation. (❺) Antibody-mediated internalization of RBCs carrying mitochondria (Mito$^+$ RBCs) induces type I interferon production upon leakage of mitochondrial DNA from the phagosome into the cytosol and activation of cGAS. Abbreviations: dsDNA, double-stranded DNA; dsRNA, double-stranded RNA; FcγR, Fcγ receptor; IFN, interferon, mtROS, mitochondrial reactive oxygen species; NET, neutrophil extracellular trap; RBC, red blood cell; RLR, RIG-I-like receptor.

efficient intracellular removal of oxidized residues prior to the externalization of mitochondrial components. This is accomplished by the internal routing of Ox mtDNA to lysosomes for degradation (95). The combination of type I interferon and anti-Sm/RNP antibodies interferes with this lysosomal routing pathway and results in mitochondrial retention and eventual extrusion of interferogenic complexes composed of Ox mtDNA bound to TFAM (transcription factor A, mitochondrial), which is responsible for coiling mtDNA into nucleoids. Accordingly, abundant Ox mtDNA is visualized within mitochondria in ex vivo SLE blood neutrophils (95). Interestingly, the release of mitochondrial components from live cells, including neutrophils, has been recently reported as an alternative quality control mechanism in migrating cells. Defective mitochondria translocate to the plasma membrane at the trailing edge, where they are incorporated into small vesicular structures that form within the retraction fibers. These structures are then left behind the migration path, in a process called mitocytosis (96). Whether a similar mechanism participates in the extrusion of mtDNA from live neutrophils requires further elucidation.

In addition to granulocytes, platelets have emerged as a source of extracellular mtDNA in SLE. In this context, immune complex–activated platelets extrude mitochondria that, unless rapidly cleared, loose their membrane integrity, causing the release of inflammatory DAMPs, including mtDNA (97). Like the case of neutrophils, the release of mitochondria from platelets requires activation through FcγRIIA, supporting an additional pathogenic role of autoantibodies and/or immune complexes in this process (**Figure 1**). It has been proposed that platelet-mediated release of proinflammatory mitochondrial components might also contribute to the cardiovascular morbidity and mortality risk of SLE patients (98).

## Mitochondria Are a Source of Intracellular Interferogenic Nucleic Acids in SLE

Mitochondria are not only a source of extracellular DAMPs. In fact, a wide array of mitochondrial stressors, including environmental insults, oxidative and proteotoxic stress, impaired autophagy, mtDNA mutations, and infection, have been reported to trigger mtDNA release into the cytosol and cell-intrinsic production of type I interferon and/or IL-1β (84). This phenomenon plays important protective roles when induced by pathogens, as it promotes an antiviral state (99), or upon genotoxic stress as it activates the DNA repair pathway (100). However, if dysregulated, the release of mtDNA into the cytosol triggers a proinflammatory response (84). For example, by studying cells lacking the mitochondrial endonuclease G (EndoG), Kim et al. (101) found that voltage-dependent anion-selective channel 1 (VDAC1) oligomerization is responsible for the release of cytosolic mtDNA. The same study showed that high levels of VDAC1 oligomers and cytosolic mtDNA are present in both MpJ-*Fas*$^{lpr}$ lupus-prone mice and SLE PBMCs. Importantly, both a type I interferon signature and levels of cytosolic mtDNA were reduced in these mice upon inhibition of VDAC1 oligomerization, which also alleviated lupus-like symptoms (101).

Mitochondria also contain dsRNA that, when relocated into the cytosol, leads to innate immune activation. Indeed, loss of the mitochondrial degradosome components helicase SUV3 and polynucleotide phosphorylase PNPase results in a massive accumulation of dsRNA that escapes into the cytoplasm. This mitochondrial dsRNA engages an MDA5-driven antiviral signaling pathway that triggers type I interferon. Consistently, patients carrying mutations in *PNPT1*, which encodes PNPase, display mitochondrial dsRNA accumulation coupled with upregulation of ISGs and additional markers of immune activation (102). Similarly, upon mtDNA breaks, BAX/BAK macropores enable the release of mitochondrial dsRNA into the cytoplasm, which triggers a RIG-I-dependent response (103). Whether mitochondrial dsRNA leakage is involved in type I interferon production in SLE patients remains to be elucidated. Interestingly, the sensing of mitochondrial dsRNA by endolysosomal TLRs, such as TLR7, has been shown to induce type I interferon production and an autoimmune phenotype in mice lacking the GTPase IRGM1, a mitophagy inducer (104) (**Figure 2**).

It is worth highlighting that both MDA5 and RIG-I-driven signal transduction pathways are mediated through the mitochondrion-localized protein MAVS (102, 103). Thus, mitochondria play a dual role in this context by being a source of innate immune stimuli (mitochondrial dsRNA) and their transducing downstream signals through MAVS. Importantly, SLE PBMCs display spontaneous MAVS oligomerization in correlation with type I interferon activity and mitochondrial oxidative stress (105), and all of these are reverted by the mitochondrion-targeted antioxidant MitoQ (105). These findings suggest that mitochondria, through both RLR-dependent and RLR-independent MAVS oligomerization, may contribute to the SLE type I interferon activity (**Figure 2**).

Overall, different mechanisms that result in loss of mitochondrial integrity and/or compartmentalization might contribute to SLE pathogenesis through the release of mitochondrial nucleic acids into the extracellular space or the cytosol. Oxidation, which takes place in the steady state

in neutrophils, enhances the proinflammatory potential of mtDNA. As discussed below, this process might contribute not only to amplifying interferogenic loops but also to skewing downstream adaptive immune responses in SLE.

## Mitochondrial Quality-Control Defects in SLE: Links with Interferon

In addition to nucleic acids, other mitochondrial DAMPs such as mitochondrial ROS (mtROS) activate innate immune responses. However, different mechanisms involved in mitochondrial quality control, such as mitophagy and activity of mitochondrion-derived vesicles (MDVs), are in place to maintain homeostasis and avoid the production and/or release of mitochondrial DAMPs. Increasing evidence supports that impaired mitochondrial quality control might be involved in SLE pathogenesis. Importantly, interferon can be upstream or downstream of these defects.

Macroautophagy, hereafter referred as autophagy, is responsible for the catabolism of cellular components through their encapsulation by the autophagosome, a double-membrane structure. Eventually, fusion with the lysosome degrades the cargo, which can be reused or catabolized for energy production. Several types of autophagy have been identified. Nonselective autophagy occurs upon nutrient deprivation to supply cells with essential metabolic building blocks. In contrast, cargo-specific autophagy occurs under nutrient-rich conditions to mediate the removal of superfluous or damaged organelles and protein aggregates. An example of cargo-specific autophagy is mitophagy (106). Genetic links between SLE and autophagy, such as polymorphisms in ATG5 and ATG7, have been described, but whether these alleles affect mitophagy remains unknown (107, 108).

Cell-specific defective mitophagy has been reported in SLE. For example, SLE $CD4^+$ T cells display mitochondrial dysfunction characterized by increased mitochondrial mass (megamitochondria) and hyperpolarization, which has been proposed to contribute to abnormal T cell activation (109). Multiple mechanisms have been invoked, including defective mitophagy (110). A potential contributor to defective mitophagy in SLE $CD4^+$ T cells is overexpression of HRES-1/Rab4, a small GTPase that regulates endosomal traffic and promotes the degradation of the mitophagy initiator Drp1. In line with this, rapamycin, a potent autophagy/mitophagy inducer, ameliorates disease severity and mitochondrial dysfunction via mTOR inhibition in $CD4^+$ T cells from both MRL/*lpr* mice and SLE patients (111). Mitophagy also controls $CD8^+$ T cell activation and function. In SLE, a pathogenic role has been proposed for the $NAD^+$ modulating ectoenzyme CD38, which negatively regulates $CD8^+$ T cell mitochondrial fitness and cytotoxic function. By reducing cellular $NAD^+$ levels, CD38 suppresses the activity of sirtuins and limits the recruitment of damaged mitochondria to the phagophore, where they are normally encapsulated within the autophagosome through the PINK1-Parkin pathway. CD38 also diminishes lysosomal acidification by reducing the expression of V-ATPase. These events result in reduced mitophagy and accumulation of damaged, depolarized mitochondria (112). Furthermore, exposure of healthy $CD8^+$ T cells to IFN-α increases $NAD^+$ consumption through the upregulation of CD38 (113). These data suggest that both cell-intrinsic as well as cell-extrinsic factors, such as type I interferons, might be upstream of abnormal mitophagy and subsequent activation of SLE T cells.

Type I interferon signaling also affects mitophagy in myeloid cells. It was described more than 20 years ago that IFN-α activity within SLE sera instructs monocytes to differentiate into DCs able to sustain the survival and expansion of autoreactive lymphocytes (114). More recently, exposure of monocytes to IFN-α was reported to alter lysosomal acidification and cause defective mitophagy, resulting in the accumulation of cytoplasmic mtDNA, STING activation and type I interferon–dependent induction of monocyte differentiation into DCs (115) (**Figure 2**).

A link between defective mitophagy and a systemic interferonopathy with autoimmunity has been recently described in *Irgm1*-deficient mice. IRGM1 is a dynamin-like immunity-related

GTPase (IRG) required for the lysosomal degradation of autophagosomes. Its deficiency promotes lysosomal dysfunction in fibroblasts, allowing the cytoplasmic activation of the cGAS-STING pathway through cytosolic mtDNA. Within macrophages, IRGM1 supports autophagosome-lysosome fusion to degrade mitochondrial dsRNA, which in turn prevents the activation of endosomal TLR7. As a result, *Irgm1*-deficient mice develop a systemic interferonopathy with Sjögren syndrome–like symptoms (104).

Besides its role in mitochondrial quality control, mitophagy also plays a fundamental role during development and differentiation of specific cell lineages. For example, during terminal lens differentiation in the eyes of vertebrates, all membrane-bound organelles, including mitochondria, undergo complete degradation (116). Likewise, mammalian erythropoiesis involves enucleation and removal of cytosolic organelles, including mitochondria, as proerythroblasts differentiate into reticulocytes and eventually mature red blood cells (RBCs). We recently reported that "physiological erythroid mitophagy" is defective in SLE, especially in patients with high disease activity, resulting in mature RBCs that retain mitochondria ($Mito^+$ RBCs) (117). The mechanism underlying erythroid mitophagy has been well-characterized in mice (118) but was missing in humans. Toward this end, we used an in vitro model of human terminal erythroid differentiation (119) starting from PBMCs from patients and controls. Using this system, we demonstrated that a metabolic switch from glycolysis to OXPHOS regulated by the degradation of hypoxia-inducible factor 2a (HIF2α) controls activation of the ubiquitin-proteasome system (UPS) and subsequently the autophagy-mediated removal of mitochondria in humans. A defect in HIF2α degradation results in mitochondrial retention within SLE RBCs. Importantly, this phenotype is not directly triggered by type I interferon (117), and the mechanisms that control HIF2α levels during human erythropoiesis remain unknown. Of relevance to SLE, $Mito^+$ RBCs carry an extra load of mitochondrial DAMPs as they leave the bone marrow. Importantly, a significant number of SLE patients display antibodies that bind RBCs and opsonize them through FcγR within myeloid cells. This triggers the production of type I interferon in trans, in a $Mito^+$ RBC mtDNA– and myeloid cGAS/STING-dependent manner (117) (**Figure 2**).

In addition to mitophagy, MDVs—small vesicular carriers that transport mitochondrial proteins and lipids—participate in mitochondrial quality control. Thus, MDVs are involved in the constitutive delivery of proteins from mitochondria to lysosomes or peroxisomes, where they are degraded or participate in peroxisomal de novo biogenesis, respectively. In addition, MDVs regulate mitochondrial motility in axons, selectively package mitochondrial proteins in extracellular vesicles, and participate in mitochondrial antigen presentation during infections (120). MDVs also play a role in neutrophil mitochondrial quality control (95). As reported in earlier sections of this review, human neutrophils extrude mitochondrial components, both during NETosis as well as in the steady state. The latter results from the neutrophil's inability to complete mitophagy in response to mitochondrial damage. Instead, Ox mtDNA is routed to lysosomes for degradation within MDV-like vesicles. This micromitophagy process is altered when human neutrophils are exposed to type I interferon and to immune complexes that activate TLR7/8, leading to mitochondrial retention and eventually extrusion of highly immunogenic nucleoids (95).

The above-outlined observations reveal important connections between defective mitochondrial quality control measures affecting different cell types and the pathogenesis of SLE. Some of these can be ascribed to dysregulation of cell-intrinsic factors (i.e., hypoxia-related transcription factors or mitophagy regulators), whereas others are associated with cell-extrinsic factors such as cytokines, especially type I interferon, and/or SLE-specific autoantibodies. In the absence of direct genetic links, these alterations might represent disease amplification phenomena rather than upstream causes of SLE. Understanding their contribution to disease in individual patients might be fundamental to enable patient stratification toward targeted therapeutic interventions.

## Mitochondrial Metabolic Defects as Therapeutic Targets in SLE

Mitochondria contribute to cellular homeostasis through maintaining ATP levels as well as generating low levels of ROS, which is important for cell signaling. These two processes are linked through the electron transport chain. The electron transport chain is composed of four multimeric protein complexes located in the IMM that transfer electrons from donors to acceptors in a process known as oxidative phosphorylation (OXPHOS). The electron donors NADH and FADH2 are produced by the Krebs cycle, which is a chain of enzymatic reactions that oxidize fatty acids, glutamine, and acetyl-CoA derived from pyruvate. Defects in electron transport chain function and/or abnormalities in the Krebs cycle leading to accumulation of metabolite intermediates as well as mtROS have been described in inflammatory diseases (121). Notably, increased oxidative stress and mtROS production are features of SLE and have been considered as therapeutic targets. Recent evidence supports that type I interferon and early complement components contribute to these metabolic alterations.

Type I interferon affects mitochondrial function and enhances oxidative metabolism in multiple cell types (122). As reviewed earlier, IFN-α-treated monocytes accumulate mtROS and cytoplasmic mtDNA due to defective mitophagy flux, inducing an inflammatory DC phenotype (115). Type I interferons also promote, through an autocrine interferon receptor–dependent pathway, changes in pDC cellular metabolism characterized by increased fatty acid oxidation and OXPHOS. Direct inhibition of fatty acid oxidation or fatty acid synthesis prevents full pDC activation (123), supporting that targeting OXPHOS could be exploited therapeutically in SLE. Type I interferons affect OXPHOS in other immune cells, including memory $CD8^+$ T cells (124). Thus, $CD8^+$ T cells treated with type I interferon or isolated from the blood of SLE patients display enlarged mitochondria and lower spare respiratory capacity, which is associated with cell death upon rechallenge with T cell receptor agonists (113).

The well-established association between SLE and complement C1q deficiency also involves $CD8^+$ T cell mitochondrial metabolism (125). Accordingly, C1q modulates mitochondrial mass and biogenesis through interaction with the globular C1q receptor (p32/gC1qR), a mitochondrion-encoded cell surface protein (25). In a chronic graft-versus-host disease model of SLE, C1q dampened $CD8^+$ T cell differentiation toward an effector phenotype and reduced responses to self-antigens, thus acting as a metabolic rheostat for effector $CD8^+$ T cells (25).

Intermediates of the Krebs cycle, such as succinate, play a crucial role in ATP generation within mitochondria. Recently, however, new roles for succinate outside metabolism have emerged (126). Upon deletion of mitochondrial complex III in regulatory T cells (Tregs), for example, a buildup in succinate alters the expression of genes associated with Treg suppressive functions. An epigenetically mediated inhibition of the ten-eleven translocation (TET) family of DNA demethylases might contribute to this phenotype (127). Succinate has also been shown to contribute to B cell responses in SLE. Thus, an IL-$10^+$/IFN-$\gamma^+$ memory $CD4^+$ T cell subset expanded in blood and kidney from pediatric SLE patients with proliferative nephritis produces mtROS as the result of reverse electron transport fueled by succinate. Importantly, these cells provide B cell help through the synergistic effect of IL-10 and succinate. Similar cells are generated in vitro upon priming naive $CD4^+$ T cells with pDCs exposed to neutrophil-derived Ox mtDNA, establishing another link between type I interferon, oxidative stress, and adaptive immune alterations in SLE (42).

Succinate stabilizes the transcription of hypoxia-inducible factors (HIFs) in different cell types, including tumor cells and activated macrophages (128). Whether this metabolite is involved in mitochondrial retention during SLE erythropoiesis as the result of HIF2α stabilization remains to be addressed (117). HIF dysfunction has also been reported in renal-infiltrating $CD4^+$ and

$CD8^+$ T cells from lupus nephritis patients (129). Overexpression of HIF1 protein levels and HIF-dependent genes is associated with altered T cell metabolism and decreased apoptosis, thus favoring kidney inflammation. Perturbation of these environmental adaptations by selective HIF1 blockade reverted this phenotype in murine lupus models (129). Notably, this phenotype differs from the one observed in interferon-treated $CD8^+$ T cells that undergo cell death due to increased $NAD^+$ consumption and impaired mitochondrial respiration (113).

It is important to highlight that although the ultimate causes of mitochondrial metabolic dysfunction in SLE are probably multifactorial, genetic factors might be at play. In humans, a single-nucleotide polymorphism (SNP) variant of the ATP6 or F0F1-ATPase gene (complex V) has been associated with SLE (130). Inhibition of this ATPase leads to mitochondrial hyperpolarization and ATP depletion, which are observed in SLE. The murine lupus susceptibility locus Sle1c2 defines the *ESRRC* gene, a known regulator of mitochondrial function. Decreased expression of this gene in mice contributes to mitochondrial dysfunction, increased mtROS, and abnormal $CD4^+$ T cell activation with increased IFN-γ production (131).

These recent advances in immunometabolism point to the importance of this area of research and the potential of testing whether Krebs cycle metabolite intermediates that play a role in immune signaling could be targeted in SLE. Itaconate, for example, is an immunomodulatory metabolite with therapeutic potential (132). In addition to enhancing the oxidation of succinate to fumarate, and therefore reducing intracellular succinate accumulation, itaconate triggers multiple anti-inflammatory pathways (133). Permeable itaconate and 4-octyl itaconate restricted type I interferon and inflammatory cytokine production by macrophages (134). When added to PBMCs from SLE patients, it activated Nrf2 signaling and decreased proinflammatory cytokines such as TNF-α and IL-6 (135). Metformin is a drug widely used to treat type 2 diabetes, as it prevents gluconeogenesis. Metformin transiently inhibits mitochondrial electron transport chain complex I, therefore decreasing OXPHOS and ATP production and indirectly leading to AMPK activation. Metformin plays anti-inflammatory roles by promoting Treg differentiation and blocking STAT3 activation, which has been attributed to either AMPK activation/mTORC1 inhibition or increased fatty acid oxidation (136). In vitro, metformin inhibits IFN-γ and promotes IL-2 production by $CD4^+$ T cells from SLE patients and lupus-prone mice (137). The combination of metformin and 2-deoxy-glucose, an inhibitor of glycolysis-derived pyruvate, reversed disease activity biomarkers in several murine lupus models in correlation with decreased $CD4^+$ T cell mitochondrial activation (137). Metformin also reduced the response of SLE $CD4^+$ T cells to type I interferon by inhibiting STAT1 phosphorylation (138), suggesting that it may benefit SLE patients with high type I interferon activity. Indeed, in randomized trials, metformin added to standard-of-care treatment reduced the risk of SLE disease flares and corticosteroid exposure, although these effects were mainly observed in patients with mild disease activity who were seronegative at baseline (139, 140).

## INTERFERON AND SLE AUTOANTIBODIES: FRIENDS OR FOES?

Autoantibodies to nuclear antigens are a hallmark of SLE, but cytosolic, cell surface, and extracellular antigens are also targeted in this disease. Among them, only a few antibody specificities are known to play a direct pathogenic role. SLE autoantibodies, however, impact interferogenic and proinflammatory pathways through a variety of mechanisms. Furthermore, a series of classic and recently described auto-specificities mimic genetic defects associated with monogenic interferonopathies and lupus. SLE autoantibodies can also target and neutralize interferon family members. This illustrates the complex role of SLE autoantibodies beyond the traditional view as passive inducers of inflammation upon immune complex deposition in target tissues.

## Prointerferogenic Autoantibodies

SLE autoantibodies contribute to activation of the interferon system through their capacity to carry and deliver, through FcγR-mediated internalization, nucleic acids otherwise inaccessible to intracellular sensors such as endosomal TLRs and cytosolic cGAS. In vitro, for example, IFN-α and ISGs are induced when unfractionated PBMCs or pDCs are cultured with SLE serum, purified serum IgG, or reconstituted immune complexes containing autoantibodies against RNA, necrotic or apoptotic cellular material (5). This effect is inhibited by Fcγ blockade or the addition of nucleases, and it is augmented by NA-binding proteins such as HMGB1 or LL37 (141, 142), which are also SLE antigenic targets (143). The in vivo significance of these findings is supported by the correlation between the presence of these auto-specificities and a type I interferon signature in SLE blood (144). Anti-RNA/RNP autoantibodies not only carry TLR7 ligands capable of inducing interferon production by pDCs but also trigger release of interferogenic Ox mtDNA from neutrophils (92, 95).

As described earlier, RBCs and anti-RBC autoantibodies act as Trojan horses in SLE. Thus, defective erythropoiesis leads to mitochondrial retention in mature RBCs from a subset of SLE patients. Opsonization of $Mito^+$ RBCs within myeloid cells and leakage of $Mito^+$ RBC–derived mtDNA into the cytosol lead to cGAS/STING activation, triggering the production of type I interferon (117) (**Figure 2**). Opsonizing antibodies might directly recognize surface antigens on RBCs or be associated with complement-fixing immune complexes through complement receptor type 1 (CR1), also on the RBC surface. Interestingly, although the direct binding of antibodies to RBCs is a hallmark of autoimmune hemolytic anemia, immune complex binding to CR1 is not associated with hemolysis (145). Indeed, overt hemolysis is rare in SLE, supporting the latter scenario. Whether additional cell types such as platelets, which carry a plethora of potential DAMPs, play an inflammatory role in SLE through similar mechanisms remains to be explored.

Another mechanism by which SLE autoantibodies contribute to interferon production is by interfering with extracellular nucleic acid degradation. For example, autoantibodies that recognize NET components protect extracellular dsDNA from DNase1–mediated degradation (146, 147), and their presence is associated with lupus nephritis (52). Recently, autoantibodies targeting DNase1L3 have been described in >50% of patients with sporadic SLE and lupus nephritis. These autoantibodies reduce DNase1L3 enzymatic activity and consequently decrease microparticle-associated dsDNA degradation (**Figure 1**). Importantly, their presence correlates with overall disease activity, supporting an upstream pathogenic role (148). Cellular debris, another source of extracellular nucleic acids, is removed by phagocytes through a process involving numerous players, including C1q. Anti-C1q antibodies reduce C1q protein levels and impair the clearance of dying cells, therefore mimicking deficiency of C1q, one of the strongest genetic risk factors for early-onset SLE (149). Whether these autoantibodies also interfere with $CD8^+$ T cell mitochondrial function remains to be addressed.

## Anti-interferon Autoantibodies

SLE autoantibodies also target the interferon pathway. Anti-cytokine autoantibodies are actually a component of the healthy immune repertoire and may regulate cytokine biology by reducing their production or by prolonging their half-life (150). In SLE patients, autoantibodies against multiple cytokines including type I and II interferons, G-CSF, TNF, IL-1, IL-6, and IL-10 have been reported (150). In fact, autoantibodies against type I and II interferons are present in sera from up to 27% of SLE patients (151). While in nearly 50% of SLE patients with anti–type I interferon autoantibodies the target cytokine is neutralized, anti–type II interferon autoantibodies do not display blocking activity. As expected, the presence of neutralizing anti–type I interferon

autoantibodies is associated with significantly lower levels of interferon bioactivity and lower disease activity (152). Surprisingly, non-neutralizing anti-IFN-γ autoantibodies correlate with greater disease activity, higher titer of anti-dsDNA antibodies, and increased ISG expression (153). Whether this is mediated through increased half-life of the target cytokine remains to be determined.

## RECENT INSIGHTS INTO THE ORIGIN OF SLE AUTOANTIBODIES

Autoreactive B cells are a hallmark of SLE. Not surprisingly, genetic studies of de novo or familial SLE cases using genome-wide approaches confirm that intrinsic B cell defects lead to SLE (83, 154, 155). Among these, homozygous missense mutations in *PRKCD*, encoding protein kinase δ (PKCδ), confer resistance to B cell receptor (BCR)- and calcium-dependent apoptosis and result in defective deletion of autoreactive B cells and lymphoproliferation (156). Variants in *BLK*, an Src B kinase relevant to signaling downstream of the BCR and type I interferon, have been implicated by GWAS in multiple autoimmune diseases. Importantly, rare damaging variants of *BLK* were found in ~12% of patients with SLE, whereas variants found in healthy controls were not deleterious. *BANK1* encodes a catalytically inactive scaffolding protein thought to recruit BLK, among other phosphokinases, to signal in response to BCR ligation. A common *BANK1* SNP has been associated with several autoimmune diseases. Similar to *BLK*, a rare variant of *BANK1* resulting in enhanced nuclear localization of IRF5 was found restricted to SLE patients (19).

In the end, regardless of whether the upstream genetic pathway leading to SLE is B cell–intrinsic (e.g., *PRKCD*, *BLK*, etc.) or –extrinsic (e.g., *C1q*, *DNASE1L3*, etc.), autoantibody specificities against antigens such as dsDNA, Smith antigen (Sm) and RNPs become prevalent. The microenvironment and the B cell pathways involved in the generation of these autoantibodies have been long debated. T cell–dependent B cell activation starts at the T cell–B cell border in secondary lymphoid organs. B cells then either migrate to extrafollicular foci, quickly expand, and become short-lived plasmablasts or enter germinal centers (GCs), which are the major sites of affinity maturation and long-term memory and plasma cell generation (157) (**Figure 3**). A load of somatic hypermutation detected in autoantibodies from SLE patients supports their GC origin (158, 159). However, quantitative longitudinal assessment reveals different antibody patterns depending on their specificities (160). Thus, levels of anti-Sm and anti-RNP antibodies are relatively stable and resistant to antiproliferative therapies, supporting a long-lived plasma cell origin. In contrast, levels of anti-dsDNA antibodies fluctuate with disease activity and decrease after antiproliferative therapy (161), supporting their short-lived plasmablast origin. Consistently, pathogenic autoantibodies have been shown to derive from extrafollicular reactions in multiple lupus mouse models (20, 162–164). Features of this response include its localization in the bridging channels of the spleen or the medullary cords of lymph nodes and its independence from Bcl6 expression in B cells.

### The Extrafollicular B Cell Compartment as a Source of SLE Autoantibodies

The recent definition of extrafollicular B cell markers has fueled interest in the contribution of this pathway to infectious (165–167) and autoimmune (40, 168, 169) diseases. In SLE, two subsets of extrafollicular B cells were reported to expand during active disease, especially in patients of African American descent: an $IgD^+CD27^-$ Mitotracker $Green^+$ activated naive (aNAV) cell population (169) and a subset within the $IgD^-CD27^-$ double negative (DN) memory compartment characterized by expression of CD11c and T-bet, lack of the follicular markers CXCR5 and CD21, and increased responsiveness to TLR7 (DN2) (40).

aNAV and DN2 cells share phenotypic markers with a subset of B cells first described in mice as aged-associated B cells (ABCs). ABCs were characterized in the spleen by the expression of CD11c

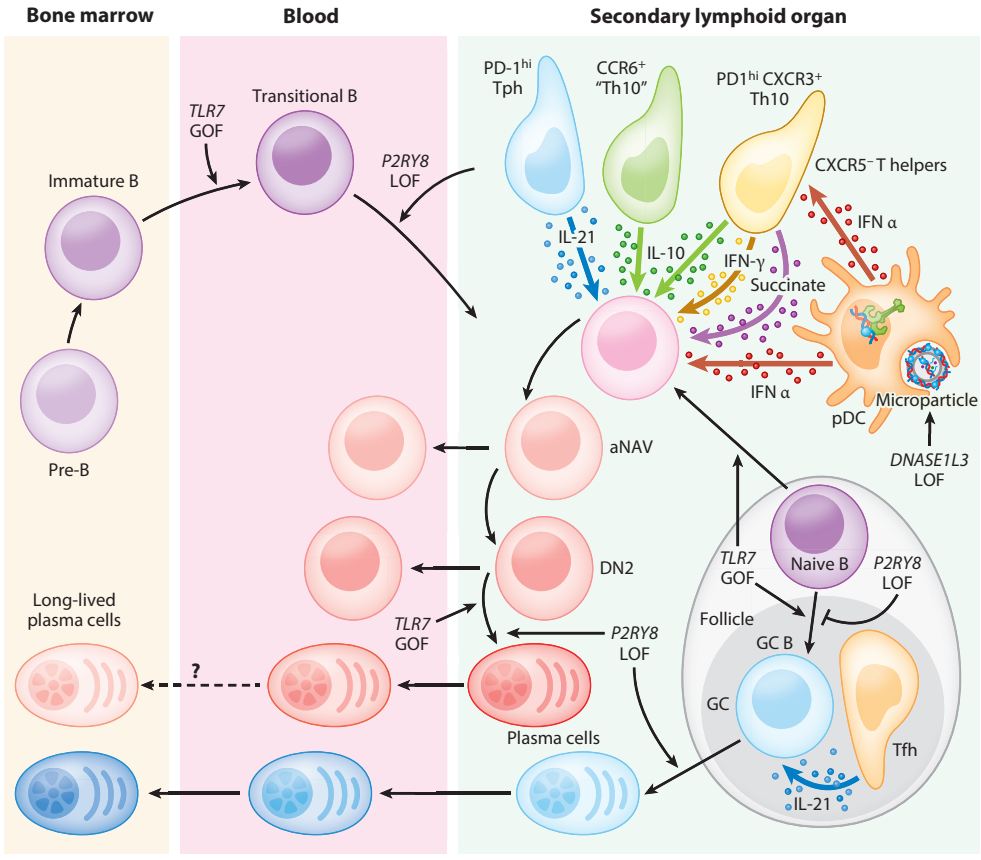


**Figure 3**

Amplification of extrafollicular antibody generation in systemic lupus erythematosus. There are multiple checkpoints of B cell tolerance along the trajectory of B cell development (*upper left*, *purple*) and two T cell–dependent B cell differentiation pathways (GC response, originated from follicle in blue; extrafollicular response, outside follicle in red). These are fueled by different subsets of T helper cells (peripheral helpers on the upper right, and follicular helpers within GCs). Checkpoints of B cell tolerance are impacted in monogenic lupus. *P2YR8* LOF leads to increased AKT and ERK activity in B cells, which disrupts B cell negative selection from transitional to follicular stage and promotes plasma cell differentiation. *P2YR8* LOF prevents GC confinement of B cells and thus disrupts GC tolerance checkpoints. *TLR7* GOF drives aberrant survival of B cell receptor–activated immature B cells and amplifies both GC and extrafollicular responses. *DNASE1L3* LOF leads to the accumulation of microparticle-associated DNA, which is sensed by TLR9 in pDCs. Type I interferon and additional pDC-derived signals amplify extrafollicular responses by promoting the differentiation of extrafollicular helper T cells (Th10 cells) as well as extrafollicular B cells. Abbreviations: aNAV, activated naive; GC, germinal center; GOF, gain of function; LOF, loss of function; pDC, plasmacytoid dendritic cell; Tfh, T follicular helper; TLR7, Toll-like receptor 7; Tph, T peripheral helper.

and CD11b, absence of CD21 and CD23, and, in ~50% of them, expression of T-bet. As their name indicates, these cells accumulate with age, especially in female mice (170, 171). Moreover, ABCs prematurely expand and play a pathogenic role in different murine lupus models (170, 172). Several human B cell subsets resembling ABCs have been described according to the expression of different markers in distinct contexts. In addition to aNAV and DN2 cells, T-bet$^{+}$CD11c$^{hi}$ B cells were reported to be expanded in SLE (173, 174), and CD27$^{-}$CD21$^{lo}$FCRL4$^{hi}$ atypical memory B cells were found expanded in malaria (175, 176) and HIV (177) patients. These cell populations, as defined by expression levels of CD21, CD11c, T-bet, or combinations thereof, are different from

$CD27^+$ conventional memory B cells expressing low levels of CD21, which are transiently induced after immunization and are thought to represent recent GC graduates (178). Healthy $CD21^{lo}$ B cells display variable levels of CD11c (179, 180). $CD11c^+$ B cells are, on the contrary, remarkably enriched in $CD21^{lo}$ expressors (181). T-bet is also enriched in $CD21^{lo}$ B cells, and to a greater extent in $CD21^{lo}CD11c^{hi}$ B cells. Moreover, single-cell proteotranscriptomics of B cells from a patient with inherited T-bet deficiency revealed an absence of ABC-like cells, establishing T-bet as an essential transcription factor for these cells' development (179). Zeb2, another transcription factor highly expressed in DN2 cells (40), might be induced by T-bet as shown in cytotoxic T cells (182).

Despite the recent advances in their phenotypic characterization, the extrafollicular origin of human ABC-like B cells requires further clarification. Expression of CXCR5, the main follicular homing chemokine receptor, is heterogeneous among $CD21^{lo}$ B cells (180) but almost uniformly downregulated in $CD21^{lo}CD11c^+$ and/or T-$bet^+$ B cells (40, 168). In the end, however, all the major B cell compartments defined by CD27 and IgD, including naive ($CD27^-IgD^+$), conventional memory ($CD27^+$), and DN ($CD27^-IgD^+$) B cells, may contain a subpopulation of T-$bet^+CD11c^+CD21^{lo}$ ABC-like cells (40, 168, 180). In the context of SLE, most ABC-like cells are found, however, within the DN2 compartment (40, 174).

Within SLE patients, aNAV cells and DN2 cells share similar transcriptomes and exhibit a substantial level of clonal connectivity. Furthermore, both are precursors of antibody-secreting cells (40). The expression levels and target genes of IRF4, a transcription factor essential for plasma cell differentiation (183), are enriched in DN2 cells (40). Consistently, BACH2, IRF8, and EST1, transcription factors that repress plasma cell differentiation (184), are downregulated in DN2 cells, and protein levels of BLIMP-1, a transcription factor that induces plasma cell differentiation, increase gradually along a naive, aNAV, and eventually DN2 cell trajectory. Similar to plasma cells, which downregulate surface BCRs, DN2 cells express 50% lower levels of surface immunoglobulin compared with their $CD27^+$ memory B cell counterparts (40). Neither their potential to differentiate into plasma cells nor their low expression levels of the lymph node homing receptors L-selectin (CD62L) and CXCR5 are, however, direct evidence that DN2 cells are generated extrafollicularly. In this context, the frequency of somatic hypermutation within aNAV and DN2 cells is at an intermediate level between naive and switched memory B cells, which could still support their GC origin if the eventual loss of follicular homing markers would preclude GC recycling and further accumulation of somatic hypermutation.

## T Cell Dependence of Extrafollicular B Cell Responses in SLE

GC responses are mostly T cell dependent, but T cell–independent GCs have been well-characterized and shown to contribute to long-lived memory and plasma cell responses, such as those to bacterial polysaccharide antigens (185). The extrafollicular response can also be dependent or independent of T cells. A fundamental B cell survival signal provided by T helper cells is CD40L. aNAV and DN2 cells downregulate TRAF5, an essential mediator of CD40 signaling, and are consequently hyporesponsive in vitro to CD40L stimulation. While in vitro generation of DN2-like cells from naive B cells proceeds in the absence of CD40L (40), BAFF, which has a redundant downstream signal with CD40L, induces DN2 differentiation from naive B cells (40). Importantly, cytokines required for DN2 induction and further antibody-secreting cell differentiation such as IL-21, IL-2, and IFN-$\gamma$ (41) are predominantly derived from T cells. Consistently, IL-21 deficiency completely abolishes the accumulation of ABCs in a SWAP-$70^{-/-}$ and $DEF6^{-/-}$ mouse model of lupus. In the same model, lack of SAP, an adaptor protein that sustains T cell–B cell interactions (186), inhibits the accumulation of ABCs (187). Recently, the blood B cell compartment of patients with monogenic defects in *IL21R*, *IFNGR1*, and *CD40/CD40L* was shown to have lower frequencies of $CD21^{lo}$ B cells (180), supporting their in vivo dependence on T cell signals.

Extensive efforts have been made to identify the T cell subset(s) involved in extrafollicular B cell help in SLE. The first well-characterized extrafollicular helper subset in humans (T peripheral helper cells) was described in the synovium of patients with rheumatoid arthritis. These cells, identified as $CD4^+$ $CXCR5^-$ $PD1^{hi}$ T cells, provide B cell help through IL-21, CXCL13, and SLAMF5 (188). T peripheral helper cells were later found expanded in SLE blood (189). Recent studies, however, support that additional T cell subsets contribute to extrafollicular B cell help in SLE. Thus, pediatric SLE blood contains an expanded $CD4^+IL\text{-}10^+IFN\text{-}\gamma^+$ T helper population (Th10) (42). As T peripheral helper cells, Th10 cells express high levels of PD1, lack CXCR5 expression, and exhibit chemokine receptors (CXCR3) and transcription factors (T-bet) related to Th1 cells. In addition, Th10 cells accumulate the metabolite succinate as the result of reverse electron transfer. Blood Th10 and T follicular helper (Tfh) cells are equally effective at inducing naive and memory B cell differentiation into antibody-secreting cells, but the helper function of Th10 cells is independent of IL-21 and dependent on IL-10 and succinate. Expansion of Th10 cells in SLE blood correlates with IgG and IgA levels and with the frequency of ABC-like cells (42). Similar cells have been reported in the context of COVID-19 vaccine responses and HIV infection (190, 191).

In addition to Th10, IL-10-producing $CD4^+$ T cells expressing CCR6 but lacking CXCR3 were described in a pristane-induced lupus model and in SLE patients. Like Th10, these cells provide B cell help in an IL-10-dependent manner (192). Notably, the cytokine profile of Th10 and $CCR6^+$ $CD4^+$ T cells overlaps significantly with that of type 1 regulatory (Tr1) $CD4^+$ T cells (193–196) and Th1 "switched" $CD4^+$ T cells (197), thus making their identification based on surface markers or cytokine profiles challenging. Further studies are required to fully understand the complexity of the extrafollicular $CD4^+$ memory T cell compartment in healthy and autoimmune scenarios.

The anatomical sites where extrafollicular help is provided in the context of SLE remain to be determined. As in mice, the human extrafollicular reaction was first described in secondary lymphoid organs (198, 199) but evidence supporting the role of inflamed tissues has emerged. $CD11c^+$ B cells and Th10-like cells are represented within lupus nephritis cellular infiltrates (42, 174). Theoretically, the interaction between T and B cells could be mediated by chemokine-chemokine receptor pairs, or these cell types could be attracted to the same location independently of each other. Whereas T peripheral helper cells in the rheumatoid arthritis synovium express CXCL13, the main Tfh cell chemoattractant for B cells, Th10 cells do not secrete this chemokine. Furthermore, both aNAV and DN2 cells lack expression of its receptor, CXCR5. Th10 cells upregulate CXCR3 (42), a receptor for the interferon-inducible chemokine CXCL10 (IP10) (200). CXCR3, also expressed by DN2 cells (40), may direct these cell types to interferon-expressing areas within the lupus kidney.

## Monogenic Lupus Provides Novel Links to the Extrafollicular Reaction

Extrafollicular responses could be a primary or a secondary pathogenic event in SLE, and these two possibilities are not mutually exclusive. pDCs and type I interferon, for example, induce the generation of Th10 cells in vitro (42) and might therefore be upstream of extrafollicular B cell help pathways in SLE. TLR7 signaling promotes GC reactions in mice, and yet it is one of the best-established extrafollicular reaction–associated pathways. Consequently, ABCs are not found in some *MyD88*$^{-/-}$ or *TLR7*$^{-/-}$ murine models, and chronic TLR7 stimulation is enough to induce ABC generation (170). Furthermore, anti-chromatin antibodies drive AM14 rheumatoid factor–encoding B cells to differentiate into plasmablasts outside follicles in the lupus-prone MRL-lpr/lpr (MRL-lpr) background (163). In this model, the extrafollicular reaction does not require T cells but depends on the combination of TLR7 and TLR9 stimulation (201, 202). In humans, DN2 cells

are hyperresponsive to TLR7, and TLR7 signaling is indispensable for the induction of these cells under some in vitro conditions (40, 41). Importantly, while patients with inborn errors of immunity due to TLR signaling defects, such as *MyD88* or *IRAK4* LOF, exhibit normal numbers of T-bet$^{+}$CD21$^{lo}$ ABC-like cells (180), a recently described TLR7$^{Y264H}$ gain-of-function mutation in a patient with early-onset SLE supports the role of TLR7 signaling in the SLE extrafollicular response (20). This variant selectively increased sensing of guanosine and 2′,3′-cGMP, a potential endogenous TLR7 ligand, and was sufficient to induce lupus in a B cell–intrinsic manner when introduced in mice. Despite prominent spontaneous GC formation in Tlr7$^{Y264H}$ mice, higher autoantibody titers and a more pronounced expansion of ABCs and plasma cells were observed upon GC ablation. Inclusion of phenotypic markers covering both ABC-like (Tbet$^{+}$CD21$^{lo}$) and DN2 (CD27$^{-}$IgD$^{-}$CXCR5$^{-}$CD21$^{lo}$CD11c$^{+}$) B cell populations in human studies might facilitate drawing conclusions about the origin and role of extrafollicular B cell pathways in disease.

In addition to TLR7, the functional characterization of recently described genes associated with monogenic lupus provides strong support for the contribution of extrafollicular pathways to human SLE (**Figure 3**). Thus, rare Mendelian or de novo LOF variants in *P2RY8*, the G protein receptor for *S*-geranyl-geranyl-L-glutathione (GGG) that drives B cell clustering inside GCs (203, 204), were identified in either lupus kindreds or patients with the related antiphospholipid syndrome (21). Interestingly, P2RY8 protein levels were also found downregulated in B cells from SLE patients who did not carry germline *P2RY8* variants, and these expression levels correlated with both the presence of lupus nephritis and increased frequencies of DN2 B cells and plasma cells. Therefore, disruption of P2RY8 signaling, and subsequently of B cell GC confinement, represents an upstream event in SLE pathogenesis. However, P2RY8 plays additional roles in B cell tolerance checkpoints, such as restraining plasma cell accumulation and limiting selection of immature self-reactive B cells into the recirculating pool (21). P2RY8 is also widely expressed by other lymphocytes (205), and the absence of a rodent homolog makes it difficult to dissect the potential contribution to SLE of additional cell types that express this protein.

As reviewed above, *DNASE1L3* LOF mutations cause monogenic lupus (69). Deficiency in this enzyme induces accumulation of microparticle-associated dsDNA and elicits a universal anti-dsDNA response that is T cell–dependent in both mice and humans (18). Ablation of TLR7-dependent GC formation in *DNASE1L3*$^{-/-}$ mice does not change the titers of anti-dsDNA autoantibodies or the number of antibody-secreting cells. In contrast, disrupting pDCs and/or TLR9-dependent type I interferon signaling decreases anti-dsDNA antibody titers and ameliorates autoreactivity (164). Therefore, the anti-dsDNA response triggered by extracellular microparticle-associated dsDNA accumulation depends on the extrafollicular response and is enhanced by TLR9 signaling and type I interferon (**Figure 3**).

Despite this and other supportive evidence (20, 40, 164), questions about the contribution of extrafollicular reactions to SLE pathogenesis remain. There is no experimental evidence, for example, that the specificities of antibody-secreting cells generated through GC or extrafollicular responses are different. In two mouse models (*DNase1L3*$^{-/-}$ and Tlr7$^{Y264H}$) mentioned above, the quantity of autoantibodies in GC-impaired mice was comparable to or even higher than that of GC-competent mice, but their quality and the ensuing autoimmune-related tissue damage were not evaluated. For example, even though the autoantibody titers and number of antibody-secreting cells were unchanged in *Dnase1l3*$^{-/-}$*Tlr7*$^{-/-}$ mice with reduced GCs, kidney manifestations, including glomerular deposition of IgG and C3, were rescued. Whether the amelioration of tissue damage is due to decreased affinity of autoantibodies produced through extrafollicular response remains to be explored. Evidence from mice suggests that long-lived plasma cells are derived exclusively from GC responses (206). As mentioned above, long-lived plasma cells contribute to distinct specificities in SLE and are resistant to B cell depletion therapies.

In conclusion, further dissecting the potentially different roles of GC and extrafollicular responses and the mechanisms that control them calls for future research. Understanding the upstream pathways and amplifiers of autoreactive T and B cell responses in individual patients will improve selection of available therapies and will guide the development of new ones targeting these pathways.

## DISCLOSURE STATEMENT

V.P. has received consulting fees from Astra-Zeneca, Sanofi, and Janssen as well as a research grant from Sanofi and a research contract from Astra-Zeneca.

## LITERATURE CITED


1. Tsokos GC. 2011. Systemic lupus erythematosus. *N. Engl. J. Med.* 365:2110–21
2. Lazar S, Kahlenberg JM. 2023. Systemic lupus erythematosus: new diagnostic and therapeutic approaches. *Annu. Rev. Med.* 74:339–52
3. Hoi AY, Morand EF. 2021. Treatment update in systemic lupus erythematous. *Rheum. Dis. Clin. N. Am.* 47:513–30
4. Almlof JC, Nystedt S, Leonard D, Eloranta ML, Grosso G, et al. 2019. Whole-genome sequencing identifies complex contributions to genetic risk by variants in genes causing monogenic systemic lupus erythematosus. *Hum. Genet.* 138:141–50
5. Bave U, Vallin H, Alm GV, Ronnblom L. 2001. Activation of natural interferon-alpha producing cells by apoptotic U937 cells combined with lupus IgG and its regulation by cytokines. *J. Autoimmun.* 17:71–80
6. Means TK, Latz E, Hayashi F, Murali MR, Golenbock DT, Luster AD. 2005. Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. *J. Clin. Investig.* 115:407–17
7. Guiducci C, Gong M, Xu Z, Gill M, Chaussabel D, et al. 2010. TLR recognition of self nucleic acids hampers glucocorticoid activity in lupus. *Nature* 465:937–41
8. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. 2002. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 416:603–7
9. Santini SM, Lapenta C, Logozzi M, Parlato S, Spada M, et al. 2000. Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity in vitro and in Hu-PBL-SCID mice. *J. Exp. Med.* 191:1777–88
10. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. 2001. Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. *Immunity* 14:461–70
11. Pascual V, Farkas L, Banchereau J. 2006. Systemic lupus erythematosus: all roads lead to type I interferons. *Curr. Opin. Immunol.* 18:676–82
12. Looney RJ. 2010. B cell-targeted therapies for systemic lupus erythematosus: an update on clinical trial data. *Drugs* 70:529–40
13. Navarra SV, Guzman RM, Gallacher AE, Hall S, Levy RA, et al. 2011. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 377:721–31
14. Bruce IN, Furie RA, Morand EF, Manzi S, Tanaka Y, et al. 2022. Concordance and discordance in SLE clinical trial outcome measures: analysis of three anifrolumab phase 2/3 trials. *Ann. Rheum. Dis.* 81:962–69
15. Crow YJ, Stetson DB. 2022. The type I interferonopathies: 10 years on. *Nat. Rev. Immunol.* 22:471–83
16. Belot A, Rice GI, Omarjee SO, Rouchon Q, Smith EMD, et al. 2020. Contribution of rare and predicted pathogenic gene variants to childhood-onset lupus: a large, genetic panel analysis of British and French cohorts. *Lancet Rheumatol.* 2:E99–109. Correction. 2020. *Lancet Rheumatol.* 2:E644

17. Charras A, Haldenby S, Smith EMD, Egbivwie N, Olohan L, et al. 2022. Panel sequencing links rare, likely damaging gene variants with distinct clinical phenotypes and outcomes in juvenile-onset SLE. *Rheumatology*. In press. **https://doi.org/10.1093/rheumatology/keac275**
18. Sisirak V, Sally B, D'Agati V, Martinez-Ortiz W, Ozcakar ZB, et al. 2016. Digestion of chromatin in apoptotic cell microparticles prevents autoimmunity. *Cell* 166:88–101
19. Jiang SH, Athanasopoulos V, Ellyard JI, Chuah A, Cappello J, et al. 2019. Functional rare and low frequency variants in BLK and BANK1 contribute to human lupus. *Nat. Commun.* 10:2201
20. Brown GJ, Canete PF, Wang H, Medhavy A, Bones J, et al. 2022. TLR7 gain-of-function genetic variation causes human lupus. *Nature* 605:349–56
21. He Y, Gallman AE, Xie C, Shen Q, Ma J, et al. 2022. P2RY8 variants in lupus patients uncover a role for the receptor in immunological tolerance. *J. Exp. Med.* 219(1):e20211004
22. Lee-Kirsch MA, Gong M, Chowdhury D, Senenko L, Engel K, et al. 2007. Mutations in the gene encoding the 3′-5′ DNA exonuclease TREX1 are associated with systemic lupus erythematosus. *Nat. Genet.* 39:1065–67
23. Briggs TA, Rice GI, Daly S, Urquhart J, Gornall H, et al. 2011. Tartrate-resistant acid phosphatase deficiency causes a bone dysplasia with autoimmunity and a type I interferon expression signature. *Nat. Genet.* 43:127–31
24. An J, Briggs TA, Dumax-Vorzet A, Alarcon-Riquelme ME, Belot A, et al. 2017. Tartrate-resistant acid phosphatase deficiency in the predisposition to systemic lupus erythematosus. *Arthritis Rheum.* 69:131–42
25. Ling GS, Crawford G, Buang N, Bartok I, Tian K, et al. 2018. C1q restrains autoimmunity and viral infection by regulating $CD8^+$ T cell metabolism. *Science* 360:558–63
26. Schejbel L, Skattum L, Hagelberg S, Ahlin A, Schiller B, et al. 2011. Molecular basis of hereditary C1q deficiency—revisited: identification of several novel disease-causing mutations. *Genes Immun.* 12:626–34
27. Xu L, Zhao J, Sun Q, Xu X, Wang L, et al. 2022. Loss-of-function variants in *SAT1* cause X-linked childhood-onset systemic lupus erythematosus. *Ann. Rheum. Dis.* 81(12):1712–21
28. Ortíz-Fernández L, Martín J, Alarcón-Riquelme ME. 2022. A summary on the genetics of systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, and Sjogren's syndrome. *Clin. Rev. Allergy Immunol.* In press
29. Langefeld CD, Ainsworth HC, Cunninghame Graham DS, Kelly JA, Comeau ME, et al. 2017. Transancestral mapping and genetic load in systemic lupus erythematosus. *Nat. Commun.* 8:16021
30. Nehar-Belaid D, Hong S, Marches R, Chen G, Bolisetty M, et al. 2020. Mapping systemic lupus erythematosus heterogeneity at the single-cell level. *Nat. Immunol.* 21:1094–106
31. Banchereau R, Hong S, Cantarel B, Baldwin N, Baisch J, et al. 2016. Personalized immunomonitoring uncovers molecular networks that stratify lupus patients. *Cell* 165:551–65
32. Perez RK, Gordon MG, Subramaniam M, Kim MC, Hartoularos GC, et al. 2022. Single-cell RNA-seq reveals cell type-specific molecular and genetic associations to lupus. *Science* 376:eabf1970
33. Arazi A, Rao DA, Berthier CC, Davidson A, Liu Y, et al. 2019. The immune cell landscape in kidneys of patients with lupus nephritis. *Nat. Immunol.* 20:902–14
34. Fava A, Buyon J, Mohan C, Zhang T, Belmont HM, et al. 2020. Integrated urine proteomics and renal single-cell genomics identify an IFN-gamma response gradient in lupus nephritis. *JCI Insight* 5(12):e138345
35. Guthridge JM, Lu R, Tran LT, Arriens C, Aberle T, et al. 2020. Adults with systemic lupus exhibit distinct molecular phenotypes in a cross-sectional study. *EClinicalMedicine* 20:100291
36. Nakano M, Ota M, Takeshima Y, Iwasaki Y, Hatano H, et al. 2022. Distinct transcriptome architectures underlying lupus establishment and exacerbation. *Cell* 185:3375–89.e21
37. Dunlap GS, Billi AC, Xing X, Ma F, Maz MP, et al. 2022. Single-cell transcriptomics reveals distinct effector profiles of infiltrating T cells in lupus skin and kidney. *JCI Insight* 7(8):e156341
38. Billi AC, Ma F, Plazyo O, Gharaee-Kermani M, Wasikowski R, et al. 2022. Nonlesional lupus skin contributes to inflammatory education of myeloid cells and primes for cutaneous inflammation. *Sci. Transl. Med.* 14:eabn2263
39. Morand EF, Furie R, Tanaka Y, Bruce IN, Askanase AD, et al. 2020. Trial of anifrolumab in active systemic lupus erythematosus. *N. Engl. J. Med.* 382:211–21

40. Jenks SA, Cashman KS, Zumaquero E, Marigorta UM, Patel AV, et al. 2018. Distinct effector B cells induced by unregulated Toll-like receptor 7 contribute to pathogenic responses in systemic lupus erythematosus. *Immunity* 49:725–39.e6
41. Zumaquero E, Stone SL, Scharer CD, Jenks SA, Nellore A, et al. 2019. IFNγ induces epigenetic programming of human T-bet(hi) B cells and promotes TLR7/8 and IL-21 induced differentiation. *eLife* 8:e41641
42. Caielli S, Veiga DT, Balasubramanian P, Athale S, Domic B, et al. 2019. A $CD4^+$ T cell population expanded in lupus blood provides B cell help through interleukin-10 and succinate. *Nat. Med.* 25(1):75–81
43. Munroe ME, Lu R, Zhao YD, Fife DA, Robertson JM, et al. 2016. Altered type II interferon precedes autoantibody accrual and elevated type I interferon activity prior to systemic lupus erythematosus classification. *Ann. Rheum. Dis.* 75:2014–21
44. Oke V, Gunnarsson I, Dorschner J, Eketjall S, Zickert A, et al. 2019. High levels of circulating interferons type I, type II and type III associate with distinct clinical features of active systemic lupus erythematosus. *Arthritis Res. Ther.* 21:107
45. Boedigheimer MJ, Martin DA, Amoura Z, Sanchez-Guerrero J, Romero-Diaz J, et al. 2017. Safety, pharmacokinetics and pharmacodynamics of AMG 811, an anti-interferon-gamma monoclonal antibody, in SLE subjects without or with lupus nephritis. *Lupus Sci. Med.* 4:e000226
46. van Vollenhoven RF, Kalunian KC, Dörner T, Hahn BH, Tanaka Y, et al. 2022. Phase 3, multicentre, randomised, placebo-controlled study evaluating the efficacy and safety of ustekinumab in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* 81(11):1556–63
47. Broggi A, Granucci F, Zanoni I. 2020. Type III interferons: Balancing tissue tolerance and resistance to pathogen invasion. *J. Exp. Med.* 217:e20190295
48. Rumore PM, Steinman CR. 1990. Endogenous circulating DNA in systemic lupus erythematosus: occurrence as multimeric complexes bound to histone. *J. Clin. Investig.* 86:69–74
49. Galluzzi L, Yamazaki T, Kroemer G. 2018. Linking cellular stress responses to systemic homeostasis. *Nat. Rev. Mol. Cell Biol.* 19:731–45
50. Gullett JM, Tweedell RE, Kanneganti TD. 2022. It's all in the PAN: crosstalk, plasticity, redundancies, switches, and interconnectedness encompassed by PANoptosis underlying the totality of cell death-associated biological effects. *Cells* 11:1495
51. Chen Q, Wang J, Xiang M, Wang Y, Zhang Z, et al. 2022. The potential role of ferroptosis in systemic lupus erythematosus. *Front. Immunol.* 13:855622
52. Hakkim A, Furnrohr BG, Amann K, Laube B, Abed UA, et al. 2010. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *PNAS* 107:9813–18
53. Doran AC, Yurdagul A Jr., Tabas I. 2020. Efferocytosis in health and disease. *Nat. Rev. Immunol.* 20:254–67
54. Elmore S. 2007. Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35:495–516
55. Martin M, Blom AM. 2016. Complement in removal of the dead—balancing inflammation. *Immunol. Rev.* 274:218–32
56. Pisetsky D. 2017. The role of microparticles in the pathogenesis of SLE: a new look at an old paradigm. *Lupus Sci. Med.* 4:e000220
57. Ding SC, Lo YMD. 2022. Cell-Free DNA fragmentomics in liquid biopsy. *Diagnostics* 12(4):978
58. Napirei M, Ludwig S, Mezrhab J, Klockl T, Mannherz HG. 2009. Murine serum nucleases—contrasting effects of plasmin and heparin on the activities of DNase1 and DNase1-like 3 (DNase1l3). *FEBS J.* 276:1059–73
59. Napirei M, Wulf S, Eulitz D, Mannherz HG, Kloeckl T. 2005. Comparative characterization of rat deoxyribonuclease 1 (Dnase1) and murine deoxyribonuclease 1-like 3 (Dnase1l3). *Biochem. J.* 389:355–64
60. Wilber A, O'Connor TP, Lu ML, Karimi A, Schneider MC. 2003. Dnase1l3 deficiency in lupus-prone MRL and NZB/W F1 mice. *Clin. Exp. Immunol.* 134:46–52
61. Chan RWY, Serpas L, Ni M, Volpi S, Hiraki LT, et al. 2020. Plasma DNA profile associated with DNASE1L3 gene mutations: clinical observations, relationships to nuclease substrate preference, and in vivo correction. *Am. J. Hum. Genet.* 107:882–94

62. Serpas L, Chan RWY, Jiang P, Ni M, Sun K, et al. 2019. Dnase1l3 deletion causes aberrations in length and end-motif frequencies in plasma DNA. *PNAS* 116:641–49
63. Odaka C, Mizuochi T. 1999. Role of macrophage lysosomal enzymes in the degradation of nucleosomes of apoptotic cells. *J. Immunol.* 163:5346–52
64. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, et al. 2004. Neutrophil extracellular traps kill bacteria. *Science* 303:1532–35
65. Stetson DB, Ko JS, Heidmann T, Medzhitov R. 2008. Trex1 prevents cell-intrinsic initiation of autoimmunity. *Cell* 134:587–98
66. Ablasser A, Hemmerling I, Schmid-Burgk JL, Behrendt R, Roers A, Hornung V. 2014. TREX1 deficiency triggers cell-autonomous immunity in a cGAS-dependent manner. *J. Immunol.* 192:5993–97
67. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. 1992. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* 356:314–17
68. Magerus A, Bercher-Brayer C, Rieux-Laucat F. 2021. The genetic landscape of the FAS pathway deficiencies. *Biomed. J.* 44:388–99
69. Al-Mayouf SM, Sunker A, Abdwani R, Abrawi SA, Almurshedi F, et al. 2011. Loss-of-function variant in DNASE1L3 causes a familial form of systemic lupus erythematosus. *Nat. Genet.* 43:1186–88
70. Batu ED, Kosukcu C, Taskiran E, Sahin S, Akman S, et al. 2018. Whole exome sequencing in early-onset systemic lupus erythematosus. *J. Rheumatol.* 45:1671–79
71. Carbonella A, Mancano G, Gremese E, Alkuraya FS, Patel N, et al. 2017. An autosomal recessive DNASE1L3-related autoimmune disease with unusual clinical presentation mimicking systemic lupus erythematosus. *Lupus* 26:768–72
72. Ozcakar ZB, Foster J 2nd, Diaz-Horta O, Kasapcopur O, Fan YS, et al. 2013. DNASE1L3 mutations in hypocomplementemic urticarial vasculitis syndrome. *Arthritis Rheum.* 65:2183–89
73. Ueki M, Takeshita H, Fujihara J, Iida R, Yuasa I, et al. 2009. Caucasian-specific allele in non-synonymous single nucleotide polymorphisms of the gene encoding deoxyribonuclease I-like 3, potentially relevant to autoimmunity, produces an inactive enzyme. *Clin. Chim. Acta* 407:20–24
74. Acosta-Herrera M, Kerick M, Gonzalez-Serna D, Myositis Genet. Consort., Scleroderma Genet. Consort., et al. 2019. Genome-wide meta-analysis reveals shared new *loci* in systemic seropositive rheumatic diseases. *Ann. Rheum. Dis.* 78:311–19
75. Knight JS, Kaplan MJ. 2012. Lupus neutrophils: 'NET' gain in understanding lupus pathogenesis. *Curr. Opin. Rheumatol.* 24:441–50
76. Papayannopoulos V. 2018. Neutrophil extracellular traps in immunity and disease. *Nat. Rev. Immunol.* 18:134–47
77. Soni C, Reizis B. 2019. Self-DNA at the epicenter of SLE: immunogenic forms, regulation, and effects. *Front. Immunol.* 10:1601
78. Li P, Jiang M, Li K, Li H, Zhou Y, et al. 2021. Glutathione peroxidase 4-regulated neutrophil ferroptosis induces systemic autoimmunity. *Nat. Immunol.* 22:1107–17
79. Kawane K, Motani K, Nagata S. 2014. DNA degradation and its defects. *Cold Spring Harb. Perspect. Biol.* 6:a016394
80. Rodero MP, Tesser A, Bartok E, Rice GI, Della Mina E, et al. 2017. Type I interferon-mediated autoinflammation due to DNase II deficiency. *Nat. Commun.* 8:2176
81. Livingston JH, Crow YJ. 2016. Neurologic phenotypes associated with mutations in *TREX1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, *ADAR1*, and *IFIH1*: Aicardi–Goutières syndrome and beyond. *Neuropediatrics* 47:355–60
82. Rice GI, Rodero MP, Crow YJ. 2015. Human disease phenotypes associated with mutations in *TREX1*. *J. Clin. Immunol.* 35:235–43
83. Omarjee O, Picard C, Frachette C, Moreews M, Rieux-Laucat F, et al. 2019. Monogenic lupus: dissecting heterogeneity. *Autoimmun. Rev.* 18:102361
84. Riley JS, Tait SW. 2020. Mitochondrial DNA in inflammation and immunity. *EMBO Rep.* 21:e49799
85. Becker Y, Marcoux G, Allaeys I, Julien AS, Loignon RC, et al. 2019. Autoantibodies in systemic lupus erythematosus target mitochondrial RNA. *Front. Immunol.* 10:1026
86. Petri M. 2020. Antiphospholipid syndrome. *Transl. Res.* 225:70–81

87. Dieude M, Senecal JL, Raymond Y. 2004. Induction of endothelial cell apoptosis by heat-shock protein 60-reactive antibodies from anti-endothelial cell autoantibody-positive systemic lupus erythematosus patients. *Arthritis Rheum*. 50:3221–31
88. Becker YLC, Gagne JP, Julien AS, Levesque T, Allaeys I, et al. 2022. Identification of mitofusin 1 and complement component 1q subcomponent binding protein as mitochondrial targets in systemic lupus erythematosus. *Arthritis Rheum*. 74:1193–203
89. Pisetsky DS, Spencer DM, Mobarrez F, Fuzzi E, Gunnarsson I, Svenungsson E. 2020. The binding of SLE autoantibodies to mitochondria. *Clin. Immunol.* 212:108349
90. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, et al. 2008. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. *Nat. Med.* 14:949–53
91. Yousefi S, Mihalache C, Kozlowski E, Schmid I, Simon HU. 2009. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death Differ*. 16:1438–44
92. Lood C, Blanco LP, Purmalek MM, Carmona-Rivera C, De Ravin SS, et al. 2016. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nat. Med.* 22:146–53
93. Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, et al. 2011. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci. Transl. Med.* 3:73ra20
94. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, et al. 2003. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J. Exp. Med.* 197:711–23
95. Caielli S, Athale S, Domic B, Murat E, Chandra M, et al. 2016. Oxidized mitochondrial nucleoids released by neutrophils drive type I interferon production in human lupus. *J. Exp. Med.* 213:697–713
96. Jiao H, Jiang D, Hu X, Du W, Ji L, et al. 2021. Mitocytosis, a migrasome-mediated mitochondrial quality-control process. *Cell* 184:2896–910.e13
97. Boudreau LH, Duchez AC, Cloutier N, Soulet D, Martin N, et al. 2014. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. *Blood* 124:2173–83
98. Linge P, Fortin PR, Lood C, Bengtsson AA, Boilard E. 2018. The non-haemostatic role of platelets in systemic lupus erythematosus. *Nat. Rev. Rheumatol.* 14:195–213
99. West AP, Khoury-Hanold W, Staron M, Tal MC, Pineda CM, et al. 2015. Mitochondrial DNA stress primes the antiviral innate immune response. *Nature* 520:553–57
100. Wu Z, Oeck S, West AP, Mangalhara KC, Sainz AG, et al. 2019. Mitochondrial DNA stress signalling protects the nuclear genome. *Nat. Metab.* 1:1209–18
101. Kim J, Gupta R, Blanco LP, Yang S, Shteinfer-Kuzmine A, et al. 2019. VDAC oligomers form mitochondrial pores to release mtDNA fragments and promote lupus-like disease. *Science* 366:1531–36
102. Dhir A, Dhir S, Borowski LS, Jimenez L, Teitell M, et al. 2018. Mitochondrial double-stranded RNA triggers antiviral signalling in humans. *Nature* 560:238–42
103. Tigano M, Vargas DC, Tremblay-Belzile S, Fu Y, Sfeir A. 2021. Nuclear sensing of breaks in mitochondrial DNA enhances immune surveillance. *Nature* 591:477–81
104. Rai P, Janardhan KS, Meacham J, Madenspacher JH, Lin WC, et al. 2021. IRGM1 links mitochondrial quality control to autoimmunity. *Nat. Immunol.* 22:312–21
105. Buskiewicz IA, Montgomery T, Yasewicz EC, Huber SA, Murphy MP, et al. 2016. Reactive oxygen species induce virus-independent MAVS oligomerization in systemic lupus erythematosus. *Sci. Signal.* 9:ra115
106. Galluzzi L, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, et al. 2017. Molecular definitions of autophagy and related processes. *EMBO J.* 36:1811–36
107. Zhou XJ, Lu XL, Lv JC, Yang HZ, Qin LX, et al. 2011. Genetic association of PRDM1-ATG5 intergenic region and autophagy with systemic lupus erythematosus in a Chinese population. *Ann. Rheum. Dis.* 70:1330–37
108. Zhang YM, Cheng FJ, Zhou XJ, Qi YY, Zhao MH, Zhang H. 2015. Rare variants of ATG5 are likely to be associated with Chinese patients with systemic lupus erythematosus. *Medicine* 94:e939
109. Caza TN, Talaber G, Perl A. 2012. Metabolic regulation of organelle homeostasis in lupus T cells. *Clin. Immunol.* 144:200–13

110. Perl A. 2013. Oxidative stress in the pathology and treatment of systemic lupus erythematosus. *Nat. Rev. Rheumatol.* 9:674–86
111. Caza TN, Fernandez DR, Talaber G, Oaks Z, Haas M, et al. 2014. HRES-1/Rab4-mediated depletion of Drp1 impairs mitochondrial homeostasis and represents a target for treatment in SLE. *Ann. Rheum. Dis.* 73:1888–97
112. Chen PM, Katsuyama E, Satyam A, Li H, Rubio J, et al. 2022. CD38 reduces mitochondrial fitness and cytotoxic T cell response against viral infection in lupus patients by suppressing mitophagy. *Sci. Adv.* 8:eabo4271
113. Buang N, Tapeng L, Gray V, Sardini A, Whilding C, et al. 2021. Type I interferons affect the metabolic fitness of $CD8^+$ T cells from patients with systemic lupus erythematosus. *Nat. Commun.* 12:1980
114. Blanco P, Palucka AK, Gill M, Pascual V, Banchereau J. 2001. Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. *Science* 294:1540–43
115. Gkirtzimanaki K, Kabrani E, Nikoleri D, Polyzos A, Blanas A, et al. 2018. IFNα impairs autophagic degradation of mtDNA promoting autoreactivity of SLE monocytes in a STING-dependent fashion. *Cell Rep.* 25:921–33.e5
116. Morishita H, Eguchi T, Tsukamoto S, Sakamaki Y, Takahashi S, et al. 2021. Organelle degradation in the lens by PLAAT phospholipases. *Nature* 592:634–38
117. Caielli S, Cardenas J, de Jesus AA, Baisch J, Walters L, et al. 2021. Erythroid mitochondrial retention triggers myeloid-dependent type I interferon in human SLE. *Cell* 184:4464–79.e19
118. Zhang J, Ney PA. 2010. Reticulocyte mitophagy: monitoring mitochondrial clearance in a mammalian model. *Autophagy* 6:405–8
119. van den Akker E, Satchwell TJ, Pellegrin S, Daniels G, Toye AM. 2010. The majority of the in vitro erythroid expansion potential resides in $CD34^-$ cells, outweighing the contribution of $CD34^+$ cells and significantly increasing the erythroblast yield from peripheral blood samples. *Haematologica* 95:1594–98
120. Sugiura A, McLelland GL, Fon EA, McBride HM. 2014. A new pathway for mitochondrial quality control: mitochondrial-derived vesicles. *EMBO J.* 33:2142–56
121. Nolfi-Donegan D, Braganza A, Shiva S. 2020. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. *Redox. Biol.* 37:101674
122. Fritsch SD, Weichhart T. 2016. Effects of interferons and viruses on metabolism. *Front. Immunol.* 7:630
123. Wu D, Sanin DE, Everts B, Chen Q, Qiu J, et al. 2016. Type 1 interferons induce changes in core metabolism that are critical for immune function. *Immunity* 44:1325–36
124. van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, et al. 2012. Mitochondrial respiratory capacity is a critical regulator of $CD8^+$ T cell memory development. *Immunity* 36:68–78
125. Manderson AP, Botto M, Walport MJ. 2004. The role of complement in the development of systemic lupus erythematosus. *Annu. Rev. Immunol.* 22:431–56
126. Mills EL, Kelly B, Logan A, Costa ASH, Varma M, et al. 2016. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell* 167:457–70.e13
127. Weinberg SE, Singer BD, Steinert EM, Martinez CA, Mehta MM, et al. 2019. Mitochondrial complex III is essential for suppressive function of regulatory T cells. *Nature* 565:495–99
128. Mills E, O'Neill LA. 2014. Succinate: a metabolic signal in inflammation. *Trends Cell Biol.* 24:313–20
129. Chen PM, Wilson PC, Shyer JA, Veselits M, Steach HR, et al. 2020. Kidney tissue hypoxia dictates T cell-mediated injury in murine lupus nephritis. *Sci. Transl. Med.* 12:eaay1620
130. Vyshkina T, Sylvester A, Sadiq S, Bonilla E, Canter JA, et al. 2008. Association of common mitochondrial DNA variants with multiple sclerosis and systemic lupus erythematosus. *Clin. Immunol.* 129:31–35
131. Perry DJ, Yin Y, Telarico T, Baker HV, Dozmorov I, et al. 2012. Murine lupus susceptibility locus *Sle1c2* mediates $CD4^+$ T cell activation and maps to estrogen-related receptor γ. *J. Immunol.* 189:793–803
132. Peace CG, O'Neill LA. 2022. The role of itaconate in host defense and inflammation. *J. Clin. Investig.* 132(2):e148548
133. Bambouskova M, Gorvel L, Lampropoulou V, Sergushichev A, Loginicheva E, et al. 2018. Electrophilic properties of itaconate and derivatives regulate the IκBζ -ATF3 inflammatory axis. *Nature* 556:501–4
134. Mills EL, Ryan DG, Prag HA, Dikovskaya D, Menon D, et al. 2018. Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. *Nature* 556:113–17

135. Tang C, Wang X, Xie Y, Cai X, Yu N, et al. 2018. 4-Octyl itaconate activates Nrf2 signaling to inhibit pro-inflammatory cytokine production in peripheral blood mononuclear cells of systemic lupus erythematosus patients. *Cell Physiol. Biochem.* 51:979–90
136. Lee SY, Lee SH, Yang EJ, Kim EK, Kim JK, et al. 2015. Metformin ameliorates inflammatory bowel disease by suppression of the STAT3 signaling pathway and regulation of the between Th17/Treg balance. *PLOS ONE* 10:e0135858
137. Yin Y, Choi SC, Xu Z, Perry DJ, Seay H, et al. 2015. Normalization of $CD4^+$ T cell metabolism reverses lupus. *Sci. Transl. Med.* 7:274ra18
138. Titov AA, Baker HV, Brusko TM, Sobel ES, Morel L. 2019. Metformin inhibits the type 1 IFN response in human $CD4^+$ T cells. *J. Immunol.* 203:338–48
139. Wang H, Li T, Chen S, Gu Y, Ye S. 2015. Neutrophil extracellular trap mitochondrial DNA and its autoantibody in systemic lupus erythematosus and a proof-of-concept trial of metformin. *Arthritis Rheum.* 67:3190–200
140. Sun F, Geng S, Wang H, Wang H, Liu Z, et al. 2020. Effects of metformin on disease flares in patients with systemic lupus erythematosus: post hoc analyses from two randomised trials. *Lupus Sci. Med.* 7:e000429
141. Tian J, Avalos AM, Mao SY, Chen B, Senthil K, et al. 2007. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat. Immunol.* 8:487–96
142. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, et al. 2007. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* 449:564–69
143. Lande R, Palazzo R, Gestermann N, Jandus C, Falchi M, et al. 2020. Native/citrullinated LL37-specific T-cells help autoantibody production in Systemic Lupus Erythematosus. *Sci. Rep.* 10:5851
144. Kirou KA, Lee C, George S, Louca K, Peterson MG, Crow MK. 2005. Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. *Arthritis Rheum.* 52:1491–503
145. Hanaoka H, Iida H, Kiyokawa T, Takakuwa Y, Kawahata K. 2018. A positive direct Coombs' test in the absence of hemolytic anemia predicts high disease activity and poor renal response in systemic lupus erythematosus. *Lupus* 27:2274–78
146. Yeh TM, Chang HC, Liang CC, Wu JJ, Liu MF. 2003. Deoxyribonuclease-inhibitory antibodies in systemic lupus erythematosus. *J. Biomed. Sci.* 10:544–51
147. Emlen W, Ansari R, Burdick G. 1984. DNA-anti-DNA immune complexes. Antibody protection of a discrete DNA fragment from DNase digestion in vitro. *J. Clin. Investig.* 74:185–90
148. Hartl J, Serpas L, Wang Y, Rashidfarrokhi A, Perez OA, et al. 2021. Autoantibody-mediated impairment of DNASE1L3 activity in sporadic systemic lupus erythematosus. *J. Exp. Med.* 218:e20201138
149. Leffler J, Bengtsson AA, Blom AM. 2014. The complement system in systemic lupus erythematosus: an update. *Ann. Rheum. Dis.* 73:1601–6
150. Howe HS, Leung BPL. 2019. Anti-cytokine autoantibodies in systemic lupus erythematosus. *Cells* 9(1):72
151. Slavikova M, Schmeisser H, Kontsekova E, Mateicka F, Borecky L, Kontsek P. 2003. Incidence of autoantibodies against type I and type II interferons in a cohort of systemic lupus erythematosus patients in Slovakia. *J. Interferon Cytokine Res.* 23:143–47
152. Morimoto AM, Flesher DT, Yang J, Wolslegel K, Wang X, et al. 2011. Association of endogenous anti-interferon-alpha autoantibodies with decreased interferon-pathway and disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum.* 63:2407–15
153. Gupta S, Tatouli IP, Rosen LB, Hasni S, Alevizos I, et al. 2016. Distinct functions of autoantibodies against interferon in systemic lupus erythematosus: a comprehensive analysis of anticytokine autoantibodies in common rheumatic diseases. *Arthritis Rheum.* 68:1677–87
154. Tusseau M, Belot A. 2022. "P2RY8-son" break of tolerance promotes SLE. *J. Exp. Med.* 219:e20211972
155. Jiang SH, Stanley M, Vinuesa CG. 2020. Rare genetic variants in systemic autoimmunity. *Immunol. Cell Biol.* 98:490–99
156. Kuehn HS, Niemela JE, Rangel-Santos A, Zhang M, Pittaluga S, et al. 2013. Loss-of-function of the protein kinase C δ (PKCδ) causes a B-cell lymphoproliferative syndrome in humans. *Blood* 121:3117–25

157. Victora GD, Nussenzweig MC. 2022. Germinal centers. *Annu. Rev. Immunol.* 40:413–42
158. Winkler TH, Fehr H, Kalden JR. 1992. Analysis of immunoglobulin variable region genes from human IgG anti-DNA hybridomas. *Eur. J. Immunol.* 22:1719–28
159. van Es JH, Gmelig Meyling FH, van de Akker WR, Aanstoot H, Derksen RH, Logtenberg T. 1991. Somatic mutations in the variable regions of a human IgG anti-double-stranded DNA autoantibody suggest a role for antigen in the induction of systemic lupus erythematosus. *J. Exp. Med.* 173:461–70
160. McCarty GA, Rice JR, Bembe ML, Pisetsky DS. 1982. Independent expression of autoantibodies in systemic lupus erythematosus. *J. Rheumatol.* 9:691–95
161. McCune WJ, Golbus J, Zeldes W, Bohlke P, Dunne R, Fox DA. 1988. Clinical and immunologic effects of monthly administration of intravenous cyclophosphamide in severe systemic lupus erythematosus. *N. Engl. J. Med.* 318:1423–31
162. William J, Euler C, Christensen S, Shlomchik MJ. 2002. Evolution of autoantibody responses via somatic hypermutation outside of germinal centers. *Science* 297:2066–70
163. Herlands RA, William J, Hershberg U, Shlomchik MJ. 2007. Anti-chromatin antibodies drive in vivo antigen-specific activation and somatic hypermutation of rheumatoid factor B cells at extrafollicular sites. *Eur. J. Immunol.* 37:3339–51
164. Soni C, Perez OA, Voss WN, Pucella JN, Serpas L, et al. 2020. Plasmacytoid dendritic cells and type I interferon promote extrafollicular B cell responses to extracellular self-DNA. *Immunity* 52:1022–38.e7
165. Woodruff MC, Ramonell RP, Nguyen DC, Cashman KS, Saini AS, et al. 2020. Extrafollicular B cell responses correlate with neutralizing antibodies and morbidity in COVID-19. *Nat. Immunol.* 21:1506–16
166. Kaneko N, Kuo HH, Boucau J, Farmer JR, Allard-Chamard H, et al. 2020. Loss of Bcl-6-expressing T follicular helper cells and germinal centers in COVID-19. *Cell* 183:143–57.e13
167. Elsner RA, Shlomchik MJ. 2020. Germinal center and extrafollicular B cell responses in vaccination, immunity, and autoimmunity. *Immunity* 53:1136–50
168. Jenks SA, Cashman KS, Woodruff MC, Lee FE, Sanz I. 2019. Extrafollicular responses in humans and SLE. *Immunol. Rev.* 288:136–48
169. Tipton CM, Fucile CF, Darce J, Chida A, Ichikawa T, et al. 2015. Diversity, cellular origin and autoreactivity of antibody-secreting cell population expansions in acute systemic lupus erythematosus. *Nat. Immunol.* 16:755–65
170. Rubtsov AV, Rubtsova K, Fischer A, Meehan RT, Gillis JZ, et al. 2011. Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c$^{+}$ B-cell population is important for the development of autoimmunity. *Blood* 118:1305–15
171. Hao Y, O'Neill P, Naradikian MS, Scholz JL, Cancro MP. 2011. A B-cell subset uniquely responsive to innate stimuli accumulates in aged mice. *Blood* 118:1294–304
172. Rubtsova K, Rubtsov AV, Thurman JM, Mennona JM, Kappler JW, Marrack P. 2017. B cells expressing the transcription factor T-bet drive lupus-like autoimmunity. *J. Clin. Investig.* 127:1392–404
173. Liu Y, Zhou S, Qian J, Wang Y, Yu X, et al. 2017. T-bet$^{+}$CD11c$^{+}$ B cells are critical for antichromatin immunoglobulin G production in the development of lupus. *Arthritis Res. Ther.* 19:225
174. Wang S, Wang J, Kumar V, Karnell JL, Naiman B, et al. 2018. IL-21 drives expansion and plasma cell differentiation of autoreactive CD11c$^{hi}$T-bet$^{+}$ B cells in SLE. *Nat. Commun.* 9:1758
175. Portugal S, Tipton CM, Sohn H, Kone Y, Wang J, et al. 2015. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. *eLife* 4:e07218
176. Weiss GE, Crompton PD, Li S, Walsh LA, Moir S, et al. 2009. Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. *J. Immunol.* 183:2176–82
177. Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, et al. 2008. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J. Exp. Med.* 205:1797–805
178. Lau D, Lan LY, Andrews SF, Henry C, Rojas KT, et al. 2017. Low CD21 expression defines a population of recent germinal center graduates primed for plasma cell differentiation. *Sci. Immunol.* 2:eaai8153
179. Yang R, Avery DT, Jackson KJL, Ogishi M, Benhsaien I, et al. 2022. Human T-bet governs the generation of a distinct subset of CD11c$^{high}$CD21$^{low}$ B cells. *Sci. Immunol.* 7:eabq3277
180. Keller B, Strohmeier V, Harder I, Unger S, Payne KJ, et al. 2021. The expansion of human T-bet$^{high}$CD21$^{low}$ B cells is T cell dependent. *Sci. Immunol.* 6:eabh0891

181. Rincon-Arevalo H, Wiedemann A, Stefanski AL, Lettau M, Szelinski F, et al. 2021. Deep phenotyping of CD11c$^{+}$ B cells in systemic autoimmunity and controls. *Front. Immunol.* 12:635615
182. Dominguez CX, Amezquita RA, Guan T, Marshall HD, Joshi NS, et al. 2015. The transcription factors ZEB2 and T-bet cooperate to program cytotoxic T cell terminal differentiation in response to LCMV viral infection. *J. Exp. Med.* 212:2041–56
183. Xu H, Chaudhri VK, Wu Z, Biliouris K, Dienger-Stambaugh K, et al. 2015. Regulation of bifurcating B cell trajectories by mutual antagonism between transcription factors IRF4 and IRF8. *Nat. Immunol.* 16:1274–81
184. Luo W, Mayeux J, Gutierrez T, Russell L, Getahun A, et al. 2014. A balance between B cell receptor and inhibitory receptor signaling controls plasma cell differentiation by maintaining optimal Ets1 levels. *J. Immunol.* 193:909–20
185. Liu X, Zhao Y, Qi H. 2022. T-independent antigen induces humoral memory through germinal centers. *J. Exp. Med.* 219:e20210527
186. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. 2008. SAP-controlled T-B cell interactions underlie germinal centre formation. *Nature* 455:764–69
187. Manni M, Gupta S, Ricker E, Chinenov Y, Park SH, et al. 2018. Regulation of age-associated B cells by IRF5 in systemic autoimmunity. *Nat. Immunol.* 19:407–19
188. Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, et al. 2017. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* 542:110–14
189. Bocharnikov AV, Keegan J, Wacleche VS, Cao Y, Fonseka CY, et al. 2019. PD-1$^{hi}$CXCR5$^{-}$ T peripheral helper cells promote B cell responses in lupus via MAF and IL-21. *JCI Insight* 4:e130062
190. Rodda LB, Morawski PA, Pruner KB, Fahning ML, Howard CA, et al. 2022. Imprinted SARS-CoV-2-specific memory lymphocytes define hybrid immunity. *Cell* 185:1588–601.e14
191. Collora JA, Liu R, Pinto-Santini D, Ravindra N, Ganoza C, et al. 2022. Single-cell multiomics reveals persistence of HIV-1 in expanded cytotoxic T cell clones. *Immunity* 55:1013–31.e7
192. Facciotti F, Larghi P, Bosotti R, Vasco C, Gagliani N, et al. 2020. Evidence for a pathogenic role of extrafollicular, IL-10-producing CCR6$^{+}$B helper T cells in systemic lupus erythematosus. *PNAS* 117:7305–16
193. Gregori S, Goudy KS, Roncarolo MG. 2012. The cellular and molecular mechanisms of immunosuppression by human type 1 regulatory T cells. *Front. Immunol.* 3:30
194. Song Y, Wang N, Chen L, Fang L. 2021. Tr1 cells as a key regulator for maintaining immune homeostasis in transplantation. *Front. Immunol.* 12:671579
195. Gagliani N, Magnani CF, Huber S, Gianolini ME, Pala M, et al. 2013. Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. *Nat. Med.* 19:739–46
196. Bonnal RJP, Rossetti G, Lugli E, De Simone M, Gruarin P, et al. 2021. Clonally expanded EOMES$^{+}$ Tr1-like cells in primary and metastatic tumors are associated with disease progression. *Nat. Immunol.* 22:735–45
197. Cope A, Le Friec G, Cardone J, Kemper C. 2011. The Th1 life cycle: molecular control of IFN-gamma to IL-10 switching. *Trends Immunol.* 32:278–86
198. Stein K, Hummel M, Korbjuhn P, Foss HD, Anagnostopoulos I, et al. 1999. Monocytoid B cells are distinct from splenic marginal zone cells and commonly derive from unmutated naive B cells and less frequently from postgerminal center B cells by polyclonal transformation. *Blood* 94:2800–8
199. Ehrhardt GR, Hijikata A, Kitamura H, Ohara O, Wang JY, Cooper MD. 2008. Discriminating gene expression profiles of memory B cell subpopulations. *J. Exp. Med.* 205:1807–17
200. Karin N. 2020. CXCR3 ligands in cancer and autoimmunity, chemoattraction of effector T cells, and beyond. *Front. Immunol.* 11:976
201. Herlands RA, Christensen SR, Sweet RA, Hershberg U, Shlomchik MJ. 2008. T cell-independent and Toll-like receptor-dependent antigen-driven activation of autoreactive B cells. *Immunity* 29:249–60
202. Sweet RA, Ols ML, Cullen JL, Milam AV, Yagita H, Shlomchik MJ. 2011. Facultative role for T cells in extrafollicular Toll-like receptor-dependent autoreactive B-cell responses in vivo. *PNAS* 108:7932–37
203. Lu E, Wolfreys FD, Muppidi JR, Xu Y, Cyster JG. 2019. *S*-Geranylgeranyl-L-glutathione is a ligand for human B cell-confinement receptor P2RY8. *Nature* 567:244–48

204. Muppidi JR, Schmitz R, Green JA, Xiao W, Larsen AB, et al. 2014. Loss of signalling via Gα13 in germinal centre B-cell-derived lymphoma. *Nature* 516:254–58
205. Wu C, Macleod I, Su AI. 2013. BioGPS and MyGene.info: organizing online, gene-centric information. *Nucleic Acids Res.* 41:D561–65
206. Toyama H, Okada S, Hatano M, Takahashi Y, Takeda N, et al. 2002. Memory B cells without somatic hypermutation are generated from Bcl6-deficient B cells. *Immunity* 17:329–39