Review

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Role of the Lyn tyrosine kinase in the development of autoimmune disease

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The Lyn tyrosine kinase plays a key regulatory role in the immune system that was first highlighted by the phenotype of Lyn$^{-/-}$ mice. These animals develop an autoimmune disease similar to the autoimmune disorder systemic lupus erythematosus. Deregulation of the Lyn pathway is also observed in lupus patient samples, validating Lyn$^{-/-}$ mice as a model of lupus, as well as providing an archetype for the testing of suitable therapeutic agents. Here, we present an overview of the role of Lyn in immune cells and autoimmunity, emphasizing the pathogenic mechanisms contributing to autoimmune disease in Lyn$^{-/-}$ mice and the deregulation of Lyn-dependent pathways in patients with lupus, and provide a perspective on the therapeutic targeting of Lyn-regulated pathways in this disease.

Keywords: autoimmune disease • glomerulonephritis • inflammation • lupus • Lyn

Lyn tyrosine kinase: from historical perspective to role in immune cells

Lyn is one of nine members of the Src family of protein tyrosine kinases (SFKs). These enzymes are commonly associated with cell surface receptors that lack intrinsic kinase activity [1]. Lyn was first described in 1987 as an Lck/Yes-related novel tyrosine kinase and hence its name was born [2]. Subsequent phylogenetic studies showed that Lyn is evolutionarily most related to the hematopoietic SFK Hck [3]. The *Lyn* gene maps to human chromosome 8q12.1 [2] and 4qA1 in mice where a pseudo gene is also found [4]. Two Lyn proteins of 53 and 56 kDa are expressed and arise from alternate splicing of exon 2 [5,6]. The two isoforms of Lyn are found in mice, rats and humans and differ by only 21 amino acids in the enzyme's N-terminal unique domain [7]. Both isoforms are co-expressed, and only recently have studies began to unravel differences in their function [8]. The nucleotide and amino acid sequences of the mouse, rat and human Lyn genes have been reported and are highly conserved [7]. Although Lyn is expressed widely in the immune system, it is not expressed in T cells [9], except under very specific and artificial circumstances [10–12]. SFKs are myristoylated, which localizes them to the plasma membrane, in close proximity to receptors and other signaling complexes [13]. In immune cells, SFK members including Lyn, are commonly associated with immunoreceptors and constitute a critical part of the signaling mechanism [14]. Upon receptor cross-linking, SFKs become activated and phosphorylate tyrosine residues on the receptor complex to recruit key signaling proteins, triggering a cascade of signaling events that lead to a physiological response (e.g., activation or proliferation). Significant homology exists between the SFKs and they are often co-expressed in the same cell. As such, functional redundancy is possible, and indeed mouse knockout studies have shown that loss of one SFK may in some cases be compensated for by another [15]. Early studies on Lyn focused on its properties relative to other members of the Src family who were known to initiate signaling cascades downstream of co-associated immunoreceptors [16] that induced cell proliferation and activation [17]. It was not until the generation of the

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Lyn$^{-/-}$ mouse in the mid-1990s that a unique inhibitory role for Lyn was discovered, challenging the belief that Lyn was primarily an activatory and pro-oncogenic Src kinase. Contrary to original expectations, Lyn$^{-/-}$ mice exhibited a progressive autoimmune disease reminiscent of systemic lupus erythematosus (SLE; lupus) [18,19], and Lyn was subsequently found to be a signaling effector molecule in both activatory and inhibitory pathways (reviewed in [20]) (Figure 1).

The kinase activity of SFKs is tightly controlled through regulated phosphorylation of two key tyrosine residues: in the case of Lyn, Tyr508 in the C-terminal 'regulatory' domain and the autophosphorylation site in the kinase domain (Tyr397). At steady-state, the negative regulatory Tyr508 is phosphorylated by the tyrosine kinase c-Src kinase (Csk; [21]) and interacts with Lyn's own Src homology 2 (SH2) domain, restraining the enzyme in a 'closed', inactive conformation (Figure 2A). Activation of Lyn occurs via dephosphorylation of Tyr508, as well as autophosphorylation of Tyr397. This allows Lyn to adopt an 'open' conformation, exposing SH2 and SH3 domains that can interact with their binding partners, while the active protein tyrosine kinase (PTK) domain is able to phosphorylate its substrate (Figure 2B). Readers are referred to the comprehensive review of Brown and Cooper on the structure, regulation and substrates of SFKs [22]. In addition to CD45 phosphatase, which can dephosphorylate both the activatory and inhibitory tyrosine residues on Lyn [23,24], the SHP-1 phosphatase is implicated in negative regulation of Lyn activity via dephosphorylation of the Lyn autophosphorylation site [25]. Mutating the C-terminal regulatory Tyr508 by replacing it with a phenylalanine residue permanently locks the enzyme into an active state (Figure 2C). In this constitutively active state Lyn-regulated proteins become hyper-Tyr phosphorylated [26,27]. The crystal structure of the kinase domain of Lyn has been reported and closely resembles that of Src, Lck and Hck [28].

In B cells, Lyn is associated with the B-cell antigen receptor (BCR) complex [29,30], and is rapidly activated upon BCR cross-linking [31]. Upon activation, Lyn is able to phosphorylate tyrosine residues on immunoreceptor tyrosine-based activation motifs (ITAMs) on

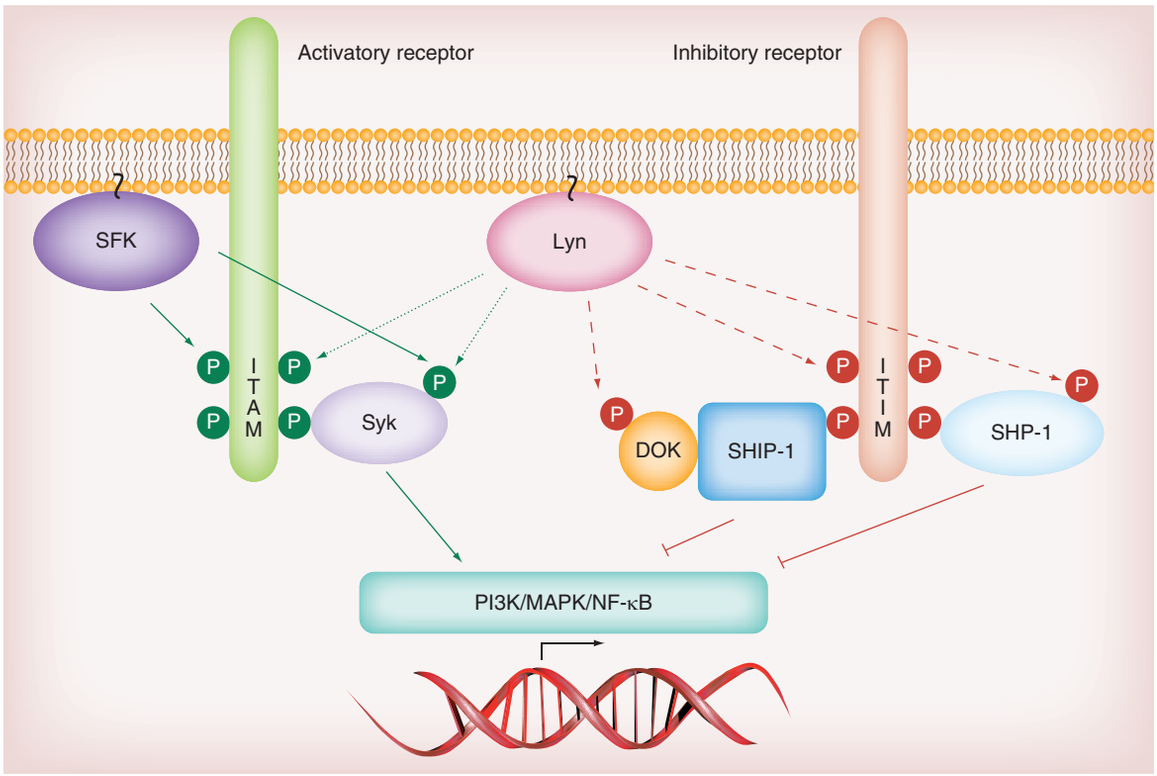

Figure 1. Schematic representation of Lyn and the Src family of protein tyrosine kinases in immune cell signaling.
The SFKs play an essential role in initiating activatory signaling from ITAM-containing immunoreceptors such as the BCR. While Lyn contributes to positive signaling, it also plays an essential nonredundant role in inhibitory signaling from ITIM-bearing inhibitory receptors. Solid lines signify positive signaling pathways, while dotted green lines indicate activatory signaling pathways that can also be regulated by Lyn. Dashed red lines specify inhibitory pathways that are regulated exclusively by Lyn.
BCR: B-cell antigen receptor; ITAM: Immunoreceptor tyrosine-based activation motif; ITIM: Immunoreceptor tyrosine-based inhibition motif; SFK: Src family of protein tyrosine kinases.

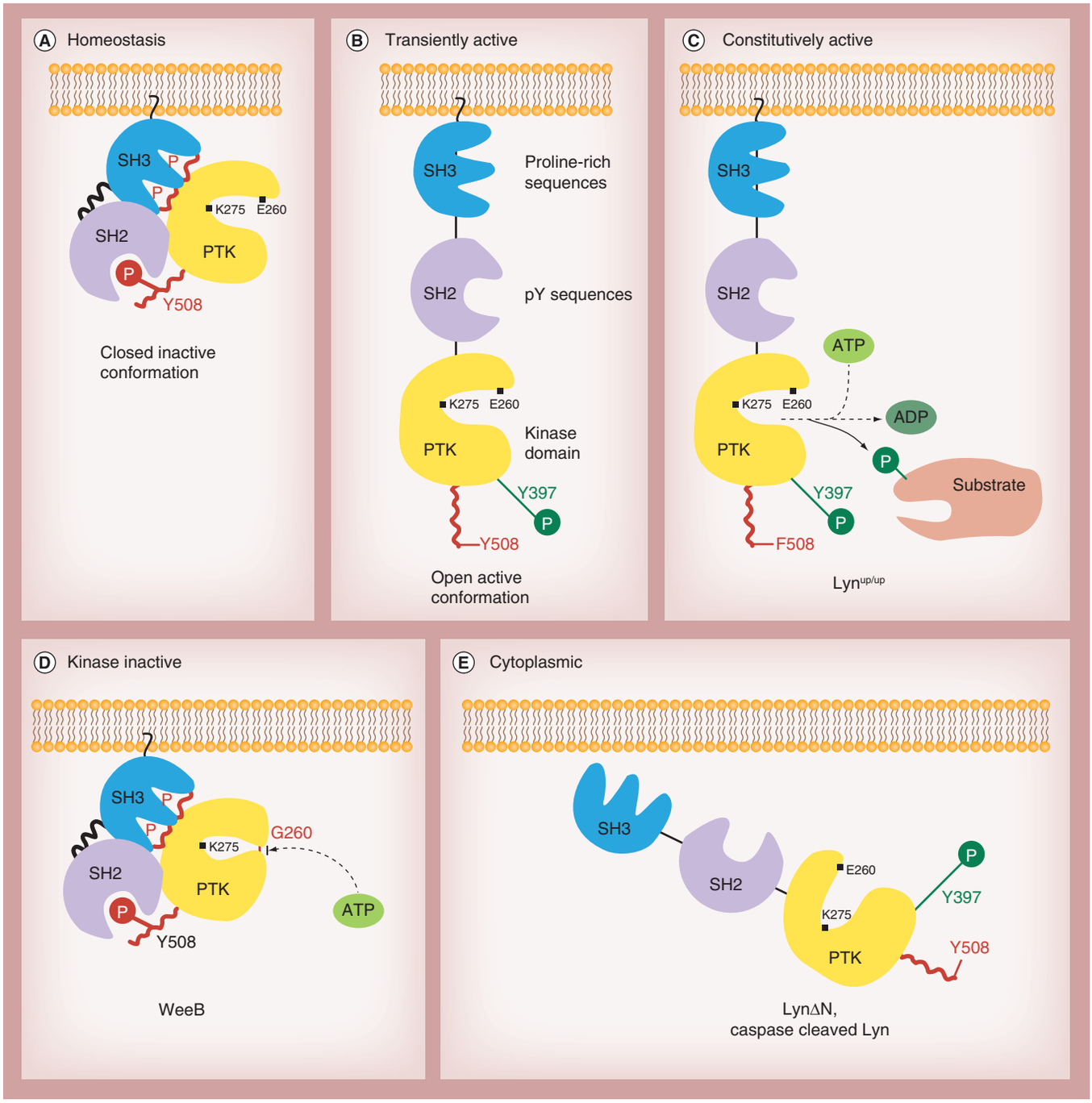

Figure 2. Structure and conformation of the Src family of protein tyrosine kinases. Representative structure of the Src family of protein tyrosine kinases (SFKs) at **(A)** steady state; **(B)** when transiently active; **(C)** when rendered constitutively active through mutation of the C-terminal regulatory tyrosine as occurs in Lyn$^{up/up}$ mice; **(D)** when rendered kinase-inactive such as in WeeB- or Mld4-mutant mice; and **(E)** when cytoplasmic as occurs in LynΔN mice. Key domains and amino acid residues indicated are: Src homology 2 (SH2) domain, SH3 domain and protein tyrosine kinase (PTK) domain; K275: ATP-binding site; Y397: autophosphorylation site; Y508: negative regulatory tyrosine; E260: site mutated in WeeB mice that renders Lyn kinase inactive.

Ig-α and -β subunits of the BCR, which serve as sites to recruit additional effector molecules via SH2 domain binding such as Syk (Figure 1) [32,33]. Once other effectors are recruited to the plasma membrane, positive signaling cascades are initiated that result in increased calcium flux, leading to survival and differentiation pathways. The phosphorylation of ITAMs can also be performed by other SFKs, such as Fyn, leading to redundancy among SFKs in activatory signaling (Figure 1) [29,34–35]. However, the initiation of inhibi-

tory signaling cascades in B cells is a feature uniquely characteristic of Lyn. Lyn is able to phosphorylate tyrosine residues in immunoreceptor tyrosine-based inhibition motifs (ITIMs) in inhibitory receptors such as FcγRIIb1, PIR-B and CD22 (Figure 1) [36–41]. Phosphorylated ITIMs serve as docking sites for SH2 domain-containing inhibitory phosphatases such as the protein tyrosine phosphatase SHP-1 and the lipid phosphatase SHIP-1, and once recruited, they can be activated by phosphorylation and serve to switch off signaling through dephosphorylation of enzymes, adaptor proteins and phospholipids [42–46]. Due to the dispensable role of Lyn in propagating signals from the BCR, yet nonredundant role in initiating and sustaining inhibitory signaling pathways, $Lyn^{-/-}$ B cells manifest a net loss in inhibitory signaling and thus exhibit a hyperactive phenotype [36–38,40–41].

In addition to its roles in BCR signaling, Lyn also participates in signaling cascades in other immune cell types, including dendritic cells (DCs) [9,47], mast cells [9,48–49] and macrophages [15,26,50], and it is also involved in myelopoiesis and erythropoiesis pathways [44]. As well as its role in signaling from antigen receptors, Lyn also participates in signaling from Fc receptors, growth factor and chemokine receptors, and integrins.

$Lyn^{-/-}$ mice as a model for SLE

SLE is a chronic, relapsing, remitting systemic autoimmune disease that predominantly affects women of child-bearing age. There are many parallels between human SLE and the SLE-like syndrome developed by $Lyn^{-/-}$ mice, the most notable of which is production of autoantibodies towards a wide spectrum of nuclear and cytoplasmic components, which deposit in tissues and initiate systemic tissue pathology. SLE is influenced by genetic and environmental factors, a feature that is also recapitulated in the $Lyn^{-/-}$ mouse. Three independent mouse models in which the *Lyn* gene has been disrupted have been developed, each demonstrating similar B-cell dysfunction and age-dependent development of autoimmune-mediated glomerulonephritis [18–19,37] comparable with human lupus nephritis. Young $Lyn^{-/-}$ mice typically exhibit a B-cell lymphopenia, an almost complete loss of marginal zone B cells and concurrent plasmacytosis, and have high serum titers of IgM, IgA and IgE [18–19,37,51–54]. Their B cells have an activated phenotype manifested as BCR downregulation and enhanced MHC class II, CD80 and CD86 expression [36,51], and signaling studies show that their B cells are hyperactive and exhibit a failure to engage inhibitory signaling pathways [36–38,40–41]. These are features that are shared with SLE patients. This B-cell dysfunction coupled with loss of B-cell tolerance leads to the production of pathogenic IgG and IgA autoantibodies targeting nuclear antigens [51,53]. Autoreactive IgG and IgA can be observed in mice as young as 8 weeks of age and progressively accumulates with age. As described in Figure 3, a key feature of older mice is the expansion of the myeloid compartment, which gives rise to splenomegaly, inflammatory cytokine imbalances, and T-cell hyperactivation, despite the fact that Lyn is not ordinarily expressed in T cells. It is these characteristics that transition the disease into a pathogenic state, leading to the production of pathogenic autoantibodies, the formation of immune complexes and their deposition in the microvessels of the glomeruli (Figure 3). This results in the fixation of complement, which catalyzes the influx of $CD45^{+}$ immune cells resulting in inflammation and glomerular damage [55]. Systemic inflammation is a key feature of disease mediated by proinflammatory cytokine production by B cells, T cells and myeloid cells (Figure 3). Deregulated IL-6 and IFN-γ production are crucial for pathogenic autoantibody production and glomerular disease, which will be discussed further in subsequent sections of this article.

Research into SLE has historically tended to focus on B cells as the main offenders, but it is becoming clear that other immune cells play an important role in promoting disease pathology. Indeed, clinical trials in SLE of numerous B-cell-specific therapies have not been as successful as anticipated [56]. Interestingly, belimumab, a monoclonal antibody targeting the B-cell survival factor BAFF is the only novel therapy approved for the treatment of SLE in the last 50 years [57]. However, the benefits obtained with belimumab are modest and only attained in patients with mild disease who are already receiving standard therapy [58]. The lack of novel therapies as well as the limited clinical use of belimumab necessitates that there be more effort towards developing new therapeutic agents for SLE. Since they were originally described, $Lyn^{-/-}$ mice have become well characterized through the study of signaling molecules, cytokines and various cell types in disease pathology as outlined below. It is now clear that these mice can be utilized as an important preclinical animal model for the trialing of therapeutics that display clinical potential, providing hope of fulfilling an unmet clinical need.

The role of Lyn in human SLE

B-cell dysfunction has long been recognized in SLE [59]; however, studies examining signaling pathway changes in human SLE have been limited. About the same time that $Lyn^{-/-}$ mice were first described, a key study reported abnormal signaling events in B cells from lupus patients [60]. The authors showed that SLE B cells exhibited augmented calcium responses and

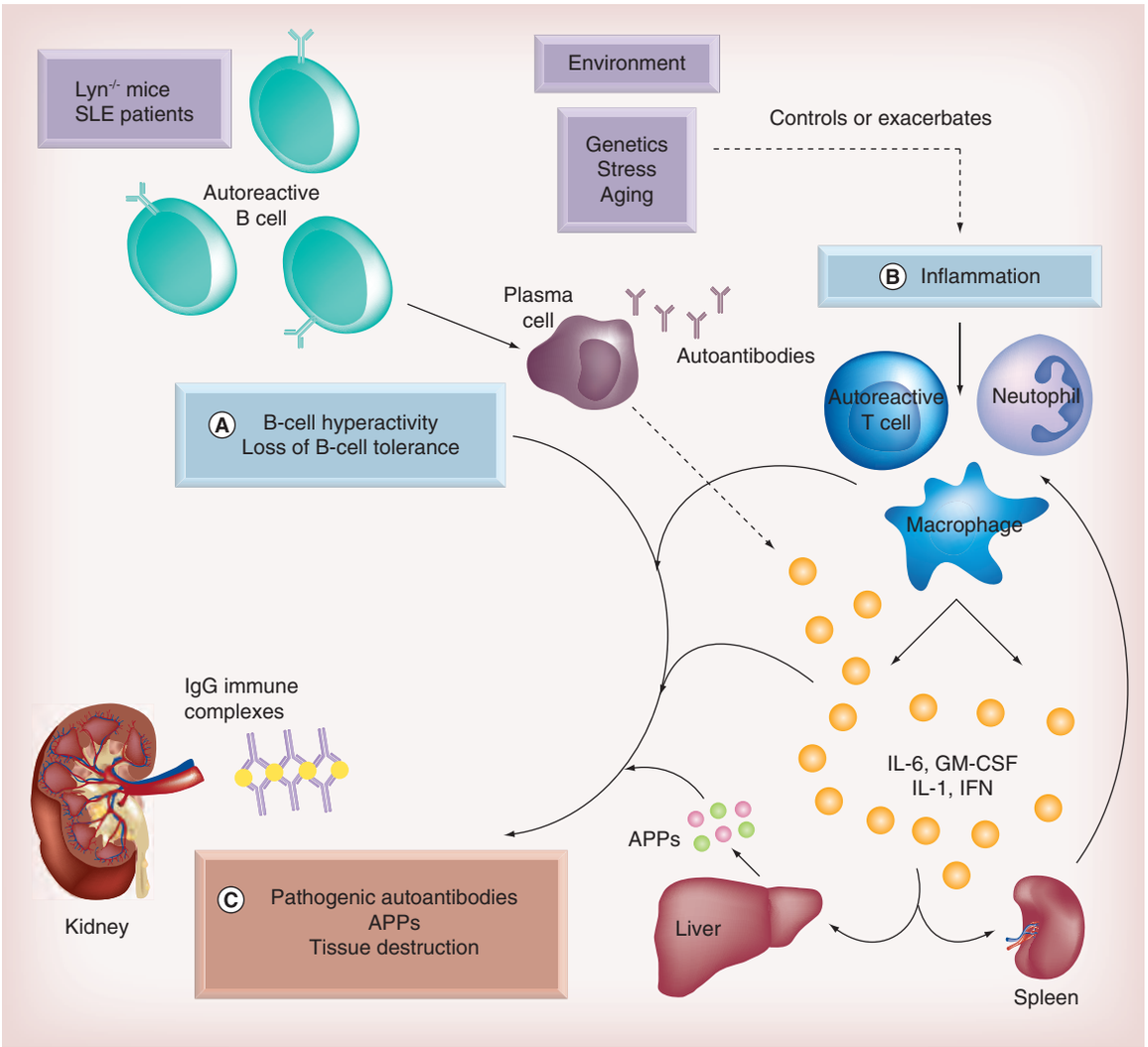

Figure 3. Inflammation plays a major role in lupus pathogenesis. (A) Genetically prone mice and lupus patients have hyperactive B cells and exhibit a loss of B-cell tolerance. They show plasma cell expansion and produce autoantibodies, which are not pathogenic unless generated in an inflammatory milieu. **(B)** Innate immune cells and plasma cells enhance inflammation. **(C)** Proinflammatory cytokines can drive T-cell activation leading to B cell class-switching and the production of pathogenic autoantibodies. Inflammation leads to expansion of innate immune cells and stimulates the production of APPs. Ensuing tissue damage is worsened by environmental factors and genetic make-up.
APP: Acute phase protein; SLE: Systemic lupus erythematosus.

enhanced phosphorylation after BCR cross-linking, congruent with B-cell hyperactivity [60], and potentially consistent with altered Lyn signaling pathway activity. Studies directly addressing the role of Lyn in human SLE have not been extensive; however, two independent studies have reported that intracellular expression of Lyn protein was significantly reduced in a majority of SLE patients [61,62]. While one study found that levels of Lyn mRNA were reduced [61], it was not speculated as to why expression of Lyn at the gene level was altered. The other study found that expression of the regulatory phosphatase CD45 was abnormal in SLE, and this extended to the expression of the inhibitory phosphatase SHP-1 as well [62]. Additional studies of the role of Lyn in SLE B cells sought to determine if not only the amount of Lyn, but also the intracellular localization and metabolism of Lyn could be involved in B-cell dysregulation in SLE patients. These studies confirmed that Lyn protein levels were reduced in the majority of SLE patients and, furthermore, showed that translocation and localization of Lyn to lipid raft signaling domains was altered [63]. In addition, altered Lyn expression was associated with hyperproliferative responses and production of anti-dsDNA autoantibodies. The authors also showed that there were increases in ubiquitination of Lyn, and suggested that this could

be a mechanism responsible for reduced levels of Lyn protein. The altered regulation of Lyn was also suggested to be due to different expression and translocation of regulatory molecules, and it was confirmed that SLE B cells displayed low expression of the CD45 phosphatase, which correlated with low Lyn expression in lipid rafts [64]. Interestingly, it has recently been reported that Lyn expression can be induced in human patient B cells by anti-TNF-α treatment and this was correlated with increased Lyn activity [65]. This is an important finding since biologics that target TNF-α can induce SLE-like autoimmunity and it may represent a corollary to studies in mice that show how increasing Lyn activity may lead to autoimmunity [27]. With the advent of genome-wide scans, polymorphisms in *Lyn* have been found to be associated with lupus [66], and a subsequent targeted scan also found *Lyn* was associated with SLE in a European population [67]. However, this study failed to show associations in African or Korean populations. In addition, other research failed to find any association in a northern European population [68]. Clearly, many more studies are required in order to fully elucidate the genetic association of Lyn with lupus. Nonetheless, correlative studies showed that polymorphisms in Csk, a regulator of Lyn activity are associated with SLE at an odds ratio of 1.32 [69]. This particular polymorphism, which was associated with increased Csk expression, resulted in enhanced inhibitory phosphorylation of Lyn. Due to their reduced Lyn activity, B cells from these individuals were hyperactivated compared with those without the haplotype as indicated by heightened BCR activation and IgM levels. In addition, polymorphisms in PTPN22, which encodes a protein tyrosine phosphatase that regulates SFK activity in T cells, B cells and myeloid cells, have been linked to human SLE [70,71]. Since PTPN22 may potentially regulate Lyn activity in B cells and myeloid cells, there may be a link between PTPN22 polymorphisms and Lyn activity in human SLE, although this is yet to be confirmed.

Further indirect evidence to support Lyn's role as a regulator of autoimmunity comes from studies of the Lyn-regulated protein FcγRIIB1. When this protein is mutated in mice, this predisposes the animals to autoimmune disease [36,72–73] and in humans, polymorphisms in FcγRIIB1 have been identified as heritable risk factors for SLE [74,75]. Although no specific genetic mutations have yet been identified in the human *Lyn* gene, it is likely that a subset of individuals will possess multiple rather than single genetic changes in Lyn-regulated pathways, for which there is already precedence in mice [36,76]. Together these cell-based and genetic studies of human SLE patients provide evidence that Lyn is putatively involved in SLE pathogenesis, and provides merit to the study of mice with alterations in Lyn as models for human SLE.

Using genetic models to elucidate the role of Lyn in SLE

The complex nature and presentation of SLE make it difficult to study many aspects of the human disease, rendering animal models of SLE an excellent way to explore underlying disease mechanisms and to provide preclinical evaluation of potential therapeutics. The $Lyn^{-/-}$ mouse has been extensively studied and manipulated since it was first described in 1995 and many important findings have been made. These studies are discussed in detail below and summarized in Table 1.

Requirement for B cells & BCR signaling in disease

The absolute requirement for B cells in autoimmune disease development in $Lyn^{-/-}$ mice has been demonstrated by the simultaneous disruption of the μ-chain gene, which leads to an early block in B-cell development. As a consequence, $Lyn^{-/-}\mu MT^{-/-}$ mice lack B cells and not surprisingly, fail to develop autoimmune disease, and as such they exhibit no inflammatory defects such as myeloid expansion, splenomegaly and T-cell activation; however, they maintain intrinsic defects in the DC compartment [51].

The simultaneous deletion of signaling molecules critical for B-cell activation also highlights the importance of BCR signaling for disease development in $Lyn^{-/-}$ mice. B-cell hyper-responsiveness, autoantibodies and glomerulonephritis were ameliorated in $Lyn^{-/-}$ mice deficient in the B-cell-specific co-receptor CD19 [77]. $Lyn^{-/-}$ mice rendered deficient for Btk, an enzyme that promotes BCR signaling and that acts downstream of Lyn, showed dramatic reductions in B-cell hyper-responsiveness and splenomegaly, and loss of autoimmunity [78,79]. $Lyn^{-/-}$ mice have also been crossed with mice expressing a transgene that expresses Btk at 25% of normal levels (Btk^{lo}). $Lyn^{-/-}Btk^{lo}$ mice displayed B-cell hyper-responsiveness; however, they failed to produce autoantibodies and did not exhibit splenomegaly characteristic of $Lyn^{-/-}$ mice, therefore demonstrating that B-cell hyper-responsiveness can be uncoupled from autoimmunity [80].

Genetics & genetic interactions in disease

In humans, genetic make-up has a major influence on autoimmune disease predisposition. It is also well known that genetic background in mice markedly influences their autoimmune susceptibility; for example, congenic regions responsible for strain differences that influence lupus susceptibility have been described [97]. The original strains of $Lyn^{-/-}$ mice were

derived on a mixed 129/Ola × C57BL/6 genetic background [18,19], and they developed severe autoimmune disease with rapid onset. Subsequent to these initial experiments, the mice have been backcrossed to both BALB/c and C57BL/6 fixed genetic backgrounds, with most recent experiments being conducted on C57BL/6 background mice. On the C57BL/6 background, $Lyn^{-/-}$ mice develop early onset autoantibodies and significant glomerular disease, but disease appears moderated compared with mixed 129/Ola × C57BL/6 background mice [51]. On the BALB/c genetic background, autoantibody development is somewhat delayed compared with C57BL/6 background mice and BALB/c $Lyn^{-/-}$ mice develop only mild glomerular disease, but interestingly, display enhanced lymphadenopathy and splenomegaly [54].

Table 1. Summary of genetic manipulations of the $Lyn^{-/-}$ mouse model.

Strain	Genetic mutation	Effect on disease	Ref.
B cells and B-cell signaling			
$Lyn^{-/-}\mu MT^{-/-}$	Lacking Lyn and B cells	Loss of T-cell hyperactivation and myeloid expansion, persistence of $Lyn^{-/-}$ DC phenotype. Ablation of autoimmune disease	[51]
$Lyn^{-/-}CD19^{-/-}$	Lacking Lyn and CD19	Loss of B-cell hyperactivation, autoantibody production and amelioration of glomerular disease	[77]
$Lyn^{-/-}Btk^{-/-}$	Lacking Lyn and Btk	Loss of B-cell hyperactivation, autoantibody production and amelioration of glomerular disease	[78,79]
$Lyn^{-/-}Btk^{lo}$	Lacking Lyn and expression of Btk reduced to 25%	Uncoupling of autoimmune disease (absent) from B-cell hyperactivity (persistent)	[80]
Genetic background and gene interaction			
129Ola x C57BL/6 $Lyn^{-/-}$	Lacking Lyn	Severe glomerular disease, possibly enhanced by epistatic modifiers of SLE on 129/Ola background	[18]
C57BL/6 $Lyn^{-/-}$	Lacking Lyn	Moderate-to-severe glomerular disease	[51]
BALB/c $Lyn^{-/-}$	Lacking Lyn	Mild glomerular disease	[54]
$Lyn^{+/-}$	Haploinsufficiency of Lyn	Delayed, mild glomerular disease	[76,81]
$Lyn^{+/-}Me^{v}/+$	Haploinsufficiency of Lyn and SHP-1	Amplification of $Lyn^{+/-}$ phenotype, myeloid compartment defects and glomerular disease	[76]
$Lyn^{+/-}SHIP\text{-}1^{+/-}$	Haploinsufficiency of Lyn and SHIP-1	Amplification of $Lyn^{+/-}$ pathogenic autoantibody production. Mild glomerular disease	[76]
Lyn-specific mutations			
$Lyn^{up/up}$	Constitutively active Lyn-Y508F	Pathogenic autoreactive antibodies and severe glomerular disease	[26]
LynMld4	Kinase-dead Lyn-T410K	Intermediate $Lyn^{-/-}$ phenotype, but no development of kidney disease	[82]
LynWeeB	Kinase-dead Lyn-E260G	Intermediate $Lyn^{-/-}$ phenotype, with late-onset glomerular disease	[83]
LynΔN	Cytosolic Lyn	TNF-α-dependent psoriasis-like skin inflammatory syndrome	[84]
Th2 environment			
$Lyn^{-/-}IL\text{-}4^{-/-}$	Lacking Lyn and IL-4	Failed to develop glomerulonephritis; kidney function rescued	[85]
$Lyn^{-/-}Igh\text{-}7^{-/-}$	Lacking Lyn and IgE	Failed to develop glomerulonephritis; kidney function rescued	[85]
$Lyn^{-/-}STAT6^{-/-}$	Lacking Lyn and STAT6	Exacerbated autoimmune traits and severe glomerular disease; uncoupling of STAT6 from expression of Th2 traits	[52]

DC: Dendritic cell; IC: Immune complex; SLE: Systemic lupus erythematosus.

Table 1. Summary of genetic manipulations of the Lyn$^{-/-}$ mouse model (cont.).

Strain	Genetic mutation	Effect on disease	Ref.
T-cell help			
Lyn$^{-/-}$CTLA4Ig	Lacking Lyn and overexpression of secreted CTLA4	Loss of IgG autoantibodies but presence of IgA autoantibodies sufficient to mediate glomerular disease	[53]
Lyn$^{-/-}$p110$\delta^{+/KD}$	Lacking Lyn and haploinsufficiency of PI3K p110δ	Moderation of T-cell signaling and activation, myeloid-derived inflammation and glomerular disease	[86]
Lyn$^{-/-}$IL-21$^{-/-}$	Lacking Lyn and IL-21	Loss of class-switched anti-DNA and histone autoantibodies, persistence of other pathogenic autoantibodies and kidney disease	[87]
Lyn$^{-/-}$TCR$\beta^{-/-}$ TCR$\delta^{-/-}$	Lacking Lyn and T cells	Greatly diminished levels of autoantibodies, however, disease not assessed	[88]
Lyn$^{-/-}$SAP$^{-/-}$	Lacking Lyn and SAP adaptor	Greatly diminished levels of autoantibodies, however, disease not assessed	[88]
Interactions between SFKs			
Lyn$^{-/-}$Fyn$^{-/-}$	Lacking Lyn and Fyn	Severe glomerular disease thought to be due to a kidney intrinsic mechanism	[89]
Lyn$^{-/-}$Fyn$^{-/-}$Blk$^{-/-}$	Lacking Lyn, Fyn and Blk	Immunodeficient; early block in B-cell development	[90]
HFL$^{-/-}$	Lacking Hck, Fgr and Lyn	Reduced inflammation and diminished glomerular disease	[91]
Inflammation			
Lyn$^{-/-}$IL-5R$\alpha^{-/-}$	Lacking Lyn and IL5Rα	Reduction in autoantibody production and very mild glomerular disease	[92]
Lyn$^{-/-}$IL-6$^{-/-}$	Lacking Lyn and IL-6	Lack of T-cell and myeloid hyperactivation, abrogation of glomerular disease. Dissociation of B-cell hyperactivity and disease	[51,81]
Lyn$^{-/-}$IFN-$\gamma^{-/-}$	Lacking Lyn and IFN-γ	Reduced production of BAFF, myeloid proliferation and T-cell hyperactivation resulting in moderated glomerular disease	[91]
Lyn$^{-/-}$sgp130Tg	Lacking Lyn and overexpression of soluble gp130	Neutralization of IL-6 transsignaling had minimal effects on B- and T-cell activation, autoantibody production and IC deposition but resulted in reduced myeloid inflammation, complement deposition and glomerular disease	[55]
Lyn$^{-/-}$IL-10$^{-/-}$	Lacking Lyn and IL-10	Exacerbation of Lyn$^{-/-}$ phenotype; marked splenomegaly and lymphadenopathy, increased proinflammatory cytokines and severe tissue inflammation	[93]
Lyn$^{-/-}$MyD88$^{-/-}$	Lacking Lyn and MyD88	Attenuation of autoantibody production and protection from glomerulonephritis	[94]
CD11c^{cre}Lyn$^{flox/flox}$	DC-specific Lyn deletion	Exacerbation of Lyn$^{-/-}$ phenotype, severe glomerulonephritis	[95]
CD11c^{cre}Lyn$^{flox/flox}$MyD88$^{flox/flox}$	DC-specific Lyn and MyD88 deletion	Abrogation of autoimmunity	[95]
CD79a^{cre}Lyn$^{flox/flox}$	B-cell-specific Lyn deletion	Similar phenotype to Lyn$^{-/-}$	[96]
CD79a^{cre}Lyn$^{flox/flox}$MyD88$^{flox/flox}$	B-cell-specific Lyn and MyD88 deletion	Abrogation of autoimmunity	[96]

DC: Dendritic cell; IC: Immune complex; SLE: Systemic lupus erythematosus.

As well as the effects of genetic background, variation in gene dosage leads to alteration in disease expression on the Lyn-mutant background. Mice carrying only one functional copy of the *Lyn* gene are susceptible to SLE-like disease development, albeit in a delayed and milder form [76,81]. The combination of Lyn heterozygosity with heterozygous mutations in the inhibitory phosphatases SHP-1 or SHIP-1 leads to accelerated autoimmunity in a synergistic manner [76]. $Lyn^{+/-}Me^{v/+}$ mice lacking one allele of *Lyn* and carrying one allele of the naturally occurring loss of function 'motheaten viable' (Me^{v}) mutation in SHP-1, show an amplification of the subtle immune cell perturbations present in either mutant alone, including B-cell deficiency, plasma cell expansion and enhanced expression of B cell co-stimulatory markers. Intriguingly, while myeloid and erythroid cell expansion was not a significant feature of individual heterozygous mutants, compound $Lyn^{+/-}Me^{v/+}$ mice displayed significant expansion of these cell compartments. Ultimately, these animals accumulated higher titers of autoantibodies and developed exacerbated glomerular disease compared with the single heterozygous mutants [76]. This synergistic effect was also observed in mice lacking one allele of *Lyn* and one allele of *SHIP-1*: $Lyn^{+/-}$ $SHIP\text{-}1^{+/-}$ mice developed higher titers of autoantibodies than single heterozygous mice and interestingly, this effect was much more striking on the C57BL/6 genetic background than on the BALB/c background [76].

Constitutive activation of Lyn & effect on disease

Our understanding of the role of Lyn in BCR signaling has been extended by the evaluation of Lyn gain-of-function ($Lyn^{up/up}$) mice. These mice carry a Tyr>Phe mutation at the enzymes critical C-terminal regulatory domain resulting in a constitutively active enzyme (Figure 2C) [26]. $Lyn^{up/up}$ mice displayed significantly reduced B-cell numbers and their B cells were hyporesponsive to BCR-mediated stimulation due to constitutive engagement of inhibitory signaling pathways; however, they also displayed hyperactivation of positive signaling molecules such as Syk and PLCγ, and exhibited an increased calcium flux [27]. Paradoxically, these mice developed autoantibodies and lethal kidney disease, speculated to be due to sustained positive signaling overriding constitutive inhibitory signaling in B cells [27]. This key study has revealed that any imbalance of Lyn activity, either up or down, can induce severe autoimmunity. This important feature, first described in mice, is now borne out in studies in humans as well [65,66].

Kinase activity of Lyn in disease

In an *N*-ethyl-*N*-nitrosourea mutagenesis screen aimed at characterizing mutant mice with leukocyte abnormalities, the *Mld4* mouse, which harbors a mutation that renders Lyn kinase-dead (T410K), was identified. These mice exhibited B-cell lymphopenia and cellular activation in a manner akin to $Lyn^{-/-}$ mice, as well as autoantibody production and immune complex deposition. Interestingly, however, severe glomerular disease did not occur, suggesting kinase-independent roles of Lyn in restraining disease [82]. At the same time, another *N*-ethyl-*N*-nitrosourea mutant harboring a *Lyn* gene mutation, the *WeeB* mouse, was described. This mouse carries a different genetic mutation to the *Mld4* mouse, but nonetheless, renders the Lyn enzyme kinase-dead (E260G; Figure 2D). *WeeB* mice displayed B-cell abnormalities similar to $Lyn^{-/-}$ mice; however, unlike *Mld4* mice they developed significant glomerular disease, albeit with delayed onset compared with $Lyn^{-/-}$ mice [83]. While these two studies are somewhat contradictory, they do suggest that mice harboring kinase-dead Lyn mutations exhibit milder autoimmune disease than mice completely lacking Lyn. Nonetheless, a more complete examination of the inflammatory phenotypes in the mutants and the biochemical basis for the disease severity difference is lacking.

Role of T cells in disease

The accumulation of class-switched pathogenic IgG autoantibodies in $Lyn^{-/-}$ mice highlights the role of T-cell help in disease development. Interestingly, despite a lack of Lyn expression in T cells, aged $Lyn^{-/-}$ mice develop significant CD4 T-cell activation and regulatory T-cell expansion, thought to be due to the inflammatory environment that is engendered as the disease progresses [51,54,91]. The contribution of T-cell costimulation to the autoimmune phenotype has been investigated in $Lyn^{-/-}$ mice by overexpressing a soluble form of the T-cell inhibitory molecule CTLA4Ig. Interestingly, $Lyn^{-/-}$ CTLA4Ig mice failed to develop IgG autoantibodies and splenomegaly; however, a form of destructive IgA-mediated glomerulonephritis was uncovered that presented in the absence of IgG nephritis [53].

A role for intact T-cell signaling was also revealed in $Lyn^{-/-}$ mice lacking one functional allele of the PI3K isoform p110δ. In these mice, dampening the PI3K signaling pathway significantly attenuated numerous traits associated with Lyn deficiency including plasma cell expansion, serum Ig titers, autoantibody development, systemic inflammation and autoimmune-mediated kidney pathology [86]. Interestingly, however, while B-cell hyper-responsiveness was retained in $Lyn^{-/-}p110\delta^{+/KD}$ mice, there were significant defects in T-cell activation and signaling. The diminution of autoimmune disease in these mice seems likely to be due to a combination of defects in inflammation and the inability of $Lyn^{-/-}$ $p110\delta^{+/KD}$ T cells to provide appropriate help signals

to B cells [86]. Importantly, this study also provides an excellent rationale for PI3K inhibition in lupus.

IL-21 is a pleiotropic cytokine that is mainly produced by activated T cells and its inhibition is currently being evaluated in Phase I clinical trials for SLE [98]. Its levels are marginally elevated in Lyn$^{-/-}$ mice, and Lyn$^{-/-}$ mice that lack IL-21 fail to produce class-switched anti-dsDNA autoantibodies, supporting a role for IL-21 in the germinal center response in these mice [87]. Nonetheless, these mice develop significant glomerular disease driven by IgG immune complex disposition similar to that in Lyn$^{-/-}$ mice, which is thought to be due to the production of IgG autoantibodies against non-DNA antigens [87]. In a very recent study, Lyn$^{-/-}$ mice rendered genetically deficient in T cells (Lyn$^{-/-}$TCRβ$^{-/-}$TCRδ$^{-/-}$) or lacking the SAP signaling adaptor molecule (Lyn$^{-/-}$SAP$^{-/-}$), produced greatly diminished levels of anti-dsDNA IgG autoantibodies providing further support for the role of T-cell help and the germinal center response in pathogenic autoantibody production [88]. However, this study did not investigate whether IgA autoantibodies were generated and if kidney pathology was present in the compound mutants to determine if disease developed with a different presentation such as occurs in Lyn$^{-/-}$ CTLA4Ig mice [53].

Interactions/redundancy with other SFKs

While it is clear that Lyn has a unique role in inhibitory signaling [20], some functional redundancy is thought to exist among the SFKs; for example, the B-cell specific SFK, Blk, is dispensable for B-cell development and function [99]. Nevertheless, when Lyn and the two other most abundant SFK that are expressed in B cells, Fyn and Blk, are simultaneously deleted, a complete block in B-cell development at the pro-B to pre-B cell stage occurs likely due to defective pre-BCR signaling [90]. Mice deficient in Lyn and Fyn have also been generated. These animals developed severe glomerular disease, despite equivalent B-cell hyper-responsiveness and autoantibody levels compared with Lyn$^{-/-}$ mice. It is thought that the synergistic effect of Lyn and Fyn deficiency is due to a kidney intrinsic mechanism in this model [89]; however, a caveat to these studies is that they were conducted on 129 × C57BL/6 mixed genetic background mice. Mice lacking the three myeloid abundant SFK members Hck, Fgr and Lyn (HFL$^{-/-}$) were originally generated to assess the interaction of the three kinases in macrophage activation and phagocytosis. While HFL$^{-/-}$ macrophages displayed normal LPS-induced activation, they showed diminished Fcγ-induced signaling and phagocytosis [15]. Autoimmune disease has subsequently been assessed in these triple-deficient mice, finding attenuated disease severity due to dampening effects on the inflammatory environment [91].

Role of inflammation in disease

The importance of inflammation in promoting autoimmune disease development was made clear with the generation of Lyn$^{-/-}$ lacking the proinflammatory cytokine IL-6; these mice remained autoimmune-prone but did not develop disease [51,81]. Lyn$^{-/-}$IL-6$^{-/-}$ mice retained features intrinsic to the Lyn$^{-/-}$ phenotype such as B-cell developmental defects and B-cell hyper-responsiveness; however, myeloid expansion, splenomegaly and T-cell activation did not occur and the production of pathogenic autoantibody and activation of innate inflammatory mechanisms that can induce kidney pathology were ameliorated. These studies were pivotal in defining the importance of IL-6-mediated inflammation in the development of SLE. More recent studies have dissected the role of classical IL-6 signaling versus IL-6 trans-signaling in autoimmune disease development in Lyn$^{-/-}$ mice revealing that classical signaling was required for T-cell activation and autoantibody production, while neutralization of trans-signaling was shown to dampen myeloid effector cell recruitment and inflammation in the kidneys [55].

Deletion of the proinflammatory cytokine IFN-γ reiterated the role of inflammation in autoimmune disease in Lyn$^{-/-}$ mice. In Lyn$^{-/-}$IFN-γ$^{-/-}$ mice, BAFF overproduction and myeloid expansion were reduced, which significantly improved glomerulonephritis. Furthermore, this study showed that BAFF levels were elevated in Lyn$^{-/-}$ mice and importantly, that their treatment with anti-BAFF antibody could ameliorate glomerulonephritis [91]. Given the key role of IL-10 in inhibiting inflammation, it is not surprising that deletion of IL-10 from Lyn$^{-/-}$ mice resulted in more severe disease manifest as marked splenomegaly and lymphadenopathy, dramatic increases in proinflammatory cytokines and severe tissue inflammation [93]. This study also showed that the inflammatory environment in Lyn$^{-/-}$ mice induced the expansion of IL-10-producing B cells. In another study, autoimmune disease in Lyn$^{-/-}$ mice was found to be significantly milder in the absence of the IL-5 receptor α-chain [92], implicating IL-5 signaling in autoantibody production.

Although cytokine-deficient Lyn$^{-/-}$ mice provide an insight into the role of cytokine-dysregulation in autoimmune disease development, Toll-like receptor (TLR) signaling has been identified as an essential pathway in inflammatory disease. In support of this, autoimmune disease in Lyn$^{-/-}$ mice was found to be dependent on the TLR signaling intermediate MyD88 [94]. Lyn$^{-/-}$ mice lacking MyD88 showed significantly attenuated titers of antinuclear antibodies and were protected

from glomerulonephritis, making an unequivocal connection between innate immunity and autoimmune disease.

Role of Th2 environment in disease pathogenesis

The autoimmune disease phenotype of Lyn$^{-/-}$ mice, promoted by IL-6 and IFN-γ, and characterized by high IgG2a/IgG2c titers and macrophage activation, lends itself to a Th1 disease classification. However, Lyn$^{-/-}$ mice represent a unique situation, where prominent Th2 traits such as atopy, mast cell hyper-responsiveness and eosinophilia are coexistent with autoimmunity [9,48]. It has recently been proposed that the Th2 environment contributes to the development of lupus in Lyn$^{-/-}$ mice [85]. Autoreactive IgE leading to basophil activation and the increased production of IL-4 was found to be a feature of Lyn$^{-/-}$ mice. This amplified the production of autoantibodies thereby contributing to disease pathogenesis [85]. This theory was supported by genetic deletion of IL-4 or IgE in Lyn$^{-/-}$ mice, which resulted in diminished pathogenic autoantibodies and glomerulonephritis [85]. In a seemingly opposing study, genetic deletion of STAT6, a key mediator of Th2 immunity downstream of IL-4 signaling, was found to significantly amplify autoimmune disease pathology in Lyn$^{-/-}$ mice, characterized by increased autoantibody titers, enhanced immune cell activation and accelerated glomerulonephritis compared with their Lyn$^{-/-}$ counterparts [52]. However, aged Lyn$^{-/-}$STAT6$^{-/-}$ mice were able to produce high titers of IgE and exhibited other Th2 traits such as increased IL-4 and IL-5, and basophil expansion, indicating that Th2 features can develop independently of the STAT6 pathway.

Cell-specific deletion of Lyn

Very recent studies have begun to elucidate the role of different immune cell types in the Lyn$^{-/-}$ phenotype. Since Lyn is expressed in most hematopoietic cells, the relative contribution of each cell type to the initiation and progression of autoimmune disease pathology is difficult to determine in the genome-wide knockout. A conditional Lyn knockout mouse has now been generated to enable the study of mice in which only specific immune cell types lack Lyn expression, while the remainder of the cells in the mouse remain Lyn-sufficient. The first study of this type assessed the contribution of Lyn$^{-/-}$ DC to the autoimmune phenotype. This study showed that DC-specific deletion of Lyn was deleterious to animals beyond global Lyn deletion, resulting in worsened serological and pathological indices due to hyperactivated and hyper-responsive MyD88 signaling pathways [95]. Deletion of MyD88 from DC-specific Lyn$^{-/-}$ led to reversal of the autoimmune phenotype. At this stage it is unclear why the disease was worsened in mice lacking Lyn in DCs only, but suggests that Lyn deficiency in other cell compartments in the global Lyn-knockout mouse may restrain disease.

While it is clear that B cells are central to the disease phenotype in Lyn$^{-/-}$ mice, it is also clear that an inflammatory environment is essential to engender their pathogenic potential [51]. To determine whether Lyn$^{-/-}$ B cells require aberrant signals from other immune cell types in a Lyn$^{-/-}$ environment or if B cells themselves are able to initiate inflammatory mechanisms that induce autoimmune disease, a second study from the same group reported on mice that lacked Lyn only in B cells. This study showed that Lyn$^{-/-}$ B cells were sufficient to induce autoimmunity in mice with an otherwise Lyn-sufficient immune system and also revealed that MyD88 signaling was necessary for this effect [96].

Cytosolic Lyn expression can induce the chronic inflammatory disease psoriasis

Lyn has been identified as a substrate for caspases 3 and 7, which are key mediators of apoptosis driven by mitochondrial pathways. The cleavage of Lyn occurs in the N-terminal unique domain after Asp18, which generates two smaller proteins (p54 and p51) and results in their relocation from the plasma membrane to the cytosol (Figure 2E). When overexpressed in immature B cells, this caspase cleaved form of Lyn, named LynΔN, behaves as an inhibitor of BCR-mediated apoptosis [100]. Transgenic mice have been developed that express LynΔN in all tissues and these mice exhibit a skin inflammatory syndrome that resembles psoriasis, which is dependent on TNF-α expression [84]. Furthermore, this phenotype was improved in a Rag1-deficient background suggesting a role for T cells in the disease. Interestingly, this caspase-cleaved form of Lyn was found to be expressed in skin biopsies from patients suffering from psoriasis [84], although more studies are required to determine if this is causal or consequential to disease.

Conclusion

Lyn plays a critical, nonredundant inhibitory signaling role in the immune system. Deletion of Lyn in mice results in hyperactivation of immune cells, loss of B-cell tolerance leading to the production of pathogenic antinuclear autoantibodies and systemic inflammation. A direct consequence of autoantibody production is the development of an SLE-like glomerulonephritis due to immune complex deposition in glomeruli, activation of the complement cascade, recruitment of myeloid effector cells to the kidney and

ensuing glomerulonephritis. The Lyn$^{-/-}$ mouse model has been used extensively to further our understanding of the disease mechanisms underlying SLE. Additional mutations in Lyn$^{-/-}$ mice have demonstrated that although B-cell hyperactivity is sufficient for autoimmunity, additional factors such as T-cell help and an inflammatory environment are essential for autoimmune disease propagation (Figure 3). Autoimmune disease is also reliant on MyD88 signaling downstream of TLRs, and aspects of both Th1 and Th2 responses are seen to contribute to disease. Lyn is deregulated in human SLE patients who present with reduced Lyn mRNA, altered protein levels and deregulated lipid raft localization correlating with the production of autoantibodies. While no specific genetic mutation in Lyn has been identified in SLE patients, Lyn is recognized as a susceptibility gene based on genome-wide association studies. As the autoimmune disease manifest by Lyn$^{-/-}$ mice closely resembles that of human SLE, Lyn$^{-/-}$ mice represent a premier preclinical model for testing of therapeutics and for further delineation of the mechanisms contributing to human SLE. Given investigations in mice and emerging studies in humans showing that any imbalance in Lyn expression or activity may lead to SLE-like autoimmunity there may only be a select few patients whose genetics have been clearly defined, where Lyn would constitute a suitable target in lupus.

Future perspective

Can targeting the SFK pathway lead to autoimmunity?

Given the susceptibility of Lyn$^{-/-}$ mice to lupus-like autoimmune disease, it is reasonable to suggest that inhibiting the Lyn signaling pathway may induce autoimmune disease in genetically susceptible individuals, and this theory is also supported by limited studies in human SLE samples. There are a number of inhibitors such as dasatinib (BMS-354825), bafetinib (INNO-406 and NS-187) and bosutinib (SKI-606) that have potent inhibitory effects on Lyn [101,102] and some of these are now being trialed in the clinic for the treatment of leukemias and solid cancers [103]. Interestingly, the increased kinase activity of SFKs including Lyn has been linked to the development of imatinib-resistant chronic myeloid leukemia and Ph+ acute lymphoblastic leukemia, and the dual acting Bcr-Abl/SFK inhibitor dasatinib has been US FDA approved for the treatment of these patients [104]. Given that disruption of Lyn activity in mice leads to autoimmunity, it is imperative to determine whether short- or long-term treatment of patients with these inhibitors has any adverse immune system effects associated with inhibition of Lyn.

Is activation of the Lyn signaling pathway a possible therapeutic for autoimmune disease?

By corollary, studies in mice suggest that a subset of autoimmune patients may benefit from enhancing Lyn pathway activity. On this note, Melior Pharmaceuticals have been developing new uses for a small molecule known as MLR-1023 that increases the kinase activity of Lyn through an allosteric mechanism. This patented agonist was originally developed by Pfizer for an unrelated chronic indication and passed through Phase II being safe and tolerated, but further development was halted for lack of efficacy. It was picked up by Melior when additional data from the trial suggested effects on blood glucose. The group showed that MLR-1023 lowered blood glucose levels in mice without increasing insulin secretion *in vivo*, and in an *in vitro* kinase screen against 47 kinases they showed that it was able to enhance Lyn kinase activity consistently by 50% [105]. They examined glucose levels in Lyn$^{-/-}$ mice finding that they had equivalent resting blood glucose levels to control mice, as well as comparable increases in blood glucose levels following an oral glucose tolerance test. Nonetheless, MLR-1023 could reduce the effects of oral glucose challenge in control mice, but not in Lyn$^{-/-}$ mice indicating that its blood glucose-lowering effects depend on the presence of Lyn kinase *in vivo* [105]. In further studies, the Melior group showed that MLR-1023 is an insulin receptor-potentiating agent that produces a rapid-onset and stable blood glucose-lowering activity in diabetic animals [106]. Now that a link has been made, it will be interesting to more closely examine the role of Lyn in metabolic diseases. MLR-1023 has very recently been licensed to Bukwang Pharmaceuticals to conduct Phase II clinical trials in Type 2 diabetes. It is not known whether MLR-1023 is also being viewed as a possible therapeutic for autoimmune disease. However, caution must be exercised when using such a drug since it is known that constitutive activation of Lyn can induce the development of autoimmune disease in mice [27], and thus it will be important to determine whether its prolonged use may lead to detrimental autoimmune side effects in genetically predisposed people.

Personalized medicine approaches for the treatment of heterogeneous diseases like SLE

Although the role of Lyn in autoimmunity has been cemented with almost 20 years of research, ongoing studies on the Lyn$^{-/-}$ model continue to reveal disease mechanisms that may ultimately aid in improving SLE therapy. As discussed in this review, great progress has already been made in determining the signaling pathways and inflammatory mediators important in autoimmune pathology regulated by Lyn. While it is

amenable to target these pathways in inbred mice, the extensive heterogeneity of human disease often makes it difficult to show efficacy of promising therapeutic targets. In this instance, it is necessary to target a central pathway that lies at the nexus of several pathogenic mechanisms. Likewise, targeting a particular group of responses or a central pathogenic pathway that is utilized by various cell types, such as inflammation, would be of benefit. By contrast, patient stratification may also be useful to delineate specific pathogenic mechanisms that are contributing to disease in particular patient subsets. This concept of personalized medicine would be of special consideration in a disease with pathologies as varied as those in SLE that are potentially due to different disease pathways. Performing immunoprofiling and signaling pathway studies on individual lupus patients to define their specific disease footprint and thus enable a directed therapeutic approach for maximum benefit, seems an obvious next step. In addition, *in vitro* studies could be performed on patient cells to determine whether nominated therapies show effects in specified readouts of cellular assays. Lyn expression and activity in human SLE patient samples could constitute a subset of this approach. Loss of Lyn activity associated with autoimmune disease, as observed in mice, may be supplemented by use of the Lyn agonist described above. These types of approaches would greatly improve how SLE is researched and treated; making a clear departure from the 'one-size-fits-all' approach that has thus far largely failed to make significant advances in SLE disease management.

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Executive summary

Lyn tyrosine kinase: from historical perspective to role in immune cells

- The Lyn tyrosine kinase is a member of the Src family of protein tyrosine kinases (SFKs), which are constitutively associated with cell surface receptors that lack intrinsic kinase activity.
- Lyn is a unique SFK that can negatively regulate signaling from inhibitory receptors; namely by directing the recruitment of inhibitory phosphatases such as SHIP-1 and SHP-1 to the plasma membrane and regulating their enzymatic activity.

Lyn$^{-/-}$ mice as a model for systemic lupus erythematosus

- Due to the unique inhibitory role of Lyn in immune cells, especially B cells, Lyn$^{-/-}$ mice display hyper-responsive B cells and accumulate autoantibodies with age in a manner akin to systemic lupuserythematosus (SLE).
- Inflammation is a key pathogenic mechanism in this model that drives disease pathogenesis.
- Many features of human SLE have been identified in the Lyn$^{-/-}$ model, providing validity to their preclinical utility.

The role of Lyn in human SLE

- Studies addressing the role of Lyn in SLE have included genome-wide association scans and biochemical signaling studies.
- Altered Lyn activity in human SLE B cells has been identified, while alterations in signaling proteins regulated by Lyn are also associated with SLE.

Conclusion

- Although SLE mortality has declined in recent times, current treatments are largely based on non-specific immunosuppression causing unwanted and often severe side effects and thus specific therapies are still needed.
- The role of Lyn in autoimmune disease has been evaluated for almost 20 years, identifying underlying pathogenic mechanisms. Future work should be aimed at utilizing the knowledge gained in the testing of therapeutic agents and defining their mechanism of action using this preclinical model.
- Any imbalance of Lyn activity can induce severe autoimmunity, questioning the utility of targeting this kinase in disease therapy.
- Recent studies have identified a Lyn agonist, which may be beneficial for increasing Lyn activity in autoimmune and inflammatory diseases associated with diminished Lyn function.
- The current use of SFK inhibitors for cancer treatment may lead to adverse side effects associated with loss of Lyn function in immune cells.
- The availability of conditional Lyn-knockout mice will enable a comprehensive study of Lyn's role in specific B-cell subsets, as well as other cell types implicated in disease such as basophils.

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