

# The Pathogenesis of Lupus Nephritis

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## ABSTRACT

Lupus nephritis is an immune complex GN that develops as a frequent complication of SLE. The pathogenesis of lupus nephritis involves a variety of pathogenic mechanisms. The extrarenal etiology of systemic lupus is based on multiple combinations of genetic variants that compromise those mechanisms normally assuring immune tolerance to nuclear autoantigens. This loss of tolerance becomes clinically detectable by the presence of antinuclear antibodies. In addition, nucleic acids released from netting or apoptotic neutrophils activate innate and adaptive immunity via viral nucleic acid-specific Toll-like receptors. Therefore, many clinical manifestations of systemic lupus resemble those of viral infection. In lupus, endogenous nuclear particles trigger IFN- $\alpha$  signaling just like viral particles during viral infection. As such, dendritic cells, T helper cells, B cells, and plasma cells all contribute to the aberrant polyclonal autoimmunity. The intrarenal etiology of lupus nephritis involves antibody binding to multiple intrarenal autoantigens rather than the deposition of circulating immune complexes. Tertiary lymphoid tissue formation and local antibody production add to intrarenal complement activation as renal immunopathology progresses. Here we provide an update on the pathogenic mechanisms that lead to lupus nephritis and provide the rationale for the latest and novel treatment strategies.

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SLE is a chronic autoimmune disease characterized by loss of tolerance against nuclear autoantigens, lymphoproliferation, polyclonal autoantibody production, immune complex disease, and multiorgan tissue inflammation.<sup>1,2</sup> SLE used to be referred to as a complex autoimmune disease of unknown etiology; however, during the last decade, a multidisciplinary approach to SLE research has built a more concise view of its pathogenesis and for lupus nephritis (LN). Here we briefly summarize an updated working model of SLE and LN, which provides a rationale for novel therapies.

## EXTRARENAL PATHOGENIC MECHANISMS OF LN

### Cell Death and Dead Cell Handling

SLE develops from a loss of self-tolerance to ubiquitous nuclear autoantigens,

which is a result of an immunization process. This observation implies two notions (Figure 1 and Table 1). First, autoreactive, long-lived plasma cells, and memory T cells memorize their immunization against nuclei. These cells cannot be deleted by current immunosuppressive therapies; hence, current treatments may suppress disease activity but do not cure SLE.<sup>2,3</sup> Second, the nuclear antigens used for immunization had to be accessible to antigen-presenting cells, a process that is normally avoided by the homeostatic mechanism of rapid dead cell clearance. In fact, SLE develops in individuals with unfortunate combinations of genetic variants that, among other immunoregulatory defects, compromise those mechanisms that normally assure low levels of chromatin in extracellular compartments, particularly mutations that alter apoptosis,<sup>4,5</sup> the

opsonization of dead cells by complement, or their removal by phagocytes.<sup>6</sup> Neutrophils undergo NETosis, which releases nucleosomes into the extracellular space.<sup>7–10</sup> This finding recently revealed an unexpected role of neutrophils in SLE.<sup>11</sup> But how do dead cell clearance defects lead to SLE?

### Induction of Antiviral Immunity

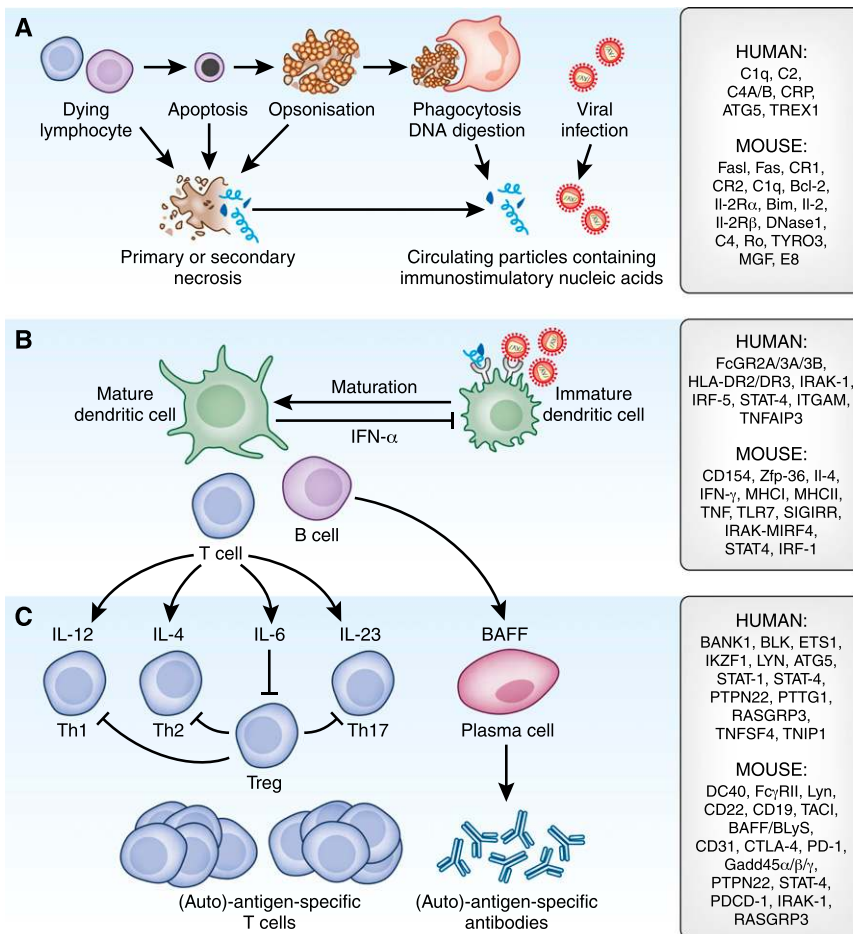
A delay of dead cell removal leads to degeneration of its components, which compromises those elements that normally distinguish self-nucleic acids from viral nucleic acids.<sup>12,13</sup> For example, nature developed the methylation of DNA and RNA as a way to inhibit RNA and DNA recognition by Toll-like receptors (TLRs) 3, 7, and 9, a set of endosomal viral nucleic acid recognition receptors that trigger antiviral immunity during viral infection.<sup>14</sup> Therefore, in SLE patients, nuclear particles are taken as viral particles that contain some protein component (antigen) as well as some immunostimulatory nucleic acid (immune adjuvant; Figure 1).

During evolution, our immune system was primed to mount potent antiviral immunity upon the recognition of viral particles, a response that is initiated against the components of virus-like nuclear particles in SLE patients. For

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**Figure 1.** Pathomechanisms of LN outside the kidney. (A) Genetic variants of homeostatic cell death (*i.e.*, Fas variants) and the rapid clearance of dead cell corpses (*e.g.*, C3/4 variants or DNases variants) result either in secondary necrosis or incomplete chromatin digestion, which both promote the exposure of nuclear particles to the immune system. (B) Nuclear particles resemble viral particles and activate the same viral nucleic acid recognition receptors on antigen-presenting cells. Genetic variants of those signaling elements are recognized to be risk factors for SLE. The activation of antigen-presenting cells changes (by costimulation) the immune interpretation of concomitantly presented antigens of the same particle. (C) Polyclonal lymphocyte expansion has multiple effects on the disease process and genetic variants further affect the differentiation of T helper cells. The complex regulation of lymphocyte activation and expansion is affected by multiple genetic variants. The susceptibility genes and genes/molecules that are involved within each biologic pathway are listed to the right: C1q, C2, C4A/B, C-reactive protein (CRP),  $\alpha$ -glucoside transporter 5 (ATG5), three prime repair exonuclease 1 (TREX1), B cell CLL/lymphoma 2 (Bcl-2), IL-2 receptor  $\alpha$  (IL-2R $\alpha$ ), tyrosine-protein kinase receptor 3 (TYRO3), mast/stem cell growth factor (MGF), Fc $\gamma$  receptor (FcGR), HLA IL-1 receptor-associated kinase (HLA IRAK), IFN regulatory factor (IRF), signal transducer and activator of transcription (STAT), integrin  $\alpha$ M (ITGAM), TNF  $\alpha$ -induced protein 3 (TNFAIP3), zinc finger protein 36 (Zfp-36), IL-4, IFN- $\gamma$ , MHCI, MHCII, TNF, TLR7, single Ig and Toll-IL 1 receptor (SIGIRR), B cell scaffold protein with ankyrin repeats 1 (BANK1), B lymphoid tyrosine kinase (BLK), IKAROS family zinc finger 1 (IKZF1), protein tyrosine phosphatase, non-receptor type 22 (PTPN22), pituitary tumor-transforming 1 (PTTG1), RAS guanyl releasing protein 3 (RASGRP3), TNF (ligand) superfamily, member 4 (TNFSF4), TNFAIP3-interacting protein 1 (TNIP1), transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI), BAFF/BLYS, cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1/PDCD-1), and Gadd45 (activated by the stress-inducible GADD45).

example, ribonucleoprotein, U1snRNP, ligates TLR7 to induce type I IFN release in plasmacytoid dendritic cells,<sup>15</sup> a process that is tightly controlled by IL-1 receptor-associated kinase-M.<sup>16</sup> RNA immune complexes activate B cells to produce antinuclear antibodies,<sup>17</sup> which is controlled by the *TIR8* gene encoding for the SIGIRR protein.<sup>18,19</sup> Nucleosomal DNA or DNA within immune complexes can activate TLR9 on plasmacytoid dendritic cells and drive B cell proliferation.<sup>20</sup> Blockade of TLR7, TLR9, or both abrogates type I IFN induction, SLE, and LN in mice.<sup>21–23</sup> This (pseudo)antiviral immune response involves all antigen-presenting cells, particularly dendritic cells and B cells, but only plasmacytoid dendritic cells secrete large amounts of type I IFNs to set off an antiviral immune response.<sup>5,24</sup> The signature for IFN receptor-dependent secondary gene expression includes multiple antiviral and proliferative genes such as *IFIT1*, *MX1*, *MX2*, *ISG15*, and the *OAS* gene family, the IFN regulatory factors IRF7 and IRF5, as well as proinflammatory chemokines CXCL10 and CXCL5, which altogether account for the nonspecific symptoms shared by viral infections and SLE, such as fever, fatigue, arthralgia, and myalgia (Figure 2).<sup>25</sup>

### Aberrant Lymphocyte Proliferation

Dendritic cells and B cells both have the capacity to process antigens and present antigens to T cells and they can substitute each other for this purpose.<sup>26</sup> Dendritic cells have a limited life span but their persistent activation by lupus autoantigens by TLR7 and TLR9 enhances their survival and renders them resistant to glucocorticoid-induced death.<sup>21</sup> Persistent activation of antigen-presenting cells turns the interpretation of autoantigens from immune ignorance and lymphocyte anergy into lymphocyte activation and proliferation, which can overcome the functional unresponsiveness or anergy of mature autoreactive B cells.<sup>27</sup> Murine double minute-2 is one of these mitogenic factors that is specifically induced by DNA recognition.<sup>28</sup> Murine double minute-2 neutralizes p53-dependent cell cycle arrest, which explains the mitogenic

**Table 1.** Pathomechanisms of LN inside the kidney

Glomerular Pathology	Tubulointerstitial Pathology
Mesangial and subendothelial, immune complex deposits, complement activation	Immune complex deposits in periglomerular vessels
Fc, Toll-like, and complement receptor activation	Complement activation
Activation of renal cells and infiltrating leukocytes (subepithelial IC causes LN class V and podocyte injury with massive proteinuria)	Activation of endothelial cells, luminal adhesion molecules
Local cytokine expression	Leukocyte recruitment
Recruitment of leukocytes	Local antibody production by B cells including tertiary lymphoid organ formation
Proliferation of endothelial and mesangial cells	Cytotoxic and Th17 T cells
Filtration barrier damage causing proteinuria and hematuria	Proapoptotic cytokines
Renal cell necrosis causing focal scarring	Proximal tubular cell damage causing proteinuria
Proliferation of parietal epithelial cells and crescent formation	Tubular/vascular atrophy
Periglomerular inflammation	Hypoxia → inflammation
Global glomerulosclerosis	Insufficient tubular and vascular repair plus ischemia promotes interstitial fibrosis

effect of endogenous DNA or DNA viruses on autoreactive lymphocytes in SLE. Other lymphocyte mitogens are B lymphocyte stimulator (BlyS, also named B cell activating factor [BAFF]) and a proliferation-inducing ligand (APRIL), which regulate B cell differentiation and Ig class switching.<sup>29</sup> The two APRIL/BlyS receptors transmembrane activator and CAML-interactor (TACI) and B cell maturation protein promote plasma cell survival; therefore, they are currently considered as potential therapeutic targets in SLE.<sup>30</sup>

Furthermore, TNF-like weak inducer of apoptosis, a proinflammatory cytokine of the TNF superfamily, regulates multiple processes in autoimmunity and tissue inflammation through activation of the fibroblast growth factor–inducible 14 receptor. B cell maturation toward autoantibody-producing plasma cells involves IFN-related factor-4.<sup>31</sup>

### Environmental Triggers of SLE Activity

Viral infections induce IFN- $\alpha$  release, which triggers antiviral immunity as well as lupus disease activity.<sup>25</sup> Bacterial infections have a nonspecific immunostimulatory effect, which involves a transient expansion of autoreactive lymphocyte clones. Furthermore, bacterial

products stimulate intrarenal immune cells and renal cells, which can trigger a transient aggravation of proteinuria and kidney damage. Another environmental trigger of SLE activity is ultraviolet light, which induces an increase in the load of dead cells by causing keratinocyte death.<sup>32</sup> In patients with a significant dead cell clearance defect, this process will lead to increased levels of extracellular nuclear material, and additional exposure of autoantigens and adjuvants to the immune system.<sup>1</sup> Drug-induced SLE involves inhibition of methyl-transferases, a process that enhances the unmasking of endogenous nucleic acids and the activation of TLR7 and TLR9.<sup>33,34</sup> Progesterone and estrogens stimulate the sex hormone–dependent immunoregulatory pathways.<sup>35</sup>

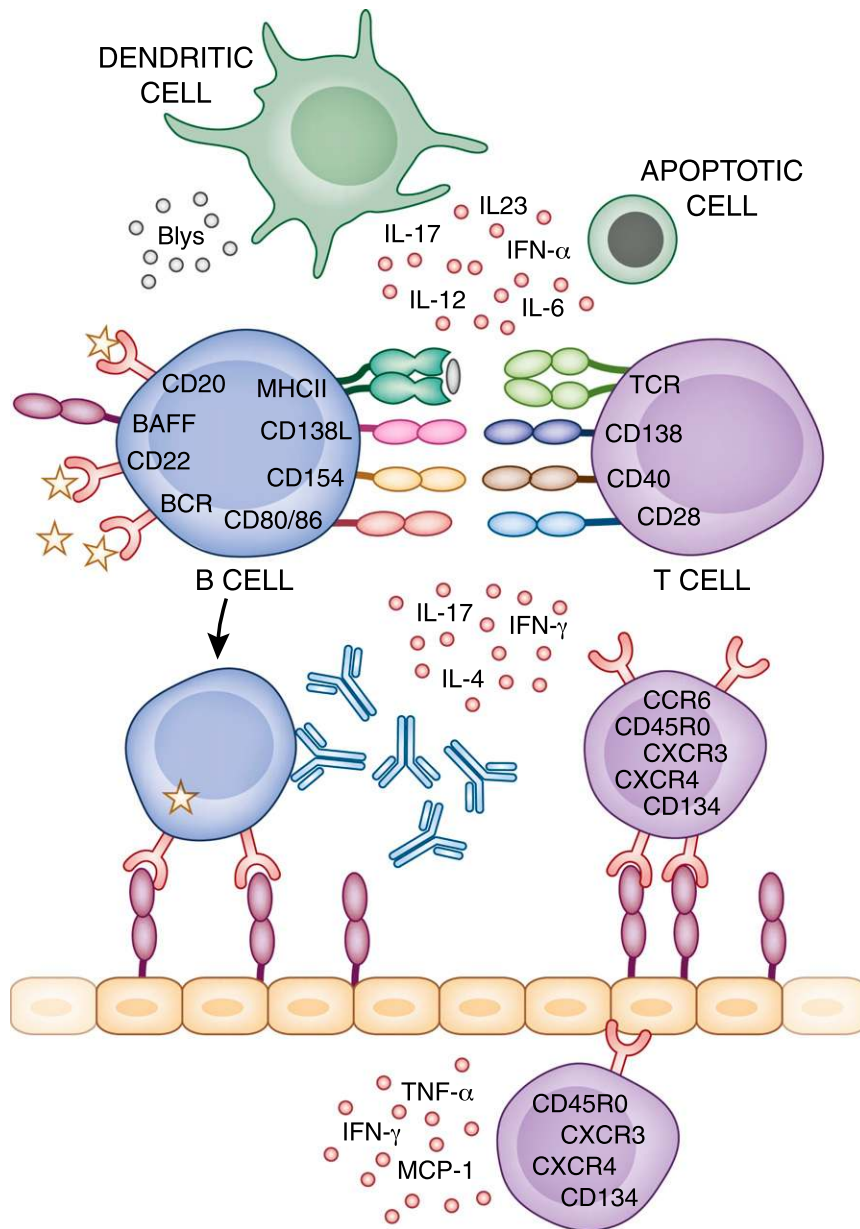
Together, SLE develops from a peculiar combination of genetic variants that impair those mechanisms that normally prevent the exposure of nuclear particles to the immune system and their capacity to activate viral recognition nucleic acid receptors (Figure 1). Therefore, an IFN- $\alpha$ –dependent (pseudo)antiviral immune response accounts for those nonspecific SLE symptoms that are shared with viral infections. The adjuvant activity of endogenous nucleic acids promotes an adaptive immune

response against the components of the nuclear particle, a process identical to vaccination. This implies the expansion of T and B cell clones with specificities for predominantly nuclear autoantigens that account for the production of anti-nuclear antibodies, immune complex disease, and T cell–dependent tissue damage. Hormonal and environmental stimuli can enhance these processes at different levels.

## INTRARENAL PATHOGENIC MECHANISMS OF LN

### Immune Complex-Mediated Renal Immunopathology

The nonspecific activation of autoreactive B cells explains the polyclonal autoantibody response leading to the diagnostic hallmark of LN, the full house pattern of IgM, IgA, and IgG deposits.<sup>36</sup> However, antibody-deficient mice still develop LN; therefore, B cells have pathogenic effects beyond antibody production,<sup>37</sup> including autoantigen presentation to activate autoreactive T cells and local proinflammatory effects.<sup>26</sup> Immune complexes deposit in the mesangium or the subendothelial and subepithelial spaces or in peritubular capillaries depending on the quality of the autoantibodies, the duration, and severity of LN.<sup>38</sup> This implies that immune complex formation in the mesangium causes class I and II lesions, subendothelial immune complex formation in class III and IV lesions, and subepithelial immune complexes in class V lesions as well as the overlapping forms III/IV and IV/V.<sup>39</sup> The traditional concept that circulating immune complexes in lupus passively deposit in the kidney has been challenged by novel data.<sup>40,41</sup> Glomerular immune complexes rather form *in situ* by secondary binding to nucleosomes from renal cells.<sup>42</sup> Another potential intrarenal source of nucleosomes are neutrophils upon NETosis,<sup>11</sup> due to the release of neutrophil extracellular traps that is initiated by anti-LL37 antibodies.<sup>7–10</sup> Heparin modulates the intrarenal effect of chromatin either by enhancing the DNA-I–dependent chromatin degradation or by preventing



**Figure 2.** Therapeutic targets for LN. Aberrant immunity in SLE involves many different cell types and cytokine mediators, which could be suitable therapeutic targets. MCP-1, monocyte chemoattractant protein-1; CCR, CC chemokine receptor; CXCR, C-X-C chemokine receptor.

chromatin binding to the glomerular basement membrane.<sup>43</sup> Anti-DNA antibodies activate endothelial and mesangial cells through different mechanisms. For example, antibodies are directly taken up inside renal cells.<sup>41</sup> This process involves cross-reactivity with  $\alpha$ -actinin or annexin II on mesangial cells,<sup>44,45</sup> but this concept could not be confirmed by recent studies.<sup>46,47</sup> In addition, intrarenal immune

complex deposits activate complement,<sup>36</sup> which demonstrates the dual role of complement factors in LN.<sup>48</sup> Complement deficiency impairs opsonization and removal of lupus autoantigens from the extracellular space, whereas complement factors also directly cause immune complex-related renal inflammation and immunopathology.<sup>49,50</sup> That immune complexes activate glomerular cells by

Fc receptor (FcR) ligation is also well established even though the data accumulated over the last few decades remain complex.<sup>51</sup> Subepithelial immune complex deposits lead to secondary membranous GN and nephrotic syndrome by damaging podocytes.

### Intrarenal Activation of TLRs and IFN Signaling

The nucleic acid component of immune complexes also activates intrarenal inflammation by TLRs in intrarenal macrophages and dendritic cells.<sup>52</sup> In addition, immunostimulatory nucleic acids activate glomerular endothelium, mesangial cells, and macrophages to produce large amounts of proinflammatory cytokines and IFN- $\alpha$  and IFN- $\beta$ .<sup>53–59</sup> The functional significance of this intraglomerular IFN signaling is poorly understood but seems to contribute to renal damage in LN and should trigger the formation of tubuloreticular structures or inclusions that represent an ultrastructural characteristic of IFN signaling.<sup>56,60</sup> Together, the ligation of TLRs, complement receptors, and FcRs activates renal cells to release proinflammatory cytokines and chemokines, and induces the luminal expression of selectins and adhesion molecules inside the microvasculature.<sup>61,62</sup>

### Chemokine-Mediated Recruitment of Different Leukocyte Subsets

Cytotoxic T cells, Th17 T cells, as well as B cells infiltrate the kidney in LN.<sup>24</sup> The members of the chemokine family specifically direct different leukocyte subsets by distinct chemokine receptors into different renal compartments.<sup>63</sup> For example, the chemokine CCL2 recruits CCR2<sup>+</sup> proinflammatory macrophages and T cells into the glomerulus and the tubulointerstitium,<sup>64,65</sup> whereas CCR1<sup>+</sup> cells only recruit to the interstitial compartment and not to the glomerulus in LN.<sup>66</sup> Other leukocyte subsets involve other chemokines and chemokine receptors for their recruitment into the kidney.<sup>63,67</sup> Leukocyte recruitment is tightly regulated. For example, renal cells produce pentraxin-3, which has the potential to directly inhibit

leukocyte recruitment by interfering with P-selectin on the surface of activated endothelial cells.<sup>68,69</sup> In lupus-like autoimmunity of C57BL/6<sup>lpr/lpr</sup> mice, the role of pentraxin-3 seems to be organ specific, because it suppresses lymphocyte recruitment to the lungs but not to the kidney.<sup>70</sup> Infiltrating leukocytes form *de novo* perivascular tertiary lymphoid organs inside the kidney, which involve the clonal expansion and ongoing somatic hypermutation of B cells in proximity to T cell aggregates.<sup>71</sup> Such B cells undergo intrarenal proliferation and activation, which contributes to local inflammation and tissue pathology in addition to their role for systemic and intrarenal autoantibody production.<sup>26,72,73</sup> These data provide a rationale for B cell-targeted therapies in LN.<sup>74,75</sup> T cell infiltrates also contribute to immunopathology in LN, particularly IL-17 producing CD3<sup>+</sup>/CD4<sup>+</sup> or CD3<sup>+</sup>CD4/8<sup>-/-</sup> T cells.<sup>76</sup> Macrophages also contribute to renal damage, particularly F4/80(hi)/CD11c(int)Gr1(lo)/Ly6C(lo)/VLA4(lo)/MHCII(hi)/CD43(lo)/CD62L(lo) macrophages.<sup>77,78</sup>

### Maladaptive Tissue Repair Contributes to CKD Progression

Damage to renal parenchymal cells triggers healing responses that contribute to renal pathology. Focal tuft necrosis is followed by a migration of parietal epithelial cells in the glomerular tuft, where they produce extracellular matrix contributing to FSGS progressing to global glomerulosclerosis.<sup>79</sup> During this process, the parietal cells maintain their polarized epithelial phenotype and lay down extracellular matrix on top of the podocytes.<sup>79</sup> In addition, cellular glomerular crescent formation results from activation of parietal epithelial cells that fill the urinary space by uncoordinated proliferation.<sup>80,81</sup> This process can be triggered by glomerular basement membrane breaks that allow plasma leakage into Bowman's space, where mitogenic plasma components such as fibrinogen trigger hyperproliferation of the parietal epithelial cells.<sup>82</sup> At later stages, the parietal epithelial cells lose their polarity and produce matrix all

around themselves, which creates honeycomb matrix deposits in Bowman's space that turn cellular crescents into fibrocellular crescents with glomerulosclerosis, also referred to as class VI LN.

### THE PRESENT AND FUTURE OF LN THERAPY

#### Nonselective Immunosuppressants

Steroids, cyclophosphamide, azathioprine, and mycophenolate mofetil remain first-line therapeutics for treatment of LN. These nonselective immunosuppressants have much improved the response rates of acute manifestations and the overall mortality of SLE; however, the long-term outcomes of LN have not further improved during the last 30 years.<sup>83</sup> High-dose steroids and cyclophosphamide are frequently associated with severe side effects, and infections contribute to the overall mortality in SLE. The same applies to the other nonselective immunosuppressants. For example, in the ALMS (Apreva Lupus Management) trial, infection-related mortality was even higher in patients treated with mycophenolate mofetil than in the group treated with high-dose cyclophosphamide. Reducing the drug dose was the first strategy to limit side effects, and some researchers wonder whether oral cyclophosphamide therapy is no longer needed.<sup>84</sup> In addition, some studies suggest that Caucasian patients may no longer need high-dose cyclophosphamide, because the Euro-Lupus trial demonstrated favorable long-term outcomes with much lower doses of cyclophosphamide.<sup>85</sup> Other immunosuppressants like dihydroorotate dehydrogenase inhibitors add to the current choices but are still nonspecific.<sup>86,87</sup> However, lupus drug developers continue to search for new drugs that more specifically modulate the aberrant immunity in SLE with fewer side effects.

#### Novel Moieties that Target Specific Leukocyte Subsets

One of the current strategies to specifically interfere with systemic autoimmunity in

SLE is to use cell type-specific drugs. For example, great hope was put into the strategy of B cell-directed therapy as B cells are the source of autoantibody production.<sup>88</sup> Meanwhile, it is clear that B cells in SLE also contribute to autoimmunity and tissue inflammation in many other ways, which increases the hope that depleting or modulating B cells would result in major benefits in SLE and LN.<sup>89</sup> This led to the development of the fully humanized anti-CD20 antibody (rituximab), the anti-CD22 antibody (epratuzumab), and to anti-BlyS (belimumab). Because the randomized placebo-controlled Lupus Nephritis Assessment with Rituximab trial failed to demonstrate a benefit of add-on rituximab for the induction therapy of incident LN class III/IV/V, this approach to B cell depletion still has questions.<sup>90,91</sup> At about the same time, the Exploratory Phase II/III SLE Evaluation of Rituximab trial also failed to demonstrate benefits on nonrenal lupus.<sup>91</sup> However, uncontrolled studies on refractory LN still document 75% responder rates and many specialists continue using rituximab successfully for these patients.<sup>92</sup>

BlyS blockade is another promising strategy to target B cell proliferation. A large randomized placebo-controlled trial suggested that add-on belimumab on top of standard maintenance therapy can significantly improve persistent disease activity up to 72 weeks.<sup>93</sup> This study led to the US Food and Drug Administration and European Medicines Agency approval of belimumab for nonrenal lupus in the United States and Europe. Patients with severe LN were excluded from the BLISS-56 and BLISS-76 trials, but data from those patients with moderate nephritis raise hope that belimumab could also be efficient in severe LN.<sup>94</sup> Such a trial is currently underway. Other B cell-directed strategies include atacicept (TACI-Ig), LY2127399 (anti-BAFF), and anti-BR3 (anti-BAFF-R).<sup>89</sup>

Dendritic cell-T cell interaction is a target of costimulatory ligand/receptor blockers such as CTLA-4-Ig (abatacept). This drug blocks the interaction of CD80 and CD86 on antigen-presenting cells with CD28 on T cells, which suppresses

T cell activation.<sup>95</sup> Abatacept suppressed lupus in mice but did not prevent flares in SLE patients.<sup>96</sup> Three trials with anti-CD40L failed to demonstrate efficacy. Abetimus is a drug that modulates autoimmunity by altering antigen recognition by T cells. Abetimus is composed of a series of linked oligonucleotides, which block the binding of anti-dsDNA antibodies to their autoimmune targets and tolerize B cells with antigen specificity for DNA. Unfortunately, the results in clinical trials have been very modest. A similar approach is followed by edratide, a peptide derived from the antigen-binding region of a human monoclonal anti-dsDNA antibody. It has been proposed that this molecule can modulate the function of DNA-reactive B cells through idiotype–anti-idiotype interactions but again, the convincing data on efficacy are still lacking.

The concept of anti-dsDNA–specific therapeutic interventions is no longer preferred because it targets only a very small subset of B cells, and no longer seems sufficient in view of the failing B cell depletion trials and the fact that dsDNA antibodies certainly contribute to SLE but remain only one of many different pathogenic elements. Finally, plasma cells now appear as an attractive therapeutic target in SLE because they harbor the long-term memory of humoral immunity and produce the lupus autoantibodies.<sup>97</sup> Rituximab does not deplete plasma cells, because these are negative for CD20.<sup>97</sup> Massive antibody production in plasma cells involves the intracellular proteasome complex for protein processing. The proteasome inhibitor bortezomib was proven to be effective in mouse models of LN,<sup>98,99</sup> but clinical trials with bortezomib in human LN is still pending.

### Additional Innovative Therapeutic Strategies

The pseudo-antiviral immunity concept is based on the molecular mimicry of endogenous nucleic acids at the viral nucleic acid recognition receptors TLR7 and TLR9.<sup>100</sup> Hence, blocking these TLRs and the subsequent IFN signaling are additive to established therapeutic targets in

SLE.<sup>101</sup> Current treatment guidelines recommend hydroxychloroquine treatment for all SLE patients, including all patients with LN.<sup>102–104</sup> Antimalarial drugs like hydroxychloroquine inhibit lysosomal acidification, which blocks the adjuvant effect of endogenous nucleic acids by TLR7 and TLR9 during the lysosomal processing of nuclear particles in endolysosomal compartments of antigen-presenting cells.<sup>20</sup> Meanwhile, more specific TLR7 and TLR9 agents have been developed that effectively suppressed LN in murine SLE models and are now being tested in clinical trials.<sup>21–23,105</sup> Antagonism of IFN- $\alpha$  is feasible with IFN- $\alpha$  antibodies. A double-blind randomized study with sifalimumab, a fully human anti-IFN- $\alpha$  mAb, demonstrated that IFN $\alpha$  drives the overexpression of IFN-dependent genes in human SLE, which is reversed by sifalimumab.<sup>106</sup> Other cytokine-directed innovative therapies include anti-Tweak (ATLAS trial) as well as anti-IL6R (tocilizumab). The current status for off-label use of these drugs was recently summarized.<sup>30,107</sup> Finally, it remains an attractive notion to add anti-inflammatory agents to immunosuppressive drugs to reduce the degree of therapeutic immunosuppression and the risk of therapy-related infections. As a proof of concept, the combination therapy of the CCL2 chemokine antagonistic Spiegelmer, mNOX-E36, and 25% of full-dose cyclophosphamide was shown to be as effective as 100% cyclophosphamide to control severe SLE in MRL/lpr/lpr mice<sup>108</sup>; thus, the cyclophosphamide-related T cell ablation and myelosuppression could be prevented, while maintaining treatment efficacy.<sup>108</sup>

### SUMMARY

The pathogenesis of LN involves extrarenal and intrarenal pathogenic mechanisms. The extrarenal factors include complex combinations of genetic variants that are different in each patient, which explains the variability of clinical manifestations. SLE develops when genetic variants compromise those mechanisms that normally assure immune tolerance

for nuclear autoantigens. Loss of tolerance becomes clinically evident by the presence of antinuclear antibodies. The nucleic acid content of nuclear particles from netting or apoptotic neutrophils activates innate and adaptive immunity by TLR7 and TLR9, which triggers an IFN- $\alpha$ –mediated antiviral host defense program that accounts for many of the nonspecific SLE symptoms. As such, dendritic cells, T helper cells, B cells, and plasma cells all contribute to the aberrant polyclonal autoimmunity. The intrarenal etiology of LN involves antibody binding to intrarenal nuclear autoantigens, local complement, and FcR activation. Tertiary lymph follicles, to some degree, form inside the kidney, which include B cells with local proinflammatory effects as well as plasma cells that secrete autoantibody inside the kidney. These insights into the pathogenesis of lupus provide the rationale for a number of novel therapeutic targets.

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### DISCLOSURES.

None.

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