

Clearing the complexity: immune complexes and their treatment in lupus nephritis

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Abstract: Systemic lupus erythematosus (SLE) is a classic antibody-mediated systemic autoimmune disease characterised by the development of autoantibodies to ubiquitous self-antigens (such as antinuclear antibodies and antidouble-stranded DNA antibodies) and widespread deposition of immune complexes in affected tissues. Deposition of immune complexes in the kidney results in glomerular damage and occurs in all forms of lupus nephritis. The development of nephritis carries a poor prognosis and high risk of developing end-stage renal failure despite recent therapeutic advances. Here we review the role of DNA-anti-DNA immune complexes in the pathogenesis of lupus nephritis and possible new treatment strategies aimed at their control.

Keywords: immune complex, systemic lupus erythematosus, nephritis, therapy

Introduction

Systemic lupus erythematosus (SLE) is a complex, heterogeneous disease of multifactorial etiology where multiple genetic, environmental and sex hormonal influences converge to break down B cell tolerance to self-antigens normally sequestered inside the cell nucleus.¹ Recent insights obtained from genetic mouse models and genome-wide association scans in large patient cohorts have enabled the identification of several key players in the multistep pathogenesis of lupus (Figure 1). These studies reveal a positive feedback loop whereby inefficient clearance of apoptotic blebs by macrophages results in positive selection of germinal center B cells, which have self-reactivity against nuclear antigens exposed on these blebs. These self-reactive B cells undergo T cell-dependent affinity maturation and isotype switching,² and differentiate into long-lived plasma cells which reside in the bone marrow. The high affinity IgG anti-DNA antibodies secreted by these cells bind to the DNA to form immune complexes which activate plasmacytoid dendritic cells (pDCs) via toll-like receptor- (TLR-) 9 to produce inflammatory cytokines such as interferon-alpha. These cytokines augment the humoral immune response and lead to further autoantibody production. The high levels of circulating DNA-anti-DNA immune complexes overwhelm the capacity of the reticuloendothelial system (RES) to clear them, and they are deposited in various tissues including glomeruli where local complement activation results in glomerular injury.³

Nephritis is a common complication of SLE, occurring in 14% to 55% of patients, with higher rates seen in Asian, African, and Hispanic populations.⁴ Histological patterns of lupus nephritis have been classified by the World Health Organization and, more recently, by the International Society of Nephrology/Renal Pathology Society

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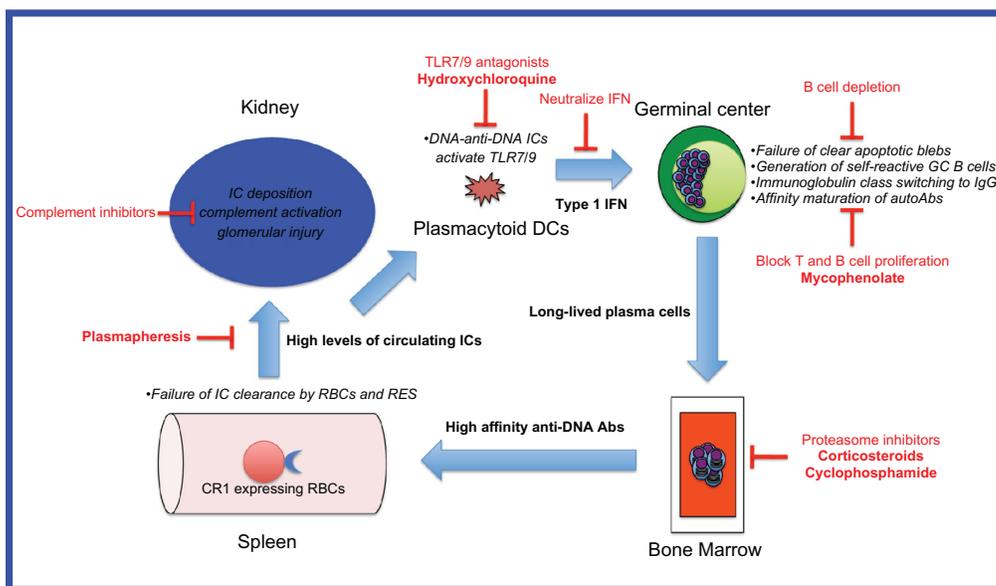


Figure 1 Model of DNA-anti-DNA immune complex generation and glomerular damage in lupus nephritis and potential therapeutic targets. **Abbreviations:** Abs, antibodies; DCs, dendritic cells; GC, germinal center; ICs, immune complexes; RBCs, red blood cells; RES, reticuloendothelial system.

(ISN/RPS) (Table 1).⁵ These histologic patterns are predictive of prognosis⁶ and provide a basis for treatment guidelines to prevent end-organ damage and improve mortality and morbidity. Despite improvements in the long-term survival of patients with SLE,⁷ patients who develop nephritis still

have a worse prognosis with a 10-year survival of only 88% compared with 94% for patients without nephritis.⁸

The mainstay of treatment for lupus nephritis has been corticosteroids, azathioprine, cyclophosphamide and, more recently, mycophenolate. These drugs are toxic with

Table 1 Classification of lupus nephritis

Class	WHO (1982)	ISN/RPS ^b (2003)
I	Normal glomeruli a. Nil by all techniques b. Normal LM, deposits by EM/IF	Minimal mesangial • Normal LM. Deposits by IF ± EM. No longer includes those with normal IF/EM
II	Pure mesangial a. Mesangial widening or hypercellularity b. Moderate hypercellularity	Mesangial proliferative
III	Focal segmental ^a a. 'Active' necrotizing lesions b. 'Active' and sclerosing lesions c. Sclerosing lesions	Focal (<50% glomeruli) ^c A. Active lesions A/C. Active and chronic lesions C. Chronic inactive lesions with scars
IV	Diffuse ^a a. Without segmental lesions b. 'Active' necrotizing lesions c. 'Active' and sclerosing lesions d. Sclerosing lesions	Diffuse segmental (IV-S) or global (IV-G) ^d (≥50% glomeruli segmental or global lesions). A, A/C, C as above
V	Diffuse membranous a. Pure membranous b. Assoc class IIa/b lesions c. Assoc class IIIa-c lesions d. Assoc class IVa-d lesions	Membranous May occur in combination with class III or IV, in which case both will be diagnosed
VI	Advanced sclerosing ^a	Advanced sclerosing (≥90% of glomeruli globally sclerosed without residual activity)

Notes: ^aPercentage was not stipulated in the 1982 modification of the WHO criteria; ^bIndicate and grade (mild, moderate, severe) tubular atrophy, interstitial inflammation and fibrosis, severity of arteriosclerosis or other vascular lesions; ^cIndicate the proportion of glomeruli with active and with sclerotic lesions; ^dIndicate the proportion of glomeruli with fibrinoid necrosis and cellular crescents.

Abbreviations: EM, electron microscopy; IF, immunofluorescence; ISN/RPS, International Society of Nephrology/Renal Pathology Society; LM, light microscopy; WHO, World Health Organization.

significant side effects and, despite their use, up to 20% of patients with nephritis will still progress to end-stage renal failure and require renal replacement therapy. It is timely therefore to re-examine the role of immune complexes in the pathogenesis of lupus nephritis and update the current status of new therapeutic strategies that target immune complexes.

DNA-anti-DNA immune complexes in the pathogenesis of lupus nephritis

Raised serum levels of circulating immune complexes have long been described in lupus, and correlate with disease activity.⁹ The role of anti-DNA antibodies in lupus nephritis is also well documented, and the evidence for the involvement of complexes containing these autoantibodies is summarized in Table 2. Despite the evidence linking DNA-anti-DNA immune complexes to lupus nephritis, the precise mechanism of renal damage is still unknown. In the prevailing hypothesis, nucleosomes released from apoptotic cells bind to autoantibodies and deposit in glomeruli, resulting in complement activation and thus tissue injury. An alternative hypothesis is that

anti-DNA antibodies cross-react with non-DNA components in glomeruli, but this is thought to be less likely.¹⁰

Doubts about the importance of DNA-anti-DNA immune complexes arise because not all patients with anti-DNA antibodies develop lupus nephritis. Furthermore, glomerular immune complex deposition may be seen without clinically overt renal disease,¹¹ suggesting that additional factors are necessary for the development of renal pathology. Particular characteristics of anti-DNA antibodies may make some more nephritogenic than others. For example, it has been postulated that the isotype and subclass of the antibody is important. In particular, the IgG isotype¹² and specifically the IgG3^{13,14} or IgG2¹⁴ subclasses present a higher risk of clinical nephritis. Although there is some evidence that avidity of anti-dsDNA antibodies may also play a role *in vitro*,^{15,16} their role *in vivo* has been questioned.^{10,17}

The specificity of anti-DNA antibodies is another important factor in pathogenicity. A specificity for nucleosomes rather than DNA,¹⁰ the presence of cationic moieties that bind to negatively charged glycosaminoglycans such as heparan sulfate,¹⁸ and cross-reactivity of antibodies with alpha-actinin¹⁹ are linked to an increased likelihood of renal pathology. Consistent with the idea of immune complex-mediated damage being central to the pathogenesis of lupus nephritis, the availability of extra-cellular chromatin¹⁷ has been identified as another factor linked to the development of nephritis. Abnormalities in DNA fragmentation as a result of reduced levels of the endonuclease DNase I have been identified in mouse models of lupus nephritis, perhaps predisposing to the deposition of chromatin in glomeruli.²⁰

Once DNA-anti-dsDNA immune complexes have been formed, they are normally cleared by the RES but defects of some of the clearance mechanisms have been described in SLE, including aberrant interactions with Fcγ receptors (FcγRs), complement and complement receptors, and anti-C1q antibodies. With respect to the first of these interactions, a particular polymorphism in FcγRIIB is associated with SLE in Asian populations.²¹ FcγRIIB has a cytoplasmic tail which mediates inhibitory functions. Therefore, FcγRIIB signaling is important in controlling the immune response, and deficiency may predispose to autoimmunity.²¹ The activating FcγRs are also involved in the pathogenesis of lupus nephritis. Immune complex binding to FcγRI and FcγRIII trigger monocytes and macrophages to release proinflammatory mediators and chemokines which recruit immune effector cells that contribute to renal damage.^{22–24} Increased expression of FcγRI on monocytes has been found to correlate with the presence of active lupus nephritis.²³ Secondly, genetic variations in C4 can

Table 2 Evidence for role of DNA-containing immune complexes in the pathogenesis of lupus nephritis

Murine models	<ul style="list-style-type: none"> • Anti-DNA antibodies bind directly to non-DNA antigenic structures in normal glomeruli^{62–64} • Immune complexes containing nucleosomes/DNA bind to glomerular basement membranes⁶⁵ and glomerular capillary walls.⁶⁶ These might serve to localize antibody-mediated injury to the glomerulus. Co-deposition of C3 complement suggests that these antibodies might initiate complement-mediated damage⁶⁵ • Transgenic mice expressing anti-DNA antibodies who are not otherwise predisposed to develop lupus, develop nephritis⁶⁷ • Infusion or transfer of anti-DNA antibodies causes nephritis^{68–70}
Human studies	<ul style="list-style-type: none"> • Chromatin colocalises with autoantibodies in glomerular-membrane-associated electron dense structures in nephritic kidneys⁷¹ • Correlation between presence^{11,72,73} and increasing levels⁷⁴ of anti-dsDNA antibodies and lupus nephritis • Correlation between presence of antinucleosome antibodies and lupus nephritis⁷³ • Persistently high titers of anti-dsDNA antibodies are a poor prognostic factor in proliferative lupus nephritis⁷⁵ • Patients with sustained reductions in anti-dsDNA antibodies 5–7 × less likely to have nephritic flares in a study of treatment with abetimus⁷⁶

Table 3 Trials of induction therapies for lupus nephritis

Class of nephritis ⁵	Study design	Intervention 1	Intervention 2
Trials of various cyclophosphamide regimens			
IV	RCT	Prednisone (av 40 mg/d). Maintenance prednisone	Prednisone (av 29 mg/day) plus PO CYC (average 107 mg/ day) for 6 months. Maintenance prednisone
WHO III, IV, Vc, Vd	RCT	IV CYC 0.5 g/m ² monthly (increased acc to nadir WBC to max 1.5 g) for 6 months followed by 2 quarterly pulses. AZA 2 weeks after last CYC	Low dose IV CYC: 500 mg fortnightly × 6 doses. AZA 2 weeks after last CYC
Not classified	RCT	Monthly IV CYC 750 mg/m ² for 6 months followed by quarterly IV CYC for 2 years	High dose (50 mg/kg) IV CYC for 4 days
Proliferative	RCT	IV CYC 10 mg/kg every 3 weeks for 4 doses. Then PO CYC 5 mg/kg for 2 days every 4 weeks for 9 months; then every 6 weeks for 12 months	PO CYC 2 mg/kg/day for 3 months then AZA 1.5 mg/kg/day
Proliferative	Phase I/II pilot study	PO CYC 0.5 g/m ² BSA monthly with SC fludarabine 30 mg/m ² on days 1–3 for 3–6 cycles	–
Trials of mycophenolate vs IV cyclophosphamide			
V	Pooled analysis of pure class V nephritis from two studies ^{83,84}	MMF 2.5–3.0 g/day	IV CYC as per NIH protocol
III, IV or V	RCT	MMF target dose 3 g/d	IV CYC NIH protocol; median dose received 0.75 g/m ²
III, IV or V	Meta-analysis of Ginzler 2005 ⁸³ and Ong 2005 ⁸⁶	MMF 1 g bid for 6 months ⁸⁶ . MMF pushed up to 3 g daily if tolerated ⁸³	IV CYC 0.75–1.0 g/m ² monthly for 6 months. ⁸⁶ NIH IV CYC ⁸³
Miscellaneous trials of conventional immunosuppressant agents			
Various	Retrospective review of Hopkins Lupus Cohort	Addition of tacrolimus to MMF in those failing MMF	–
WHO III, IV, Vc, Vd	RCT	AZA 2 mg/kg/day and pulse MP (3 × 3 pulses of 1 g over 2 years)	IV CYC 750 mg/m ² (13 doses over 2 years)
III or IV	RCT	CSA 4–5 mg/kg/d for 9 months, gradually decreasing (3.75–1.25 mg/kg/d) over next 9 months	IV CYC 8 doses of 10 mg/kg IV over 9 months, then 4–5 × PO at same dose ever 6–8 weeks
Trials of rituximab			
III, IV, V	Systematic review including 9 uncontrolled studies and 26 case reports (not including other papers listed in this table)	Various regimens of RTX. 52% had concomitant IV CYC	–
III or IV	RCT	RTX monotherapy. 1000 mg IV 2 doses 2 weeks apart	RTX + IV CYC. As for group I but with IV CYC 750 mg following the first dose of RTX
WHO IV or V	Retrospective study of refractory LN	RTX 375 mg/m ² 2 doses 2 weeks apart accompanied by IV CYC 500 mg each time	–

Table 3 (Continued)

Number of LN patients (intervention 1 vs 2)	Duration of follow-up	Outcome	Reference
26 vs 24	4 years	Lower relapse rate in PO CYC group (48% vs 14%) with steroid sparing effect of PO CYC	Donadio et al ⁷⁷
46 vs 44	10 years	Similar outcomes for renal remissions, renal flares, death, doubling of creatinine (12%), ESRD (7%)	Houssiau et al (Euro-Lupus Nephritis Trial) ^{78,79}
26 vs 21	30 months	64% vs 20% complete renal response (P = 0.08)	Petri et al ⁸⁰
16 vs 16	3.3 years	No difference in efficacy	Yee et al ⁸¹
13	2.6 to 6.7 years	Severe myelosuppression – study terminated	Illei et al ⁸²
42 vs 42	6 months	Similar outcomes for urine protein, change in urine protein, complete and partial remission rates	Radhakrishnan et al ⁸⁵
185 vs 185	24 weeks, maintenance phase reported below	Similar response rate (56% vs 53%)	Appel et al (ALMS group) ⁸⁴
90 vs 94	6 months ⁸⁶	Complete remission rate after induction therapy higher in MMF group	Zhu et al ⁸⁷
7	2–54 months	Frequent toxicity, infrequent success (1 patient achieved complete renal remission)	Lanata et al ⁸⁸
37 vs 50	5.7 years	Relapses more frequent in AZA group (RR8.8). Higher chronicity and activity indices on repeat biopsy in AZA group	Grootcholten (Dutch Working Party on SLE) ^{89,90} ; Chan ⁹¹
19 vs 21	18 months	CSA as effective as CYC	Zavada et al (Cyclofa-Lune study) ⁹²
103 with lupus nephritis (188 SLE in total)	17 months	Renal response 91%. CRR 67%, PRR 33%. Higher response rate in those having concomitant CYC than those who did not. Lymphoma regimen (375 mg/m ² × 4 doses) appeared more effective	Ramos-Casals et al ⁹³
9 vs 10	48 weeks	No difference in CRR (21%) or PRR (58%). Rituximab effective as induction therapy	Li et al ⁹⁴
7 with refractory LN	18 months	3/7 had CRR, 4/7 had PRR. Most had disease flares 6–12 months after B cell repopulation	Lateef et al ⁹⁵

(Continued)

Table 3 (Continued)

Class of nephritis ⁵	Study design	Intervention 1	Intervention 2
WHO III or IV (not all biopsied)	Observational	RTX 1000 mg days 1 and 15. Added to current immunosuppressive treatment	–
WHO III–V	Retrospective	RTX 275 mg/m ² weekly for 4 doses; IV CYC 500–100 mg 3 weeks apart for 2 doses	–
ISN III or IV	RDBPCT	RTX 1000 mg on days 1 and 15; repeated at 6 months. Background MMF target dose 3 g/day	Placebo + MMF target dose 3 g/day
ISN III–V	Prospective observational registry	RTX, various protocols	–

affect the handling of immune complexes. Deficiency in C4A relative to C4B is common in SLE, and has also been associated with the development of lupus nephritis. C4A prevents immune complex precipitation, and therefore deficiency could result in increased deposition. A number of other variants of C4 may promote or protect against immune complex damage.²⁵ Thirdly, immune complexes that bind and activate complement can also be cleared by high affinity complement binding receptor type 1 (CR1, CD35). SLE patients have reduced expression of CR1 on their erythrocytes, perhaps contributing to defective clearance of immune complexes.²⁶ Fourthly, the presence of anti-C1q antibodies can influence the handling of immune complexes. These autoantibodies have been associated with the presence and activity of lupus nephritis.^{27,28} Infusion of anti-C1q antibodies results in deposition in glomeruli in mice, which are not pathogenic unless C1q-fixing anti-glomerular basement membrane antibodies (at subnephritogenic doses) are also present.²⁹ It is thought that anti-C1q antibodies amplify the complement cascade by themselves fixing and activating complement, recruiting further anti-C1q antibodies, and thus increasing the risk of renal damage.

Immune complexes containing DNA signal via TLR-9 and can activate plasmacytoid dendritic cells,³⁰ which then process and present chromatin-derived peptides to costimulate T helper cells. Chromatin-specific T cells interact with dsDNA-specific B cells, facilitating the secretion of anti-DNA antibodies. Renal damage results from a combination of complement activation and cellular inflammation. A Th1 response is associated with diffuse proliferative lupus nephritis, while a Th2 response is associated with the membranous form.³¹ Thrombotic microangiopathy caused by antiphospholipid antibodies may also contribute to the final pathology.

Therapies of lupus nephritis targeting immune complex formation

The mainstays of treatment of lupus nephritis are corticosteroid therapy combined with cyclophosphamide or mycophenolate for induction therapy, and corticosteroids combined with azathioprine or mycophenolate for maintenance therapy. The evidence for the use of these agents is summarized in Tables 3 and 4. These agents are not specifically targeted at the reduction of DNA-anti-DNA immune complexes per se. However, a reduction in autoantibody formation and hence immune complex generation occurs after the broad immunosuppression caused by these agents, and a decrease in serum anti-dsDNA antibody levels accompanied clinical improvement in most studies listed.

Specific strategies for targeting immune complex formation include: reducing autoantibody production (targeting B cells), reducing the binding of autoantibodies, reducing the availability of nucleosomal material, increasing the clearance of immune complexes, and interfering in the feedback loop (Figure 1). This is a theoretical framework, and while the mechanisms of action of some of the currently used treatments for lupus nephritis may fall into these categories, further research is necessary in each of these areas to understand their mechanisms and potential clinical efficacy.

Reducing autoantibody production (targeting B cells and plasma cells)

Theoretically, autoantibody production may be reduced by depletion of B cells (either by targeting B cell surface molecules or by removing factors required for B cell survival); interfering with the development or function of plasma cells; or by inducing B-cell tolerance.

Table 3 (Continued)

Number of LN patients (intervention 1 vs 2)	Duration of follow-up	Outcome	Reference
13 Hispanic with active lupus nephritis	6 months	38% CRR, 38% PRR	Garcia-Carrasco et al ⁹⁶
28 (WHO III and IV) and 15 (WHO V)	12 months	Membranous and proliferative LN respond similarly to rituximab	Jonsdottir et al ⁹⁷
72 vs 72	–	No difference in renal response despite better serological response in rituximab group	Furie et al (LUNAR) ³³ ; Looney ³⁴
42	>3 months	CRR in 45%, PRR in 29% (total renal response rate 74%)	Terrier et al (French Autoimmunity and Rituximab Registry) ³⁶

Note: All studies are with corticosteroids in both arms, unless specified.

Abbreviations: AZA, azathioprine; bid, twice daily; CRF, chronic renal failure; CRR, complete renal response; CSA, cyclosporine A; CYC, cyclophosphamide; ESRD, end stage renal disease; IV, intravenous; LN, lupus nephritis; MMF, mycophenolate mofetil; PO, per oral; PRR, partial renal response; RCT, randomized controlled trial; RDBPCT, randomized double-blinded placebo-controlled trial; RTX, rituximab.

B cell depletion

Anti-nucleosome and anti-dsDNA antibodies are modestly reduced by anti-CD20 mAbs such as rituximab, which effect B cell depletion. The reduction in these titers suggests that these autoantibodies are produced by a B cell population with more rapid turnover than cells that produce anti-ENA, anti-tetanus or antipneumococcal antibodies, which persist. However, their incomplete reduction may reflect the presence of longer-lived plasma cells which do not express CD20.³² Trials of rituximab, however, have yielded conflicting results on clinical endpoints (see Table 3). Uncontrolled, observational, and retrospective studies seemed to demonstrate benefit in lupus nephritis, but two major randomized trials failed to find benefit. The LUPus Nephritis Assessment with Rituximab (LUNAR) trial, which specifically included patients with proliferative lupus nephritis, did not demonstrate any difference in the proportion of patients obtaining a renal response to rituximab compared with placebo.³³ However, the use of mycophenolate rather than cyclophosphamide as background therapy in this trial has been criticized, as it is thought that the effects of rituximab may be enhanced, or synergistic, with cyclophosphamide.³⁴ Further, given that all participants were treated with mycophenolate, any effect of rituximab may have been masked. The other major trial, Rituximab in patients with Severe Systemic Lupus Erythematosus (EXPLORER),³⁵ excluded major organ threatening disease and thus <2% of the patients had renal involvement. This trial found no difference in the rituximab compared with placebo-treated groups, but given its patient characteristics, this finding cannot be applied to patients with lupus nephritis. Contrary to these findings, prospective follow-up of 31 patients with lupus nephritis from a cohort of 136 patients entered in the French Autoimmunity and Rituximab registry,³⁶ demonstrated renal

response in 74% of patients, with complete response in 45%. Unfortunately, trials of another anti-CD20 agent, ocrelizumab (BELONG), for lupus nephritis have been halted due to concerns over serious and opportunistic infections.³⁷

A plethora of other anti-B cell therapies is on the horizon, targeting all aspects important for B cell existence and function, such as survival factors, differentiation factors, co-stimulatory factors, cell-signaling pathways, and homing factors.³⁸ Most of these studies are in preliminary phases, or have not been evaluated in human lupus nephritis. Belimumab, a fully human recombinant monoclonal antibody that binds to and inhibits B lymphocyte stimulator (BLyS, also known as B cell activating factor or BAFF) has been shown to reduce anti-dsDNA titers by 29%, but patients with lupus nephritis were excluded from early trials.^{39,40} More recently, the large phase III studies, BLISS-52 and BLISS-76, have shown promise with improvements in the SLE responder index, though more information on lupus nephritis and belimumab is awaited. The Food and Drug Administration has granted this drug a priority review designation as a potential treatment for SLE (GSK press release August 19, 2010). Epratuzumab, targets CD22, a surface molecule involved in regulating B cell receptor signaling, and modifies B cell function. A phase II study has had promising results, with improvements in BILAG scores despite lack of reduction in anti-dsDNA levels, although there were too few patients with lupus nephritis to draw any conclusions about efficacy in this domain.⁴¹

Targeting plasma cells

If B cell depletion does indeed reduce immune complexes it may do so indirectly by killing the precursor germinal center B cells that give rise to antibody-secreting plasma cells. To reduce autoantibody production more effectively,

Table 4 Trials of maintenance therapies in lupus nephritis. All received glucocorticoids unless otherwise specified

Class of nephritis ⁵	Study design	Induction	Maintenance strategy 1	Maintenance strategy 2
WHO III, IV, Vb	RCT	IV CYC 0.5–1.0 g/m ² monthly for 7 doses	AZA 1–3 mg/kg day	MMF 1.5 g/day for 12 months then weaned Maintenance strategy 3: IV CYC 0.5–1.0 g/m ² every 3 months
III–V	Retrospective study	IV CYC	AZA 2 mg/kg/day	MMF 1.5–2.0 g/day
Proliferative	RCT	Eurolupus IV CYC (500 mg × 6 fortnightly doses). Maintenance Rx started at week 12. Renal response not required prior to commencing maintenance	AZA 2 mg/kg/day target dose	MMF 2 g/day target dose
III–V	RDBPCT	MMF vs IV CYC. Patients who achieved partial or complete response re-randomized at week 24	AZA 2 mg/kg/day	MMF 2 g/day
WHO IV, Vc, Vd	RCT	PO CYC 1–2 mg/kg/day for 3 months	AZA 2 mg/kg/day for 1 month then optional reduction to 1.5 mg/kg/day if well controlled	CSA 4 mg/kg/day for 1 month then weaned to 2.5–3.0 mg/kg/day keeping trough level of 75–200 ng/mL

agents targeting plasma cells specifically may be more useful. Indeed, corticosteroids may well exert their beneficial effect by this mechanism, among others. Proteasome inhibitors have been introduced into the therapeutic armamentarium for multiple myeloma due to their ability to cause apoptosis of plasma cells.⁴² The use of proteasome inhibitors in SLE has been promising in mouse models, eliminating autoreactive plasma cells, reducing anti-dsDNA antibody levels, and preventing nephritis;⁴³ human trials are underway.

Induction of B cell tolerance

Induction of tolerance would be the ultimate way to reduce anti-dsDNA antibody concentrations. Although murine models have provided hope, human trials have again been unimpressive. Regular injections of nucleosomal peptide autoepitopes in lupus-prone mice reduced autoantibody levels and delayed the onset of nephritis by the induction of TGF-producing regulatory T cells.⁴⁴ However, abetimus, a conjugate composed of 4 identical strands of dsDNA, did not show any benefit in reducing renal flares in human SLE. Interestingly, abetimus did reduce the level of anti-dsDNA antibodies, possibly due to the formation of soluble complexes that were rapidly eliminated and, possibly, by tolerizing B cells and reducing autoantibody production.⁴⁵

Reducing the binding of autoantibodies

The mechanism of action of the antimalarials chloroquine and hydroxychloroquine in SLE has recently been revisited, because of the recognition of their inhibition of TLR-9

binding to DNA, by preventing acidification of the lysosome. However, hydroxychloroquine, as one of its many mechanisms of action, also affects the affinity of binding of antibodies to their targets. Hydroxychloroquine interferes with the binding of antiphospholipid antibodies in vitro, and causes a reduction in the levels of these autoantibodies as measured by commercially available ELISAs.⁴⁶ We recently demonstrated that the binding of anti-dsDNA antibodies as measured by the modified Farr assay is reduced by the addition of hydroxychloroquine in vitro.⁴⁷ This effect is likely to be due to the high protein-binding capacity of hydroxychloroquine,⁴⁸ and intercalation of DNA (if sharing this property with chloroquine),^{49,50} potentially modifying critical autoepitopes. Whether this affects the pathogenesis of human lupus nephritis is unknown.

Reducing the availability of DNA and nucleosomal material

Material for anti-dsDNA and antinucleosome antibodies to bind may originate from tissue damage in the kidneys, resulting in situ formation of complexes or, alternatively, from damage remotely, resulting in the formation of circulating immune complexes, which then deposit in glomeruli (reviewed by Fisman et al⁵¹). A phase Ib trial of recombinant human DNase I (rhDNase) to hydrolyze extracellular DNA in patients with lupus did not reduce anti-dsDNA levels, the concentrations of circulating immune complexes, nor change other serological markers.⁵² No further studies of rhDNase have been published.

Table 4 (Continued)

Number of LN patients (strategy 1 vs 2 vs 3)	Duration of follow-up	Outcome	Reference
19 vs 20 vs 20	72 months	Relapse free survival highest in MMF group (77%) vs AZA (57%) and IV CYC (43%). MMF and AZA better for composite endpoint of death or CRF	Contreras et al ^{98,99}
15 vs 17	41 months	CRR similar (60% vs 58%)	Sahin et al ¹⁰⁰
52 vs 53	14 months	Renal relapse rate similar (25% vs 19%)	Houssiau et al (MAINTAIN trial) ¹⁰¹
227	Not yet published in full	MMF superior to AZA in delaying time to treatment failure (composite of death, serious renal damage, renal relapse)	Wofsy et al (ALMS group) ¹⁰²
33 vs 36	4 years	Similar rates of SLE flare (13.4 vs 10.6 flares per 100 patient years), proteinuria and creatinine clearance	Moroni et al ¹⁰³

Abbreviations: see Table 3.

Increasing the clearance of immune complexes

Plasmapheresis is able to lower the titer of anti-dsDNA antibodies but does not necessarily result in sustained clinical remission once withdrawn, possibly due to compensatory increased production by pathogenic B cell clones (rebound effect).⁵³ Removal of pathogenic anti-dsDNA antibodies physically by plasmapheresis may improve outcomes for those receiving intravenous (IV) cyclophosphamide. A combination of plasmapheresis and IV cyclophosphamide results in higher rates of complete renal remission than IV cyclophosphamide alone.^{54,55} However, not all trials have found benefit, and larger randomized trials are required to confirm these findings. Other small studies and case reports have also demonstrated benefit with immunoadsorption plasmapheresis,^{56,57} but further investigation is required to clarify the role of this treatment.

Breaking the feedback (amplification) loop

Signaling of immune complexes containing RNA and DNA via TLRs 7 and 9, respectively, activates plasmacytoid dendritic cells to produce large amounts of type I interferon. Type I interferons activate B cells and enhance antibody responses to soluble proteins, thereby completing a feedback loop resulting in the increased production of immune complexes.⁵⁸ One of the mechanisms of action for antimalarial drugs such as hydroxychloroquine in lupus is thought to be the inhibition of nucleic acid interaction with

intracellular TLRs 7 and 9, possibly as a result of an increase in pH in microsomal compartments.⁵⁹ Novel treatments aimed at blocking TLR7 and TLR9 are being developed.⁶⁰ A phase I study of an anti-interferon- α monoclonal antibody has been completed,⁶¹ and a phase II study is underway.

Conclusion

Immune complexes containing IgG anti-dsDNA antibodies and DNA play a significant role in the complex pathogenesis of lupus nephritis. Although strategies specifically aimed at reducing immune complexes in SLE are mostly novel, they provide a fertile area for further research. Disappointments with early trials of new therapeutics strengthen the argument that a combination of strategies aimed at different pathogenic mechanisms is likely to be necessary to improve the prognosis of this disease.

Disclosure

The authors report no conflicts of interest.

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