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Novel Therapeutic Approaches to Lupus Glomerulonephritis: Translating Animal Models to Clinical Practice

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Abstract

Systemic lupus erythematosus is a chronic autoimmune disease frequently affecting the kidney. Renal involvement is characterized by glomerular immune complex deposits, and proliferative glomerulonephritis progressing to glomerulosclerosis and kidney failure. Development of systemic lupus erythematosus is genetically regulated and lupus susceptibility genes have been linked to immune hyper-responsiveness and loss of immune regulation. In addition to the systemic immune defects, recent studies in animal models show that susceptibility to lupus nephritis is influenced by intrinsic renal factors. Thus, renal cell responses to immune-mediated glomerular injury determine disease outcome. This supports the idea that future treatments for lupus nephritis need to focus on regulating end organ responses. The feasibility of this approach has been demonstrated in animal models of kidney disease. For over 50 years, the emphasis in management of lupus nephritis has been suppression of autoimmune responses and systemic control of inflammation. This review describes recently developed targeted drug delivery technologies and potential targets that can regulate glomerular cell responses offering a novel therapeutic approach for lupus nephritis.

INDEX WORDS

Mesangial cells; glomerulonephritis; immunoliposomes; glomerular targeting; mouse models; gene therapy; lupus nephritis

BACKGROUND

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of circulating immune complexes and serum antibodies to nuclear and cytoplasmic antigens1. Deposition of auto-antigen-antibody complexes in different tissues and the ensuing inflammatory response is a major cause of organ damage. In the kidney, immune complex deposits are detected in the glomerular mesangium and on the basement membrane. This initiates an inflammatory cascade that leads to progressive kidney disease in

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susceptible individuals. Suppression of systemic auto-immune responses is the primary treatment strategy; therefore, immuno-suppressive and anti-inflammatory agents like cyclophosphamide, mycophenolate and corticosteroids have been obvious choices to induce disease remission in lupus nephritis for the past several decades2–3. However, evidence in mouse models of lupus-like glomerulonephritis (GN) suggests a critical role for the end organ in disease progression. This review will discuss the role of end organ responses in lupus-like GN and strategies for modulating these responses as a novel therapeutic approach.

CASE VIGNETTE

A 40-year-old African-American female was referred to the nephrology clinic for one month history of lower extremity edema and 5.7 grams of proteinuria. Urine microscopy showed 10–20 red blood cells per high power field, most of them dysmorphic, and a few red blood cells casts. Results of laboratory investigation are shown in Table 1. Her clinical picture was compatible with lupus nephritis so a kidney biopsy was performed. On light microscopy there was mild mesangial hypercellularity but no glomerular crescents, fibrinoid necrosis or glomerulosclerosis. There was a mild interstitial lymphocytic infiltrate, but no significant tubular atrophy, interstitial fibrosis or vascular inflammatory lesions. Immunofluorescence microscopy had a fine granular capillary loop staining and a “full-house” picture. Electron microscopy showed frequent electron dense supepithelial and rare subendothelial and paramesangial deposits. Pathologic diagnosis of class II and V lupus nephritis with no tubulointerstitial, or vascular activity, and no chronic damage was made.

After discussing the risks and benefits of different options, treatment with mycophenolate mofetil (MMF) 250 mg twice a day and prednisone 60 mg once a day was initiated. The dose of MMF was increased to 500 mg twice a day, but no further increases were possible because of neutropenia. Eight months after the initial biopsy she had an episode of acute kidney injury with maximum serum creatinine of 3.4 mg/dL (300.56 μmol/L). A kidney biopsy confirmed acute tubular necrosis and interstitial nephritis due to the use of high dose non-steroidal anti-inflammatory drugs. There were no active glomerular lesions. Once her white cell count normalized, the dose of MMF was increased to 750 mg twice a day. After over 4 years of treatment and almost 6 months after increasing MMF dose, she was in remission (month 51, Table 1). However, soon after reducing MMF and prednisone doses, she had a relapse with hypocomplementemia, increased dsDNA antibody titers and proteinuria.

PATHOGENESIS

Human SLE Is a Complex Autoimmune Disease

SLE affects multiple organ systems with clinically heterogeneous outcomes making it a difficult disease to study in humans. A systemic autoimmune response is the hallmark of SLE, which has been attributed to a failure of tolerance mechanisms[4], aberrant T cell signaling pathways[5], and reduced thresholds for immune cell activation[6]. Renal involvement is a major cause of morbidity and mortality in lupus. Up to 60% of all patients with SLE have involvement of the kidneys at the time of presentation or some time during the course of their disease. Individuals with lupus nephritis have lower survival rates compared to those without renal involvement [7]. A study by Pollock and Pirani on 87 SLE patients showed that even within the subset of patients with lupus nephritis, the natural history of disease is significantly diverse[8]. During an 8 year follow up, patients with evidence of glomerulonephritis on the first biopsy showed significant deterioration of kidney function. Of the others, 27 patients showed minimal glomerular changes with only focal mesangial deposits, and endothelial hypercellularity. Most of these 27 patients had significantly better outcomes; however, 2 of these 27 showed a rapid progression to kidney failure. Thus,

disease severity at presentation and rate of progression vary widely among patients and are regulated by factors not completely understood. The complexity of SLE is also reflected in the large numbers of candidate susceptibility genes identified by genome wide analyses[9], yet all these account only for ~15% of SLE heritability.

Serum autoantibodies are a dominant feature associated with lupus. Studies by Yurasov et al. showed that mature, naïve autoreactive B cells persist and expand in patients even during remission10. In addition to autoantibody production, B cells are also important for antigen presentation to autoreactive T cells11. Therefore, B cell depletion would be expected to alleviate progressive SLE. Rituximab is a mouse-human chimeric antibody that recognizes CD20, a surface protein on developing and mature B cells12. It is approved for treatment of follicular B cell non-Hodgkin's lymphoma refractory to other treatments and is well tolerated. Compared to placebo, treatment with rituximab in addition to mycophenolate moefetil induced B cell depletion and was associated with significantly greater reduction in anti-double-stranded DNA antibody titters (69% vs. 50%, $p < 0.01$) and greater increase in C3 complement component (37.5 vs. 25.9 mg/dL, $p < 0.03$), but failed to affect the primary outcome of the study of partial or complete remissions at week 52 ($p = 0.55$)13–14. These results are similar to the findings of the EXPLORER (Exploratory Phase II/III SLE Evaluation of Rituximab) trial, in which treatment of patients with moderate to severe SLE (no kidney disease) with rituximab versus placebo was associated with reduction of anti-dsDNA antibody and an increase in complement levels but no difference in clinical outcomes 15.

Experiments in mouse models showed that B cells are more sensitive to cyclophosphamide and show a rapid reduction following treatment[16]. However, the B cells that re-emerge after cessation of treatment are selectively enriched for the pathogenic, high affinity anti-DNA autoantibodies[16]. A similar effect, if present in humans, may explain the low efficacy of rituximab in lupus.

B cell reconstitution following cyclophosphamide treatment is dependent on B cell activating factor (BAFF) belonging to the TNF family Thus, inhibition of BAFF prevents B cell stimulation, proliferation and prevents autoantibody production. In mice, treatment with an anti-BAFF antibody delays disease progression[17]. These studies in mouse models have been successfully extended to humans and an anti-BAFF antibody (belimumab) has recently been approved by the FDA for therapy 18. This is a major breakthrough for lupus treatment and clinical use will help define the subsets of patients that will benefit from belimumab treatment 19.

As shown in the case presented here, high anti-dsDNA antibody levels, hypocomplementemia and glomerular immune complex deposits persist despite significant improvement in kidney disease. In addition, the rituximab trials show that reduction of anti-dsDNA antibody titers is not associated with a corresponding decrease in pathology. These data emphasize the dissociation between anti-dsDNA antibody and kidney disease. This feature has been replicated in several animal models and the salient features of the spontaneous and induced models of lupus-like GN are discussed in the next section.

Models of Spontaneous SLE

Animal models have provided a better understanding of SLE pathogenesis. As discussed above, they have aided the design and evaluation of new candidate therapeutic agents. Some of the most commonly used mouse strains include the New Zealand (NZ) derived NZ Black × NZ White (NZB/W) F1, NZ Mixed (NZM) 2328, NZM2410, MRL lpr/lpr, and BXSB[21–23]. All these strains spontaneously develop anti-dsDNA antibodies, glomerular immune complex deposits and fatal GN (figure 1). Like the human disease, the kidney pathology

manifests over a long duration (5–9 months of age) and shows features of chronic progressive GN. No single mouse model can recapitulate all the characteristics of the human SLE spectrum; however, the kidney disease and immune responses in each mouse strain would represent a subpopulation of human patients. In addition to insights into pathogenesis of proliferative lupus nephritis, these murine models of spontaneous SLE have been used extensively to investigate genes dictating susceptibility to autoantibody production, proliferative GN, disease progression and mortality [22, 24, 25]. The mechanistic differences between murine and human SLE, specifically with reference to the role of Fc Receptors, complement and immune complex clearance has been previously reviewed 26. However, there are also significant similarities between the spontaneous lupus nephritis-like disease in mice with human lupus nephritis (Table 2). A critical feature to note is that while studies in humans focus on systemic immune responses based on peripheral blood with or without a kidney correlate, inbred mouse strains allow dissection of factors affecting systemic autoimmunity from those causing kidney disease. Another significant advantage of mouse models over human studies is the ability to study initiation of early renal change and the role of individual pathogenic factors affecting disease progression prior to the manifestation of clinical decreased kidney function.

Models of Immune Complex GN

Induced models of GN in rats and mice are also a powerful resource for the study of kidney disease. Lupus nephritis is considered a prototype immune complex mediated disease. In mouse models, injection of mouse antibodies reactive with glomerular components induces a transient proteinuria, but rarely progresses to glomerular disease[27]. To induce glomerulonephritis, rats or mice are immunized with heterologous IgG in complete Freund adjuvant, typically from sheep or rabbit[28–29] (Figure 1). This is followed by infusion of hyper-immune anti-GBM sheep or rabbit serum respectively, leading to glomerular IgG deposits. The endogenous anti-rabbit or sheep IgG responses reacting with the anti-GBM antibody leads to glomerular immune complex deposits and GN. In some models, immunization is not required and infusion of sheep anti-GBM serum alone is sufficient to induce immune-complex GN. Immune complex GN induced using these protocols causes a rapid loss of kidney function indicated by severe proteinuria, elevated serum urea nitrogen, and serum creatinine. The two major features distinguishing induced models from spontaneous lupus GN are the absence of circulating autoantibodies, and the lack of progression to chronic GN. However, a careful comparison of early glomerular changes, cellular infiltrates and cytokine profiles has established the validity of induced immune complex GN models as representatives of early pathology in proliferative lupus nephritis [28].

RECENT ADVANCES

Over the last decade, studies in animal models show that glomerular immune complex deposition alone without ensuing inflammation or susceptible genetic background is insufficient for the development of GN and its progression to kidney failure. There is a better understanding of participation by local factors including activation in regional lymph nodes, and synthesis of inflammatory mediators by the kidney. Studies demonstrating a dominant local (renal) influence on induction and progression of GN are discussed below.

Regional Immune Responses in Lupus Nephritis

Mesangial expansion and immune cell recruitment is the hallmark of proliferative lupus nephritis. A careful analysis of GN in MRL lpr/lpr mice at different ages shows that immune complex deposition is associated with a rapid increase in production of MCP1 (monocyte chemoattractant protein 1; encoded by the *CCL2* gene) and RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted; encoded by the *CCL5* gene) by the

glomerular mesangial cells[30]. Thus, the mesangial cells are the "first responders" to immune complex injury. MCP1 and RANTES are potent chemoattractants and initiate subsequent inflammatory cell infiltration. Macrophages, dendritic cells, and CD4+ T cells are the dominant cell types seen in and around the glomerulus[31–33]. In later stages, some mouse models and patients show CD8+ T cell, B cell and plasma cell infiltrates in the tubulointerstitial regions, correlating with prognosis of the disease.

CD4+ T cells are critical for lupus nephritis, and T cell depletion using anti-CD4 antibodies can prevent GN [34]. In NZM2328 female mice, proliferative GN is associated with increased CD4+ T cell activation in the regional kidney draining lymph nodes [33]. CD4+ T cells are also increased in the intra- and peri-glomerular regions. With disease progression, there is an antigen-specific expansion of a limited T cell repertoire preferentially in the regional lymph nodes but not in non-draining lymph nodes. Significantly, this repertoire is also expanded within the kidney. Although the antigens have not been identified, a study of one of the antigenic peptide binding regions on the T cell receptors show similar profiles in the regional lymph node and the kidney suggesting that they recognize similar, potentially local antigens. This also explains the prevention of kidney disease in MRL/lpr mice treated with FTY720, a drug that binds sphingosine-1 phosphate receptor on immune cells and prevents their egress from lymph nodes into tissues [35]. Inhibition of T cell activation in severely nephritic NZB/W F1 mice also significantly delays fatal GN [36]. B7.1 and B7.2 molecules on antigen presenting cells like dendritic cells and B cells bind CD28 on T cells resulting in cytokine production and proliferation. CTLA4 Ig is a recombinant fusion protein that binds B7.1 and B7.2 preventing T cell activation. NZB/W F1 mice with severe proteinuria treated with CTLA4 Ig and suboptimal doses of cyclophosphamide showed a significant increase in survival with 93% of mice surviving 13wks compared to 36% of control mice that received cyclophosphamide alone. All these studies show that local T cell activation and infiltration into the kidney are important events in disease induction and progression. Another study showed that protection by CTLA4Ig did not affect glomerular immune complex and C3 deposition[37]. Thus, similar to the case, lack of kidney disease progression does not lead to a corresponding reduction in immune complex deposits. A model for the pathogenesis of lupus nephritis is shown in figure 2.

In addition to local activation of T cells, the susceptibility of the kidney to injury is regulated by other factors. In NZM2328 mice, depletion of thymus derived CD25+ regulatory T cells led to increased autoantibody responses, glomerular immune complexes and early onset of severe proliferative GN in male and female NZM2328 mice by 20wks of age [38]. However, by 30 wks only female mice progressed to chronic GN, while males did not. This suggests that besides immune responses, progression of kidney disease is determined by gender dependent end organ factors.

End Organ Responses in Immune Complex GN

Elegant studies in immune complex GN models have explored the factors dictating susceptibility to immune mediated glomerular injury. The importance of genetic susceptibility in development of GN is well established in studies using different strains in both rat and mouse models. Following injection of nephrotoxic serum, Wistar Kyoto (WKY) rats rapidly develop crescentic GN in 80% glomeruli by day 10 while Lewis rats remain resistant to GN [39]. These strains share the same MHC haplotype. Bone marrow transfer from WKY into Lewis rats resulted in GN in 35% of the glomeruli suggesting that bone marrow derived cells partially regulated development of GN. To study the contribution of the kidney in disease susceptibility, (WKY × Lewis) F1 rats were transplanted with kidney from either parental strain. Immune complex GN was induced in the F1 recipients and disease severity in the parental kidney transplants was compared. WKY kidneys in the F1 recipients had greater macrophage infiltration and glomerular crescent formation compared to Lewis

kidneys. There was no difference in GN in the host F1 kidneys. This difference in parental kidney disease correlated with the ability of WKY mesangial cells to produce higher amounts of MCP1 compared to Lewis mesangial cells. Thus, chemokine production by mesangial cells in response to glomerular immune complex deposits influences kidney disease.

Mohan and colleagues have studied a large panel of inbred mouse strains to investigate the genetic susceptibility to lupus-like GN [40]. Mice from a number of different strains including B6, BALB/c, A/J, DBA/1, C3H, C3HeN, C3HeJ, NZW, 129/SvJ, and SWR were immunized with rabbit IgG emulsified in complete Freund adjuvant. Anti-GBM rabbit polyclonal serum was injected and the mice studied for kidney function in the form of proteinuria, serum urea nitrogen, serum creatinine, glomerular immune complexes, renal histo-pathology and immune responses. Despite comparable responses to rabbit IgG immunization, DBA/1, NZW, 129/SvJ, C58 developed severe GN while SJL/J, SWR, NOD did not. To identify the end organ factors dictating susceptibility, 3 susceptible (DBA1, NZW, and 129/SvJ) and 2 control (BALB/c and B6) mouse strains were injected with anti-GBM antibody and kidneys harvested 10 days later [41]. RNA was isolated and gene expression evaluated by microarray analyses. Of the 50 genes consistently down-regulated in all the susceptible mouse strains, 10 genes belonged to the kallekrein (*Klk*) family of genes. The kallekreins are a family of serine proteases with diverse physiologic processes. They act through generation of bradykinins that bind bradykinin receptors. Inhibition of bradykinin receptor activity exacerbated GN in mouse strains resistant to nephritis. The molecular pathways between bradykinin receptor activation and kidney disease are unclear. In mice, the *Klk* gene locus in on chromosome 7 and falls within a genetic segment linked to lupus susceptibility. In humans, the kallekrein genes are on the orthologous chromosome 19q13 locus which has been previously implicated in lupus susceptibility. Analysis of SNPs from different SLE patient cohorts showed association of SNPs between the *KLK1* and *KLK3* genes, possibly the *KLK3* promoter region. Thus, expression of kallekreins in the kidney may be one of the factors regulating susceptibility to GN.

Targeted Delivery to Renal Glomeruli

Based on the studies discussed above, there is a need to revisit the strategy for treatment of lupus nephritis. While regulating the systemic autoimmune responses is important, increased emphasis on controlling end-organ responses would be a synergistic therapeutic approach. Targeted drug delivery increases concentrations of therapeutic agents at the site of action, maximizing efficacy while minimizing side effects [42]. Liposomes are versatile carriers for targeted drug delivery (Figure 3). Liposomes are composed of cholesterols and phospholipids forming a micelle, capable of transporting water soluble compounds in the central core, and lipid soluble compounds within the hydrophobic bilayer 43. The surface of liposomes can be conjugated to antibodies or receptors to target specific cell types. In addition, liposomes can be coated with polyethylene glycols preventing uptake by macrophages and increasing half life in circulation. The unique architecture of the renal glomerulus lends itself to targeted delivery using liposomes. The glomerular capillary is lined by a layer of endothelium with 70 to 130nM fenestrations. In the central portion, the endothelium does not have a basement membrane and rests directly on the mesangium[44]. Thus, the vascular compartment has a direct communication with the mesangial space. At these sites, liposomes with a diameter less that 130nM can leave the vasculature and deposit in the mesangial space. Away from the center, the glomerular endothelium rests on the basement membrane. The slit diaphragm (pore size of 10–70nM) in the glomerular basement membrane prevents exit of the ~100nM liposomes into the urinary space. Modalities for glomerular delivery of drugs or oligonucleotides capable of regulating local inflammatory responses in animal models are discussed in the below.

Gene Therapy to Regulate Local Inflammation

Activation and proliferation of mesangial cells are the pathologic characteristics of lupus-like GN. Inhibition of proteins in mesangial cells that regulate these processes have been described in rodent models (Table 3). Liposomes incorporated with UV inactivated Sendai Virus or Hemagglutinating Virus of Japan (HVJ) have been used to deliver decoy oligonucleotides [53–54]. Decoy oligonucleotides consist of synthetic transcription factor specific sequences. Flooding the cell with decoy oligonucleotides prevents binding of the transcription factor with specific sequences on promoter regions on target genes. E2F is a transcription factor in mesangial cells that is important for activating proliferating cell nuclear antigen and cyclin dependent kinase 2 in the cell cycle pathway 46. Thus, injection of HVJ liposomes loaded with decoy oligonucleotides to E2F prevented mesangial cell proliferation in a rat model of immune complex GN. Small interfering RNAs and anti-sense oligonucleotides that prevent translation of target proteins have also been successfully used in models of GN and are listed in Table 3. Potential target pathways for intervention in human disease include nuclear factor-κB (NFκB), platelet-derived growth factor (PDGF), and TGFβ [55].

HVJ liposomes have been used extensively for DNA delivery in gene therapy [53–54]. The HVJ proteins on the liposomes bind cell surface sialic acid receptors and induce cell fusion. A disadvantage is the lack of tissue specificity in targeting. Therefore, glomerular delivery of HVJ liposomes requires cannulation of the renal artery, clamping of the proximal segment, followed by slow infusion of the DNA liposome solution. After injection, the clamps are removed to resume normal flow. Although this route of administration is shown to preferentially deliver DNA to the mesangial cells, it is currently not a realistic method for human application. However, these studies demonstrate the possibility that modulating glomerular responses is a viable therapeutic approach for kidney disease.

Immunoliposomal Delivery Systems

Antibodies to cell surface molecules can be conjugated to the surface of liposomes to make immunoliposomes. In rats, mesangial cells express Thy1.1 glycoprotein on their surface[56]. Therefore, liposomes with antibody to Thy1.1 on their surface (anti-Thy1.1 immunoliposomes) can target specifically to the glomerular mesangium in rats. However, no unique surface markers have been identified on murine (or human) mesangial cells to date. To develop a strategy for liposomal delivery to murine (and human) mesangium, we screened a panel of molecules expressed on the surface of mesangial cells and selected alpha 8 (α8) integrin as a potential candidate target [57]. The α8 integrin combines with β1 integrin forming a heterodimer that interacts with extracellular matrix proteins 58. Unlike other integrin molecules, α8 integrin is not expressed on vascular endothelium and has a restricted tissue distribution.

Antibody to α8 integrin was conjugated to the surface of liposomes and the anti-α8 integrin immunoliposomes (anti-α8 immunoliposomes) passed through filters to obtain ~100nM particles [57]. When injected into mice, anti-α8 immunoliposomes loaded with a red fluorescent dye could be seen to preferentially accumulate in the glomerular mesangial space. Localization of the fluorescent dye in the glomerulus showed uptake of liposomes into the mesangial cell cytoplasm. Thus, anti-α8 immunoliposomes can be used for delivery of drugs into the mesangial space and also into the mesangial cell cytoplasm. This strategy has also been successfully adapted to deliver proteins to the glomerular mesangium in normal mice 59. Significantly, there is no reduction in expression of α8 integrin on mesangial cells even with the development of GN. Thus, anti-α8 immunoliposomes are viable carriers of therapeutic agents to diseased glomeruli.

Another strategy used for glomerular delivery of drugs is to target immunoliposomes to adhesion molecules like E-selectins on activated endothelial cells[50]. Anti-E-selectin antibody conjugated to the surface of dexamethasone loaded liposomes was injected in an induced model of immune complex GN. A modification here was co-injection of recombinant TNF-α to further induce upregulation of E selectin on the endothelial cells. The dexamethasone loaded anti-E selectin ILs were detected mainly in the kidney with some accumulation in the liver and heart. Severity of kidney disease was lowered in anti-E selectin loaded with dexamethasone compared to free dexamethasone. In systemic diseases, the anti-E-selectin immunoliposomes would not be restricted to glomerular drug delivery but potentially target all other inflamed tissues associated with activated endothelium in systemic diseases.

immunoliposomes targeting tumor antigens are currently in clinical trials for delivery of cytotoxic drugs in cancer [60]. However, the ability to deliver drugs to the site of inflammation in chronic kidney disease offers a novel approach. The ability for targeted glomerular delivery by anti-α8 immunoliposomes has potential application in human therapy. Local delivery will reduce the drug doses required for therapy. Significantly, this approach may be extended to all glomerular diseases.

SUMMARY

The heterogeneity of presentations and diversity in the natural course of disease makes the study and treatment of lupus nephritis challenging. Current treatments in lupus nephritis focus on treatment with anti-inflammatory and immunosuppressive drugs. Animal models have been valuable in providing insights into the underlying pathogenic mechanisms. Recent data suggest an important role for the end organ/glomerular responses in dictating disease progression. Regulation of these responses locally presents a novel therapeutic approach. Immunoliposomal systems that can be used for delivery of drugs specifically to the glomeruli have been developed. These delivery systems can potentially be adapted for human therapy and require critical evaluation.

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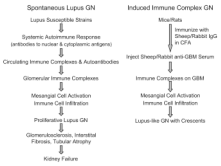

Figure 1.

A schematic comparing spontaneous and induced animal models of lupus-like glomerulonephritis (GN). Abbreviations: CFA, complete Freund adjuvant; IgG, immunoglobulin G; GBM, glomerular basement membrane.

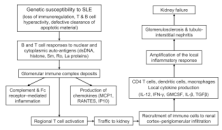

Figure 2.

A model for the pathogenesis of spontaneous lupus nephritis based on studies in mice. Abbreviations: SLE, systemic lupus erythematosus; dsDNA, double-stranded DNA; MCP1, monocyte chemoattractant protein 1; RANTES. Regulated upon Activation, Normal T-cell Expressed, and Secreted; TGFβ, transforming growth factor β; GMCSF, granulocyte-macrophage colony stimulating factor; IL, interleukin; IFN-gamma, interferon gamma.

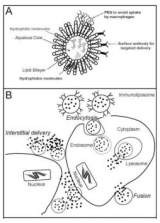

Figure 3.

(A) Illustration showing the liposome, consisting of a lipid bilayer that allows incorporation of hydrophobic molecules and a central aqueous core for hydrophilic molecules. A coat of polyethylene glycol (PEG) prevents uptake by macrophages and cells of the reticulo-endothelial system. Conjugation of antibody on the surface allows preferential targeting to cells of interest. (B) Liposomal contents are delivered into the target cells by fusion of the lipid bilayer with the cell membrane and by receptor or antibody mediated endocytosis. Liposomal contents are also released into the extracellular spaces in the vicinity of the target cell due to destabilization of the lipid bilayer in the interstitium.

Table 1

Laboratory values and treatment provided over 5 years of follow up

	Day 1	Month 5	Month 12	Month 17	Month 27	Month 51	Month 60
SCr (mg/dL)	0.8	1.0	1.8*	1.3	1.4	1.1	1.1
eGFR (mL/min/1.73 m^2)**	96	79	40	58	53	70	69
Protein (g/dL)	7.8		6.9	6.7	6.2	6.3	5.7
Albumin (g/dL)	2.3	3.8	4.3	4.0	4.0	4.0	3.2
UPCR	5.7	1.9	0.6	0.5	0.6	0.3	1.8
ANA	1:640						
Anti-dsDNA	1:640	1:40	1:40	1:40	Neg		
Anti-dsDNA (IU/mL, reference Range: <5)						20	14
Anti-RNP	Neg						
Anti-Smith	Neg						
Anti-ENA	Pos						
Anti-SSA/Ro	Pos						
Anti-SSB/La	Pos						
C3 compl (mg/dL, reference Range: 83 – 156)	36	55	67	66	86	83	73
C4 compl (mg/dL, reference Range: 10 – 38)	4	6	9	8	13	11	8
Hepatitis panel	Neg						
HIV Antibody	Neg						
Hb (g/dL)	7.6	12	12.8		13.9		9.2
Hct (%)	24.5	38.2	40.9		47.0		29.2
Mycophenolate	x	x	x	x	x	x	
Prednisone	x	x	x	x	x	x	

Note: Conversion factors for units: serum creatinine in mg/dL to µmol/L, ×88.4; eGFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, ×0.01667; protein in g/dL to g/L, ×10; albumin in g/dL to g/L, ×10; hemoglobin in g/dL to g/L, ×10. No units conversion necessary for C3 complement and C4 complement in mg/mL and g/L.

Abbreviations: SCr, serum creatinine; eGFR, estimated glomerular filtration rate, UPCR, urine protein to creatinine ratio; ANA, antinuclear antibody; dsDNA, double stranded DNA; RNP, ribonucleoprotein; ENA, extractable nuclear antigen; Neg, negative; Pos, positive; Hct, hematocrit; Hb, hemoglobin; HIV, human immunodeficiency virus.

*After partial recovery from an episode of NSAID-induced AKI 4 months earlier.

** calculated using the MDRD Study equation.

Table 2

A comparison of characteristics of LN in humans and mice

Features of human LN	**Spontaneous disease in lupus mice***
Female predominance	NZB/W F1, NZM2328
Serum autoantibodies	
anti-nuclear, anti-dsDNA	All strains
anti-Sm	MRL/lpr
Kidney Pathology	
Glomerular Immune complexes	All strains
Proliferative GN	All strains
Type V membranous nephropathy	None**
Glomerulosclerosis	All strains
Tubulointerstitial inflammation	All strains
Cellular infiltrates	
Neutrophils	NZM2328
Macrophages	All strains
Dendritic cells	All strains
NK cells	MRL/lpr
T cells (CD4/CD8)	MRL/lpr, NZM2328, NZB/W F1
B cells	NZB/W F1
Plasma cells	NZB/W F1
*Kidney failure****	All strains
Other clinical features	
Hypertension	None reported
Systemic involvement	skin, MRL/lpr; salivary glands, NZB/W F1

Abbreviations: dsDNA, double-stranded DNA; LN, lupus nephritis; NZB, New Zealand Black; NZW, NZ White; NZM, NZ Mixed; NK, natural killer; GN, glomerulonephritis.

* includes NZB/W F1, NZM2410, NZM2328, MRL/lpr, BXSB strains

** can be induced by immuno-modulation

*** elevated serum creatinine, serum urea nitrogen, proteinuria

Table 3

Potential therapeutic targets/pathways for modulation of pathogenic glomerular responses in glomerular diseases

MODEL	Target	Agent	Therapeutic effect in kidney	Reference
Thy1.1 induced GN	EGR1	Antisense ODN	reduced mesangial cell proliferation	49
	E2F	Decoy ODN	reduced PCNA and cdk2 kinase	50
	AP1	Decoy ODN	reduced TGFβ, PAI, mesangial cell proliferation, ECM production	51
	MAPK1	siRNA	Reduced TGFβ, glomerulosclerosis, PAI, ECM production	52
anti-GBM nephritis	NFκB	Decoy ODN	Reduced IL-1β, ICAM, inflammation, proteinuria	53
	Anti-E selectin immunoliposomes	dexamethasone	Reduced ICAM, inflammation	54,55
Streptozotocin induced diabetic nephropathy	TGFβ	Antisense ODN	Reduced TGFβ in urine and kidney	56
	SP1	Decoy ODN	Reduced type IV collagen, fibronectin, α-smooth muscle actin	57

Abbreviations: cdk2, cyclin dependent kinase 2; ODN, oligodeoxynucleotide; PCNA, proliferating cell nuclear antigen; EGR1, early growth response 1; GN, glomerulonephritis; siRNA, small interfering RNA; TGFβ, transforming growth factor β; GBM, glomerular basement membrane; IV, intravenous; ECM, extracellular matrix; NFκB, nuclear factor-κB; MAPK, mitogen-activated protein kinase; PAI, plasminogen activator inhibitor; IL-1β, interleukin 1β.