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Mesangial pathology in glomerular disease: targets for therapeutic intervention

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Abstract

The glomerulus is the filtration unit of the kidney. Disruption of glomerular function may be caused by primary glomerular pathology or secondary to systemic diseases. The mesangial, endothelial and epithelial cells of the glomerulus are involved in most pathologic processes. Animal models provide an understanding of the molecular basis of glomerular disease. These studies show that mesangial cells are critical players in initiation and progression of disease. Therefore, modulation of mesangial cell responses offers a novel therapeutic approach. The complex architecture of the kidney, specifically the renal glomerulus makes targeted drug delivery especially challenging. Targeted delivery of therapeutic agents reduces dose of administration and minimizes unwanted side effects caused by toxicity to other tissues. The currently available modalities demonstrating the feasibility of mesangial cell targeting are discussed.

Keywords

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

Introduction

Systemic administration of anti-inflammatory agents has been the primary focus for treatment of glomerular diseases. Recent studies by us and others suggest a critical role for glomerular cell responses in progression of renal disease. Therefore, delivery of drugs to the renal glomeruli inhibiting local inflammatory/ pathogenic responses will be expected to yield better therapeutic outcomes. Targeted therapies have shown to lower drug dosing and thereby minimize side effects. This is especially attractive in chronic renal diseases requiring treatment over extended periods. This article discusses the glomerular mesangial cell, its role in glomerular disease and some cellular pathways that are potential targets for therapy. Animal models demonstrating therapeutic potential and new strategies of mesangial targeting are discussed.

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1. Mesangium, mesangial matrix and mesangial cells

The mesangium forms the central region of the renal glomerulus and provides support to the glomerular tuft [1,2]. It consists of mesangial cells (MC) embedded in an extracellular matrix (ECM). The ECM is produced by MCs and contains collagens type IV and V, laminin A, B1, and B2, fibronectin, heparan sulfate and chondroitin sulfate proteoglycans, entactin, and nidogen. The MCs constitute 30–40% of the total glomerular cell population [3]. Two different types of MCs have been described. Vascular smooth muscle like cells containing smooth muscle actin and myosin form >90% of the MC population. Processes from these MCs connect to the glomerular basement membrane and the juxta-glomerular apparatus either directly or through the extracellular microfibrillar proteins. Contraction of MCs can constrict the capillary lumen causing alteration of blood flow into the glomerular tuft influencing glomerular filtration [4]. A smaller population of bone marrow derived MHC II positive, macrophage-monocyte like phagocytic cells have been described in rats and constitutes 3–10% of MCs. These cells are not seen in normal glomeruli in human [5]. The mesangium is separated from the vascular compartment by a fenestrated endothelium without an intervening basement membrane (Figure 1). Thus, MCs are housed in a unique environment that communicates between the vasculature and the interstitium. MCs are exposed to changes in glomerular blood flow, plasma components and macromolecules percolating through the endothelial fenestrae. In addition to maintaining the glomerular hemodynamics, MCs perform a large number of critical functions and have been reviewed extensively [1]. Of specific relevance to this review is the ability to MCs to clear circulating immune complexes, to produce pro-inflammatory mediators, and to regulate the formation and breakdown of the mesangial matrix in glomerular disease.

3. Role of MCs in glomerular pathology

Glomerular diseases manifest as diverse clinical syndromes and etiologies are not clearly understood. Glomerular changes are complex, involving all glomerular cell types including MCs, endothelial cells, podocytes, parietal epithelial cells and infiltrating inflammatory cells [2,6–9]. Deposition of immunoglobulins (Ig) or immune complexes in the mesangium is one of the causes of glomerular injury and is seen secondary to diseases like Systemic Lupus Erythematosus (SLE) [10] or primary diseases like IgA nephropathy [11]. Immunoglobulin aggregates activate MCs by signaling through surface Fc Receptors. Mesangial changes seen following glomerular injury include production of chemo-attractants for inflammatory cells, proliferation of MCs, loss of mesangial matrix (mesangiolysis), followed by excessive production of ECM (mesangial expansion). The ease of obtaining primary mesangial cell cultures has allowed extensive investigation into responses of MCs in glomerular injury. In vitro cultures cannot replicate the interactions between MCs, ECM, endothelial cells, and podocytes and the constantly changing hemodynamic state of the glomerulus, all of which influence mesangial cell responses. However, despite several differences, the mesangial cell cultures demonstrate significant similarities with the responses in vivo [12].

SLE is characterized by circulating autoantibodies to cytoplasmic and nuclear antigens. Renal involvement in SLE is associated with deposition of IgG containing immune complexes in the mesangium and along the glomerular basement membrane (GBM). These immune complexes may develop in situ by reactivity of circulating autoantibodies to negatively charged nuclear antigens deposited in the GBM and mesangial matrix [13]. Another source of mesangial immune complexes in SLE is direct deposition of immune complexes formed in circulation. The progression of renal disease in SLE has been investigated in inbred mouse strains susceptible to fatal, lupus-like glomerulonephritis. In MRL lpr/lpr mice, a model of lupus-like glomerulonephritis, the onset of mesangial immune-complex deposition was associated with an increase in renal expression of the pro-

inflammatory chemokines CCL2 (monocyte chemoattractant protein (MCP-1) and CCL5 (RANTES) and chemokine receptors CCR2 and CCR5 [14]. The source of these chemokines was identified to be MCs. Thus, MCs are primary responders to glomerular immune injury in SLE. In New Zealand Mixed (NZM) 2328 mice, IgG immune complexes are first seen in the mesangial regions [15]. Compared to a normal glomerulus (Figure 2A), there is an increase in mesangial size and cellularity (Figure 2B) associated with inflammatory cells like neutrophils, dendritic cells, macrophages and T cells in the glomeruli and peri-glomerular regions. In female NZM2328 mice, this acute proliferative phase progresses to chronic glomerulonephritis (Figure 2C). Glomerular sclerosis and fibrosis are prominent along with interstitial inflammation in the renal cortex. Lupus mesangio-proliferative glomerulonephritis is associated with increased levels of pro-inflammatory cytokines like IL1 β , TNF α , IL6, GM-CSF in the kidney. A significant increase in TGF β is seen with the onset of chronic change. The production of TGF β as a critical mediator of glomerular sclerosis and fibrosis is well established in the final common pathway of end stage renal disease [16,17].

Immune mediated glomerular injury is also seen in IgA nephropathy characterized by Immunoglobulin A (IgA) deposits in the glomerular mesangium as the diagnostic feature. Although IgA nephropathy has a wide range of clinical presentations, the most common pathologic feature is glomerular hypercellularity [18]. Patients with the familial form of IgA nephropathy produce abnormally glycosylated IgA, which deposits in the mesangium as multimers or immune complexes [19]. Treatment of human mesangial cell lines with IgA from asymptomatic relatives of patients' shows enhanced binding to MCs inducing increased production of IL6, TNF α and MCP1 compared to relatives of sporadic IgA nephropathy patients. [20]. Proinflammatory chemokines and cytokines secreted by MCs act as chemoattractants to recruit inflammatory cells into the glomerulus. In addition, chemokines also cause activation of the endothelial cells in the glomerular capillaries and upregulation of adhesion molecules, further facilitating inflammation. In another study, atypically glycosylated IgA or serum IgA from patients could induce platelet activating factor in MCs that can act on podocytes and lead to loss of nephrin [21]. In vivo, this would be expected to compromise glomerular filtration.

An example of metabolic glomerular injury is seen in diabetes mellitus. Diabetic nephropathy is associated with the involvement of all glomerular cells with mesangial matrix expansion representing a major component [8,22,23]. High extracellular glucose levels, and increased expression of the facilitative glucose transporter GLUT1 leads to activation of metabolic pathways resulting in oxidative stress. This results in accumulation of advanced glycation end (AGE) products and excessive production of ECM proteins like fibronectin, collagen types I, III and IV by MCs. These changes are accompanied by dysregulated activity of matrix metalloproteinases and their inhibitors that are critical for ECM remodeling [24]. The ensuing glomerulosclerosis and fibrosis progressing to end stage renal disease is a final common pathway for chronic glomerular disease. Aberrant remodeling of the ECM is another cause of mesangial expansion as seen in light chain related glomerular disease associated with increased synthesis of tenascin by the MCs [25]. Thus, MCs play a critical role in initiation of glomerular inflammation and its progression to chronic disease (Figure 3).

4. Potential targets for intervention

Animal models have been used to test therapeutic efficacy of novel targets. In particular, induced or spontaneous renal disease in rat and mouse models can mimic human glomerular diseases. The most commonly used models for immune injury include anti-Thy1.1 nephritis [26], nephrotoxic nephritis [27,28], and spontaneous lupus glomerulonephritis [29]. Diabetic

nephropathy is studied in an streptozotocin induced diabetes mellitus [30]. In rats, anti-thymocyte serum or anti-Th1.1 antibody reacts with mesangial cells and causes a rapid phase of mesangiolysis [26]. This is followed by mesangial expansion and hypercellularity. Nephrotoxic nephritis is a model of immune complex mediated injury in mice and rats. Mice are immunized with rabbit (or sheep) IgG emulsified in adjuvant. This is followed by injection of rabbit (or sheep) anti-serum to glomerular basement membrane. The resulting GBM immune complexes and glomerular disease mimics human immune complex GN [27,31]. Both these models are associated with clinical characteristics of renal disease in the form of proteinuria, elevated serum creatinine and blood urea nitrogen.

The genetics and pathology of lupus glomerulonephritis has been extensively studied in inbred mouse strains [32]. MRL lpr/lpr, BXSB, NZBxNZW F1, NZM2328 and NZM2410 mice are genetically susceptible to SLE and spontaneously develop autoimmune responses and renal pathology closely mimicking the human disease. In addition, like the clinical presentation, these models are chronic and the disease develops over 5–9 months of age.

Rodent models of diabetic nephropathy are induced by treatment with a N-acetyl glucosamine analog, streptozotocin [33]. Streptozotocin is transported by glucose transporter GLUT2 into pancreatic cells leading to irreversible beta cell apoptosis. The resulting insulin deficiency and hyperglycemic state mimics metabolic injury of diabetes mellitus. In addition, the renal pathology of mesangial expansion and glomerular scarring is characteristic of diabetic nephropathy.

Three major pathways have been targeted for therapies tested in animal models discussed above. NF κ B is important for cytokine production, and for inflammatory cell recruitment [34–38], PDGF for mesangial cell proliferation [39–41] and TGF β for glomerulosclerosis and fibrosis [16,17]. It should be noted that multiple stimuli can activate each pathway. There is significant cross talk between these pathways and they are rarely activated in isolation. An example of this cross talk between TGF β and PDGF through the transcription factor Egr1 has been depicted in a review by Bagavant and Fu [42]. Thus, inhibitors dominantly affecting a single pathway can have a significant impact on disease in rat or mouse models of mesangio-proliferative glomerulonephritis

4.1 Nuclear Factor kappa B (NF κ B)

NF κ B is a transcription factor that plays a key role in coordinating many cellular responses [34]. NF κ B is present in the cytosol as a complex with inhibitor of NF κ B (I κ B). In MCs, stimuli like IL1, TNF α , immunoglobulin aggregates, and reactive oxygen intermediates lead to phosphorylation of I κ B, its release from NF κ B and degradation [34]. The released NF κ B proteins translocate to the nucleus where they bind the cognate DNA sequences and regulate transcription of a large number of genes. In MCs, it is known to upregulate MCP-1, ICAM, IL6 and iNOS. Ability of agents to inhibit NF κ B has been explored extensively for their use as anti-inflammatory agents [35,36] Dehydroxymethyl-epoxyquinomicin (DHMEQ), a novel NF- κ B activation inhibitor, was effective in treatment of anti-thy1.1 antibody-induced glomerulonephritis [37]. DHMEQ treatment resulted in marked glomerular inhibition of NF κ B, decreased proteinuria, preserved creatinine clearance and decreased glomerular cell proliferation. The anti-inflammatory effects of glucocorticoids (dexamethasone, prednisone and methyl prednisolone) are also mediated partly through NF κ B inhibition [38].

4.2 Platelet Derived Growth Factor (PDGF)

Platelet Derived Growth Factor (PDGF) is a potent mitogen and a key survival factor for MCs [2]. The expression of PDGF ligands and PDGF receptors are low in the normal adult kidney, but are increased during renal development and during the progression of renal

fibrogenesis. Activation through the PDGF receptor induces stimulation of mesangial cell proliferation [39], increased extracellular matrix synthesis, and increased expression of the pro-sclerotic cytokine, TGF- β [40]. Blocking the PDGF activation pathway is effective in mouse and rat models of mesangioproliferative nephritis. Imatinib, a tyrosine kinase inhibitor of PDGF receptor was able to ameliorate renal dysfunction in (NZB/W)F1 mice that spontaneously develop fatal lupus nephritis [29]. It was shown that mice treated with imatinib survived significantly longer than vehicle treated mice. At 8 months of age, 100% of mice given the drug were alive as compared with 53% of controls. Imatinib protected the kidney against glomerular hypercellularity, tubulointerstitial damage, and accumulation of F4/80-positive mononuclear cells. Therapeutic effects of imatinib may also be due to inhibition of the TGF β pathway. Effects of another PDGF receptor tyrosine kinase inhibitor, signal transduction inhibitor 571 (STI- 571) was studied in rats with Thy1.1 glomerulonephritis [41]. PDGF receptor tyrosine kinase blockade with STI-571 showed significant reductions in mesangial cell proliferation and glomerular type IV collagen deposition.

4.3 Transforming Growth Factor β (TGF β)

TGF β is a critical mediator of glomerulosclerosis and fibrosis leading to end stage renal disease. Binding of TGF β to its receptor leads to phosphorylation of Smad2 and Smad3 [43]. The Smad2/3 dimer translocates to the nucleus with Smad 4 and up-regulates transcription of alpha collagen leading to increased collagen synthesis. In addition, Smad independent pathways lead to phosphorylation of the transcription factor Egr1 through casein kinase 2 [28]. Egr-1 is a master transcription factor that can regulate multiple molecules including mitogens like PDGF, adhesion molecules like ICAM, and cell cycle molecules like cdk2 kinases. Activation by TGF β causes increased ECM synthesis, mesangial proliferation and glomerular fibrosis. Therefore, the TGF β pathway is a critical target for glomerular therapies. Treatment of rats with anti-TGF β antibody ameliorated disease in a progressive anti-thymocyte serum model of GN [43]. Vardenafil is an inhibitor of phosphodi-esterase 5 and thrombospondin 1, which is known to activate TGF β production [44]. Vardenafil treatment inhibited mesangial cell proliferation and ECM expansion in nephritic rats.

The few representative examples discussed above show that systemic inhibition of NF κ B, PDGF or TGF β was able to inhibit mesangial expansion and glomerulosclerosis. The therapeutic approaches demonstrate the importance of these pathways in initiation and progression of glomerular disease. However, these studies do not address the protective effect of delivering the drug to the end organ alone, a primary goal of targeted drug delivery systems.

5 Mesangial cell targeting in treatment of glomerular disease

Targeted drug delivery has been of special interest in cancer treatments to increase local drug concentrations at the site of action and minimize systemic toxicity. Many glomerular diseases are either secondary to a systemic ailment like SLE, Diabetes Mellitus, IgA nephropathy or are of unknown etiology. However, recent studies show that development of renal disease is dictated by genetic susceptibility of the end-organ [45–47]. Our study in NZM2328 lupus mice showed that removal of the thymus on day 3 after birth could accelerate autoimmune responses and proliferative glomerulonephritis in both male and female mice [46]. However, only female mice developed chronic glomerulonephritis and renal failure implicating gender dependent end-organ factors dictating disease progression. In an anti-GBM nephritis model, different strains of mice developed comparable antibody titers against rabbit IgG [48]. Despite this, only some strains like DBA/J, 129SvJ and not A/J, SJL/J, SWR/J developed renal disease following infusion of rabbit anti-GBM antiserum. These studies suggest that the outcome of glomerular injury is also dictated by the end organ

response. Since MCs are major players in the initiation and progression of glomerulopathies, modulation of MC responses will be a novel therapeutic methodology.

Introduction of DNA that could down-regulate proliferative or pro-fibrotic mesangial cell responses offers elegant proof that mesangial cell targeted therapies are viable. Use of liposomes as efficient drug delivery systems is a well established technology. Liposomes incorporated with UV inactivated Hemagglutinating Virus of Japan (HVJ or Sendai virus) have been used as for delivery of DNA in gene therapy [49–50]. HVJ proteins bind to cell-surface sialic-acid receptors induce cell fusion. Intravenous injection of HVJ-liposomes incorporated with antisense TGF β 1 oligodeoxynucleotides (ODN) into rats with streptozotocin (STZ) induced diabetic nephropathy resulted in reduced levels of TGF β 1 in tissues and urine 13 days after treatment [51]. However, the TGF β 1 inhibition was not cell specific since intravenous injected HVJ-liposomes travel to all tissues including liver, lung, and spleen. In the kidney, liposomal uptake by intravenous injection was predominantly in the peri-tubular capillaries, endothelial cells and proximal tubular cells.

Injection of HVJ- liposomes directly into the renal artery results in uptake into glomerular cells. Tomita et al demonstrated this new concept in renal targeting using co-encapsulation of plasmid DNA encoding the SV40 large T antigen (pACT SVT) with high mobility group 1 (HMG1) protein into HVJ liposomes [52]. The HVJ facilitates membrane fusion while HMG1 a nuclear protein binds DNA and transports it to the nucleus. Injection of this liposomal formulation into the renal artery led to increased expression of SV40 large T antigen in glomeruli, four days after transfection. This targeting strategy has been used in gene therapy for modulating mesangial cell responses.

Incorporation of DNA containing the cis element of transcription factors compete for binding to the transcription factors. Thus, the DNA introduced acts as decoys and prevents binding of the specific transcription factors to promoter elements, thereby down regulating gene expression. This strategy was used to deliver decoy ODNs to the transcription factor E2F in a rat model of Thy1.1 induced mesangio-proliferative glomerulonephritis [53]. E2F plays an important role in the transactivation of the cell cycle regulatory genes, proliferating-cell nuclear antigen (PCNA) and cdk2 kinase. Compared to controls, E2F decoy ODN treated animals showed specific inhibition of PCNA and cdk2 kinase mRNA expression in kidneys. This was associated with a significant decrease in number of glomerular cells in S phase and attenuation of glomerular injury.

To extend the stability and life of ODNs in vivo, HVJ-liposomes containing a ring type decoy ODN to pro-fibrotic transcription factor Sp1 were injected into the renal artery of STZ treated rats. Rats studied at days 1, 7 and 14 after transfer showed lower renal type IV collagen, fibronectin and α -smooth muscle actin expression associated with reduced ECM and fibrosis [54]. HVJ liposomes were also used by Ahn et al [55] to suppress the activity of activator protein AP1, which has been implicated in transcriptional regulation of a wide range of genes participating in cell survival, proliferation, and apoptosis in the kidney. Decoy AP1 ODN inhibited TGF β 1 and Plasminogen Activation Inhibitor (PAI) expression and prevented mesangial cell proliferation and ECM production [55]. Similar results were seen with inhibition of Egr1 transcription using anti-sense ODN to Egr1 in Thy1.1 induced glomerulonephritis [28]. These studies add credence to the thesis that regulation of local, glomerular/ mesangial cell responses is a feasible approach to treatment of glomerular disease. A significant caveat of these studies is the complex route of administration required for specific targeting to the glomerular mesangium. The route of administration involves cannulation of the renal artery, ligation of the proximal segment, followed by slow injection of the DNA-liposome solution. After completion of the injection, the ligatures are removed

to allow resumption of the renal blood flow. Therefore, it has been important to develop newer systems for delivery of therapeutic agents to the renal glomerulus.

6 Systems for targeted delivery to MCs

Carrier systems for delivery to glomeruli and specifically to MCs by tail vein injections have been developed in rat and mouse models. Nanoparticles (150nm in diameter) made of isobutylcyanoacrylate injected intravenously, were found to concentrate in the rat MCs compared to other renal cells [56]. However, isobutylcyanoacrylate nanoparticles were also efficiently taken up by macrophages limiting the specificity of targetting. Addition of Polyethylene glycols (PEG) to liposomes increases their half life in circulation and prevents uptake by macrophages [57]. Evaluation of different liposomal formulations showed that addition of a cationic lipid TRX-20 (3,5-dipentadecyloxybenzamidinium hydrochloride), to the PEGylated liposomes enhanced uptake by MCs in vitro and in vivo. This was related to the binding of TRX-20 to chondroitin sulfate proteoglycans on the cell surface and ECM [58]. Rhodamine labeled TRX-20 PEGylated liposomes loaded with prednisone phosphate were injected intravenously in rats with anti-Thy1.1 induced glomerulonephritis [59]. Control rats were treated with prednisone phosphate, either free drug or loaded in PEGylated liposomes. The TRX-20 PEGylated liposomes preferentially trafficked into the renal cortex. This was associated a significant suppression of mesangial activation. Dose response curves showed that to achieve the same therapeutic effect, dose of prednisone needed in PEGylated liposomes was 10 times and free prednisone phosphate was 100 times greater than TRX-20 PEGylated prednisone delivery.

Recently, Shmizuizu et al have recently developed a methodology to delivery of siRNAs to glomeruli using poly ethylene glycol (PEG) – poly L-lysine (PLL) based vehicles [60]. PEG-PLL copolymers mixed with fluorescence-labeled siRNA were used to make 10 to 20nM siRNA/nanocarrier complexes and injected intra-peritoneally into mice and rats. Although both naked siRNA and the siRNA/nanocarrier showed similar distribution in all tissues following injection, the siRNA/nanocarrier persisted in circulation and in the renal cortex for a significantly longer time. PEG prevented uptake by cells of the reticuloendothelial system, while the small size of the nanoparticle allowed it to pass into the mesangium. While naked siRNA was excreted into the urine within 10 mins of injection the siRNA delivered by the nanocarrier was detected after one hour. In rats, the siRNA delivery by nanocarriers colocalized with glomerular Thy1.1 expression, conforming mesangial distribution. In MRL/lpr mice, a murine model of spontaneous lupus nephritis, repeated intraperitoneal injection of a mitogen-activated protein kinase 1 (MAPK1) siRNA/nanocarrier complex suppressed glomerular MAPK1 mRNA and protein expression. This was associated with improved kidney function, reduced proteinuria, and glomerular sclerosis. MAPK1 acts as an upstream regulator of TGF- β 1 in glomeruli and a consequent modulator of glomerulosclerosis via the expression of extracellular matrix components and PAI-1. This is one of the few reports of the successful use of siRNA methodology in renal disease.

Specificity of cellular targeting and preferential drug delivery can be enhanced by conjugating the surface of liposome with antibodies to cell surface markers like Thy1.1 on rat MCs [61]. Anti-Thy1.1 antibodies (OX7) were conjugated to HVJ- liposomes and loaded with fluorescein isothiocyanate. The anti-Thy1.1-HVJ immunoliposomes injected IV could traffic into 80% of the glomeruli. The ease of administration for this immunoliposomal formulation offers a significant advantage over HVJ-liposome gene transfers, where injection into the renal artery was required for delivery to the MCs [49]. Rats were immunized with rabbit IgG in Complete Freund's Adjuvant followed by passive transfer of rabbit anti-GBM serum to induce glomerulonephritis. Injection of anti-Thy1.1 - HVJ

immunoliposomes loaded with NF κ B decoy ODN reduced renal injury measured as glomerular IL1 β production, adhesion molecule ICAM expression, inflammation and proteinuria. This was not seen in control rats treated with scrambled decoy ODN.

Tuffin et al conjugated an anti-Thy1.1 antibody to PEGylated liposomes for delivery of drugs to rat MCs [62]. The unique anatomic structure of the renal glomeruli was used to design the anti-Thy1.1 immunoliposomes. The glomerular mesangium is freely accessible to contents in blood through endothelial fenestrations. The size of these fenestrations ranges from 130 to 170nm. The slit pores on the GBM are 30–70nm. Thus, circulating immunoliposomes sized between 70 and 130nm can pass into the mesangium and but cannot enter into the urinary space (Figure 1). Concentration in the mesangium was further facilitated by the anti-Thy1.1 antibody reacting with the mesangial cell surface. Delivery of a cytotoxic drug, doxorubicin into the glomerular mesangium was successfully achieved by the sized, anti-Thy1.1 immunoliposomes. Despite the expression of Thy1.1 in other organs like brain cortex and striatum, thymus, spleen and moderately in the collecting ducts of kidney, lung tissue and spleen, the liposomal delivery was specific to the renal glomerulus, possibly due to the antigen density on the MCs. [63].

7 Mesangial cell targeting in mice

Rat models have successfully established the feasibility of specifically targeting MCs and the therapeutic efficacy of treating glomerular disease by regulating local responses. Thy1.1, a cell surface marker on rat MCs has been used as the primary target in these formulations. In humans, Thy1.1 or CD90 is expressed on T cells, thymocytes, and neurons [63]. Moreover, human MCs do not express Thy1.1. Currently, there is no known unique mesangial cell specific surface marker in humans. This is also true for mouse mesangial cells. Thus, while anti-Thy1.1 immunoliposomes provide proof of concept for specific mesangial delivery, they cannot be used in humans or in experimental mouse models.

In our laboratory, we have developed an immuno-liposomal system to specifically target mouse MCs [64]. MCs express multiple integrin proteins including α -1, -3, -5 and -8 that combine with β 1 and interact with ECM proteins. Of these, the α 8 integrin is most restrictive in its distribution. Specifically, it is not detected on endothelial cells [65,66]. α 8 integrin is constitutively expressed on MCs and can be detected in the presence of mesangioproliferative nephritis ensuring its accessibility in diseased kidney. Using the immunoliposomal design strategy described by Tuffin et al [62], we developed anti- α 8 integrin immunoliposomes (anti- α 8-ILs) [64]. Anti- α 8-ILs loaded with a red fluorescent dye, 1,1V-dioctadecyl-d,d,d,d_-tetramethylindocarbocyanine (DiI), and injected IV in the tail vein of mice were able to preferentially traffic to the glomerular mesangium. The glomerular DiI co-localized with vimentin, an intracellular protein in glomerular cells but not with a podocyte marker, synaptopodin. Thus, the anti- α 8-ILs could successfully deliver their contents to the glomerular mesangium and also into the mesangial cell cytoplasm. DiI delivery was not detected in other regions in the renal interstitium or other organs, demonstrating specificity of this delivery system. Flow cytometry of cell suspensions from different tissues showed uptake by a small proportion of CD11b positive cells, i.e. macrophages and neutrophils. The ability of anti- α 8-ILs to deliver therapeutic agents was tested in a rapid model of inflammation. Mice injected intraperitoneally with poly (I:C) show rapid increase of proinflammatory cytokines like IL6 and TNF α in all tissues [67]. Mice pre-treated with anti- α 8-ILs loaded with dexamethasone phosphate showed a significant reduction of proinflammatory cytokines in the kidney compared to dexamethasone loaded rabbit IgG conjugated ILs (Figure 4). Glomerulus specific delivery with anti- α 8-ILs was seen in lupus susceptible NZM2328x NOD F1 mice in the presence of glomerular immune complex deposits and established lupus-like nephritis. This indicates

that anti- α 8-ILs are viable carriers for therapeutic agents in the presence of glomerular disease. This is the first study showing mesangial cell specific delivery following systemic administration in mice. Significantly, human MCs also express α 8 integrin on their surface, making it an attractive potential candidate for extrapolation to human.

Concluding Remarks

Glomerular diseases may be primary, affecting only the kidney, or secondary to a systemic disorder. However, there is a significant overlap in the pathologic processes underlying both groups. There is an increasing body of evidence supporting the role of end organ in dictating renal disease progression. This favors the possibility of developing gene therapy for modulating local responses in human disease. In mouse lupus, severity of disease was linked to lowered renal expression of kallekrein genes [68]. This was confirmed in cohort of patients, where polymorphisms in kallekrein genes were linked to lupus. Thus, despite the inability of animal models to recapitulate the complexity of human disease, they have provided a better understanding of the cellular and molecular basis of glomerular injury, and thereby novel therapeutic approaches. Glomerular fibrosis and glomerulo-sclerosis are final common pathways leading to renal failure. Thus, glomerular delivery of TGF β , PAI, PDGF and NF κ B inhibitors are obvious candidates for therapy. In addition, inhibition of signaling molecules in these pathways have shown efficacy in animal models, and are targets for gene therapy in humans. In patients with an underlying systemic disorder, glomerular delivery of specific therapeutics will be adjunct to treatment of the systemic disease. Significantly, long lasting local therapies are particularly attractive to prevent relapses in patients of chronic diseases like lupus nephritis.

A caveat of gene therapy described above is the rapid degradation of the ODNs by nucleases. Therapeutic effects over longer periods of time have been achieved by using circular over linear anti-sense ODNs. Chemical modifications of the phosphodiester backbones increase resistance to nuclease and improve stability. The requirement of repeated treatments in chronic glomerular disease adds to the risk of immunogenicity associated with incorporation of viral proteins in the liposomal preparations. Therefore, formulations that will avoid viral proteins, facilitate cellular uptake and yet maintain tissue specific targeting would be ideal candidates for glomerular delivery in disease. Development of newer strategies that will facilitate ease of administration, increase specificity of targeting and lower systemic toxicities offering significant advantages. In conclusion, targeted drug therapy for glomerular disease shows promise for future application for human disease.

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Abbreviations

MCs	mesangial cells
ECM	extracellular matrix
GN	glomerulonephritis
IL	Immunoliposomes

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Figure 1.

Schematic of a glomerular capillary loop showing the glomerular filtration assembly with the capillary lumen surrounded by a fenestrated endothelium, glomerular basement membrane and podocyte foot processes. Note: Absence of basement membrane between mesangium and endothelium. Immunoliposomes (ILs) ~100nM in diameter can traverse through the fenestrated endothelium directly into the mesangial space. Antibodies on ILs recognizing surface mesangial cell markers allow preferential retention as well as cellular uptake. **Inset:** Electron micrograph of sized liposomal preparation showing unilamellar vesicles. (This figure has been previously published in Scindia et al. *Arthritis & Rheum* 58 (2008) 3884–3891).

Figure 2.

Representative glomeruli from NZM2328 mice showing histopathology of lupus nephritis. (A) Normal glomerulus. (B) Immune complex deposition is followed by increase in size and cellularity. Expansion of the mesangium (arrows) is evident. (C) Glomerulosclerosis ensues with loss of the glomerular tuft, surrounded by fibrosis and inflammatory cell infiltrates. (20× magnification)

Figure 3.

A schematic showing mesangial cell responses (dashed arrows) to common modes of glomerular injury (solid arrows) and their effect on glomerular pathology in renal disease. Note that mesangial cells induce cytokines causing inflammatory cell recruitment. In turn, cytokines produced by infiltrating cells act on mesangial cells perpetuating the pathogenic response.

Figure 4.

Mice were injected with dexamethasone-loaded immunoliposomes followed by Poly(I:C), a TLR3 agonist. Kidneys were harvested 3 hours later and expression of proinflammatory cytokines IL6 and TNF alpha genes was studied by QPCR. Data are presented as relative gene expression in mice treated with dexamethasone loaded in control rabbit IgG ILs (open bars) or in anti- α 8 integrin ILs (filled bars).