Role of podocytes in lupus nephritis

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Much attention has been focused on the complex pathology of lupus nephritis (LN) in an attempt to develop specific therapies targeted to this serious manifestation of systemic lupus erythematosus (SLE). The classification of LN depends on the findings at histology according to the International Society of Nephrology (INS) and Renal Pathology Section (RPS) classification criteria [1] and involves deposition of immunoglobulin in glomerular and tubular basement membrane-enhanced inflammatory response and renal fibrosis [2]. Clinically, proteinuria and haematuria are characteristic features in patients with LN and have traditionally been thought to be the result of immune complex deposition and endocapillary proliferation causing a disruption to the filtration barrier. However, in a subset of proteinuric lupus patients, there is no evidence of the typical immune complexes and instead there appears to be extensive podocyte effacement [3]. The effacement of the foot processes as result from podocyte injury has been associated with the development of proteinuria and the nephrotic syndrome. In addition, podocytes have been identified in the population of cells comprising crescentic forms of LN.
review discusses the various stress responses of podocytes during LN including differences in podocyte motile behaviours that underlie the podocyte foot process effacement and crescent population.

Podocytes are highly differentiated cells that line the outside of the glomerular capillary and are formed of a body with extending major processes that further branch into foot processes separated by a slit diaphragm. They play an important role in the generation of urine by separating proteins from the aqueous part of the blood. Podocytes are anchored to the glomerular basement membrane via α3β1 [4–6], αvβ3 [7] and αv/β3-dystroglycans [8]. The past decade has seen much progress with regard to the understanding of general podocyte pathology. Molecules and pathways have been identified that serve as explanation for the development of podocyte foot process effacement, proteinuria and progression of renal disease.

Proteinuric kidney diseases are often linked with slit diaphragm disruption and/or foot process effacement resulting from the rearrangement of the podocyte microfilament system [9]. Recent work has enhanced our understanding of the molecular framework underlying podocyte structure, primarily through the analysis of hereditary proteinuria syndromes and genetic models [10] as well as through adequate podocyte cell culture models [11]. In addition, studies also suggest novel mechanisms of disease for the more commonly acquired proteinuric diseases, that involve the presence of cytosolic cathepsin L located in podocytes [12,13].

Table 1. Table showing the ISN classification of lupus nephritis along with the degree of proteinuria and evidence for podocyte involvement

<table>
<thead>
<tr>
<th>ISN class</th>
<th>Summarized description</th>
<th>Proteinuria range</th>
<th>Podocyte pathology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Minimal mesangial lupus nephritis</td>
<td>Non-nephrotic →</td>
<td>No evidence FP effacement</td>
<td>[17,55]</td>
</tr>
<tr>
<td>II</td>
<td>Mesangial proliferative lupus nephritis</td>
<td>Nephrotic →</td>
<td>Identified in crescents</td>
<td>[21]</td>
</tr>
<tr>
<td>III</td>
<td>Focal lupus nephritis</td>
<td>Nephrotic</td>
<td>Identified in crescents</td>
<td>[6,7]</td>
</tr>
<tr>
<td>IV</td>
<td>Diffuse lupus nephritis</td>
<td>Nephrotic</td>
<td>Identified in crescents</td>
<td>[6,7]</td>
</tr>
<tr>
<td>V</td>
<td>Membranous lupus nephritis</td>
<td>Nephrotic</td>
<td>Identified in crescents</td>
<td>[6,7]</td>
</tr>
<tr>
<td>VI</td>
<td>Advanced sclerosis lupus nephritis</td>
<td>Nephrotic</td>
<td>Identified in crescents</td>
<td>[6,7]</td>
</tr>
</tbody>
</table>

Cell-mediated podocyte injury leading to LN

Crescent formation is a characteristic feature of class IV diffuse LN. The composition of cellular crescents does not appear to be consistent and tends to be dependent on their location and the stage of disease severity. Cells found to be present include parietal epithelial cells, macrophages and myofibroblasts [19]. Whether podocytes are present has been a matter of debate. On the one hand, it was argued that podocytes are unlikely to be present in crescents, a view supported by the lack of traditional podocyte markers in crescentic cells [20]. However, using genetic tags in mouse models, it has been reported that up to 50% of the cells within crescents may originate from podocytes [21,22]. More specifically, with regard to LN, Bariety and colleagues reported the presence of podocytes in crescents identified by immunostaining for podocyte antigens in human glomerulonephritis including type IV lupus glomerulonephritis [23]. The difficulties in studying podocyte identity in cellular injury is due at least in part to their ability to undergo a phenotype switch from mesenchymal-type cells to epithelial cells during disease. This dedifferentiation process can consequently lead to a loss of their normal epitopes [23] and the development of new epitopes characteristic of macrophage or myofibroblast origin [24,25]. However, recent work has identified Nestin as a potentially stable podocyte marker as it persists in podocytes when glomerular disease is present [26]. Thorne and colleagues [21] found consistently identified Nestin-positive cells in glomerular crescents in human renal biopsies in a variety of renal pathologies including LN classes III and IV. The
contribution to further renal injury by the presence of podocytes in crescents remains unclear.

**Immune-mediated podocyte injury leading to LN**

The important question of how signals from the immune system can lead to podocyte damage is being intensely studied. Podocytes were shown to express MHC classes I and II and ICAM-1 in a murine model of necrotizing crescentic glomerulonephritis and following IFN-λ treatment in vitro suggesting that cytokines induce podocytes to allow antigen presentation to inflammatory cells [27].

Podocytes are also known to express receptors for IL-4, 10 and 13 [28], the presence of which can disrupt podocyte function.

CD8⁺ T cells have been identified (although their role not fully explained) in periglomerular infiltrates in LN [29]. In a recent study using an experimental transgenic mouse model expressing ovalbumin and hen egg lysozyme antigens in podocytes, the authors demonstrated that coinjection of CD8⁺ cytotoxic T cells and CD4⁺ T helper (Th) cells reproduced these infiltrates leading to structural and functional kidney damage. They also suggested crosstalk between tubulointerstitial dendritic cells and Th cells to explain intrarenal cytokine and chemokine production [30]. Liu and colleagues have recently identified ubiquitin C-terminal hydrolase-L1 (UCH-L1) to be expressed on podocytes with an increase in expression in proliferative glomerulonephritis including LN compared to non-immune complex-mediated glomerulonephritis [31]. Again its significance is yet undetermined but it is known that the ubiquitin–proteasome pathway has a number of functions including involvement in immune and inflammatory responses.

**The podocyte—another immune cell?**

B7–1 is usually found on B cells and involved in T-cell costimulation but its expression is induced on diseased podocytes [32]. We demonstrated its upregulation in podocytes by Lipopolysaccharide (LPS) acting via TLR4, and its role in reorganizing the actin cytoskeleton and modulating the slit diaphragm, leading to proteinuria. Interestingly, while the secretion of B7–1 has been found to be elevated in urine of patients with minimal change nephrotic syndrome, the levels were not affected in urine during LN [33] despite strong podocyte B7–1 induction [32] suggesting that different release mechanisms are operative in reversible and non-reversible forms of podocyte injury.

The slit diaphragm itself, just like any other cell junction, may also function to arbitrate signalling through molecules like nephrin, that for example can be involved in actin reorganization via a phosphoinositol 3-kinase pathway [34,35]. The Src family of kinases are involved in nephrin phosphorylation and bind the SH2-SH3 domain containing the Nck adaptor proteins that are thought to regulate actin dynamics [36,37]. Transgenic mouse experiments have shown that deletion of Nck from podocytes results in defective foot process development and nephrotic syndrome.

Abnormal T-cell-mediated immune responses have been implicated in minimal change disease in which podocyte effacement and proteinuria are well described [38]. T-cells have also been implicated in the pathogenesis of LN and may lead one to hypothesize that they follow a similarly disrupted signalling pathway resulting in effacement and proteinuria. In a different model of immune complex-mediated kidney injury (MPGN), TLR4 was identified and found to be more abundant on podocytes of cryoglobulinaemic MPGN compared to wild type [39]. TLR4 was upregulated in active disease and once stimulated lead to the release of chemokines. The implications of this can potentially be extended into other immune complex-mediated disease, e.g., LN.

Another pathway that highlights a similarity between podocytes and immune cells come from studies that clarified the anti-proteinuric action of calcineurin inhibitor cyclosporine A [54]. Calcineurin is a ubiquitously expressed serine/threonine phosphatase [40]. The best-characterized function of calcineurin is the regulation of the nuclear factor of activated T-cells (NFAT) signalling. The immunosuppressive action of the calcineurin inhibitor CsA stems from the inhibition of NFAT signalling in T-cells [41]. CsA can also induce a remission of proteinuria caused by diseases including MCD and FSGS [42]. T-cell dysfunction is commonly associated with some forms of MCD in children. Therefore, the anti-proteinuric effect of CsA is often related to the inhibitory effects of NFAT signalling in T-cells [42]. However, CsA can also reduce proteinuria in human [43] and experimental [44] Alport’s syndrome, a non-immunological disease, and LPS-induced proteinuria can develop independent of T-cells [32,45]. Faul and colleagues shed light on the mechanisms underlying the anti-proteinuric effect of CsA by showing that calcineurin regulates the dephosphorylation and thus stability of the podocyte protein synaptopodin and that the CsA benefit on glomerular filter function is independent of T-or B-cells [54].

**Podocyte motility patterns**

Podocyte foot process effacement as well as the migration of podocytes into cellular crescents requires a number of biological cellular events including a reorganization of the podocyte cytoskeleton, movement of the podocytes over the basement membrane and reconstruction of the slit diaphragm. Insights into the mechanisms of podocyte motility can be gained through studies in cultured podocytes during injury [13,46] where cellular motility is a surrogate for foot process dynamics in vivo. There, podocytes stay attached to the GBM, but changes in altered foot process dynamics result in foot process effacement and proteinuria. In some forms of inflammatory glomerular diseases, such as crescentic glomerulonephritis, podocytes can move out of their microenvironment into areas of crescentic glomerular damage [22]. This concept of dual mode podocyte motility (Figure 1) is supported by data from the 1970s, when studies showed that infusion of polycations can change foot
Fig. 1. Two different types of podocyte motility exist. Podocyte foot process effacement that represents motility of foot processes over the glomerular basement membrane seen for example in minimal change nephrotic syndrome (blue box) and podocyte migration into cellular crescent for example during lupus nephritis (red box), (green: uPAR; red: synaptopodin). The schematic is adapted from [56].

process dynamics and cause their effacement [47,48] as well as tyrosine phosphorylation of nephrin [37]. Due to the inability to image foot process dynamics continuously in living animals, the studies serve as a surrogate for the highly dynamic podocyte foot process system. Based on this concept, increased foot process motility underlies the onset of proteinuria [13]. Polycation-induced foot process effacement involves the active reorganization of actin filaments [49,50], and disruption of the actin cytoskeleton by cytochalasin can prevent polycation-induced foot process effacement [51].

Recent work is emerging on the role of molecules such as cathepsin L that is involved in the regulation of the podocyte cytoskeleton that has been shown in vitro to be required for the increased podocyte motility observed during podocyte injury leading to foot process effacement and proteinuria [13]. The podocyte’s role in crescents during some forms of LN [22] can be studied with the NZB/W mouse model of SLE. Work from our group has shown that uPAR (urokinase plasminogen activator receptor) is involved in the cellular motility required for LPS-induced proteinuria but also in podocytes that populate crescents [6]. It will be interesting to see if and how uPAR cooperates with other podocyte motility markers such as the recently described protein KIBRA known to associate with the polarity protein PATJ [52].

**Future perspective**

LN can entail different forms of podocyte damage patterns, thus offering a unique challenge, but also potential, to better understand the function of the podocyte in general and during injury. Future work needs to focus on further characterization of the role of podocytes in LN and identify the signaling mechanisms involved in inducing podocyte migration in order to develop more specific therapies that could potentially reverse or at least decrease the degree of foot process effacement, proteinuria and injured podocytes found in crescents. Therapies altering podocyte motility such as shown by injecting the small molecule drug cyclo-RGDiV, a specific inhibitor of αβ3 [53] into proteinuric mice are giving reasonable promise for more effective ways to combat proteinuria by interfering with podocyte motility [6].

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**References**


Nocturnal versus conventional haemodialysis: some current issues

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Introduction

The burden of kidney disease continues to grow in the United States, and more patients are faced with the need for dialysis [1]. While haemodialysis is a life-saving form of renal replacement therapy, the long-term outlook for patients on the conventional dialysis regimen of 4 h per session three times per week is grim: mortality four times higher than the general population for dialysis patients under 30 and six times higher than the general population for dialysis patients over 65 [2]. The number of patients on home haemodialysis—both conventional haemodialysis (CHD) and nocturnal haemodialysis (NHD)—represents a small fraction of the end-stage renal disease population (ESRD) in the USA and Canada, ∼1500 in 2003, <0.4% of all dialysis patients in the USA [3]. A survey of dialysis machine manufacturers in 2007 estimated that ∼1% of ESRD patients in the USA were doing home dialysis [4].

Home haemodialysis enjoyed early success in the USA with ∼40% of all dialysis patients treated at home at one point [5]. Scribner and colleagues carried out the first nocturnal dialysis in Seattle in the 1960s, using a pumpless plate dialyser [6]. De Palma and colleagues undertook the earliest daily dialysis with a reusable coil dialyser and blood pump [7]. But their early experiment in daily and nocturnal dialysis failed because ‘economic considerations—not quality machinery and ergonomics—came first’ [4]. Others attributed the decline in home haemodialysis and NHD to the advent of continuous ambulatory peritoneal dialysis in the 1970s, improvements in cadaveric transplant survival with the advent of cyclosporine in the 1980s and increased use of living donor kidneys in the 1990s [3].

The USA initially funded home haemodialysis programmes to counter growing costs of in-centre dialysis, but home dialysis fell further out of popularity with passage of amendments to the Social Security act on dialysis payment in 1972 since in-centre dialysis was able to deliver minimum adequacy with three sessions per week [8].

Home NHD is now a little used modality. According to data from the US Renal Data System, only 0.2% of the incident ESRD patients in 2004 started on home haemodialysis of any sort. Of the 472,999 prevalent patients, only 0.4% were on home haemodialysis [9]. In 2005, 428 of 104,018 incident dialysis patients started on home haemodialysis, and 2105 of 340,057 prevalent dialysis patients were on home haemodialysis. In the USA, home haemodialysis patients are less likely to be African American and Hispanic than in-centre dialysis patients [10].

A survey of all known daily dialysis programmes in the USA found 13 centres in North America performing daily NHD as of January 2001, caring for 115 patients [11]. The International Quotidian Dialysis Registry in Ontario recorded 229 patients in 2007, up from 199 in 2006 [12].

Yet data have accumulated showing advantages to more frequent daily or nocturnal dialysis. Based on his experiences observing patients in the intensive care unit [13], Robert Uldall in Toronto initiated a 2-year pilot study with a grant from the government of Ontario to evaluate what he called simplified nocturnal home haemodialysis [14]. He reasoned that frequent long dialysis sessions produced fewer symptoms than short intermittent treatments; at-home dialysis was less expensive than in-centre dialysis; nocturnal dialysis was less disruptive than daytime dialysis.

The programme started enrolling the first of 36 patients in 1994. The 30 patients enrolled at 5 years showed improvements in blood pressure, mineral metabolism and cognitive functioning. There was no difference in haemoglobin levels or erythropoietin use [15]. By 2003, the group had trained 90 patients and was dialysing 48 patients nightly and 5 every other night for a total experience of 230 patient-years [16].