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Role of TWEAK in Lupus Nephritis: A bench-to-bedside review

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Abstract

There is significant unmet need in the treatment of lupus nephritis (LN) patients. In this review, we highlight the role of the TWEAK/Fn14 pathway in mediating key pathologic processes underlying LN involving both glomerular and tubular injury, and thus the potential for renal protection via blockade of this pathway. The specific pathological mechanisms of TWEAK – namely promoting inflammation, renal cell proliferation and apoptosis, vascular activation and fibrosis – are described, with supporting data from animal models and in vitro systems. Furthermore, we detail the translational relevance of these mechanisms to clinical readouts in human LN. We present the opportunity for an anti-TWEAK therapeutic as a renal protective agent to improve efficacy relative to current standard of care treatments hopefully without increased safety risk, and highlight a phase II trial with BIIB023, an anti-TWEAK neutralizing antibody, designed to assess efficacy in LN patients. Taken together, targeting the TWEAK/Fn14 axis represents a potential new therapeutic paradigm for achieving renal protection in LN patients.

Introduction

Lupus nephritis (LN) is a common and serious complication of systemic lupus erythematosus (SLE), and a major cause of morbidity and mortality. Up to 50% of unselected adult patients with SLE have signs of renal involvement early in the course of their disease, and up to 60% of adults may eventually develop overt kidney disease [1, 2]. The clinical course of LN varies from mild subclinical disease to an aggressive course that may progress to end-stage renal disease (ESRD).

The introduction of currently available therapies for LN including the use of broadly immunosuppressive therapies, namely glucocorticoids, cyclophosphamide, azathioprine and mycophenolate mofetil (MMF), has improved outcomes of the disease. However, response to therapy is slow and incomplete, with less than 10% of patients achieving complete

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remission by 6 months and around 60% by 3 years [3]. Complete normalization of renal function cannot always be achieved, in particular when renal scarring has already occurred despite therapeutic intervention. Also, the majority of subjects experience a relapse within 5 years despite continued immunosuppressive therapy [4]. Furthermore, although the survival rates for patients with LN treated with the currently available immunosuppressive agents has improved to 88% at 10 years, around 10–20% of these individuals will develop ESRD [5].

Patients with active LN who do achieve a complete renal response after induction therapy have excellent overall and renal survival compared with those patients with no renal response [6, 7]. Achieving partial renal response has also been demonstrated to have a beneficial effect on patient and renal survival compared to non-responders. A study of 86 subjects with diffuse proliferative LN showed after 10 years of follow up that patient survival was 95% for patients achieving complete remission, 76% for patients achieving partial remission and 46% for non-remission subjects, and that renal survival (as defined by development of ESRD) was 94% for patients achieving complete remission, 45% for patients achieving a partial remission and 13% for non-remission [8]. Therefore, the treatment of LN should aim to increase the number of patients with a complete response, as well as to reduce the number of non-responders by converting them into partial or complete responders.

Numerous clinical trials conducted over the last 10 years assessing induction therapy for LN [9-11] have demonstrated improved safety and tolerability of newer therapeutic protocols, but failed to show a substantial improvement in renal response rates compared to established regimens of high dose cyclophosphamide and corticosteroids. In addition, the number of patients achieving a complete renal response within a short time frame remains relatively small. Importantly, current treatments for LN and some of the therapies investigated in clinical trials are broadly immunosuppressive and do not target specific pathways responsible for the development of renal disease. Moreover, these regimens are associated with poor treatment adherence, and may result in significant side effects and, occasionally, death [12-14]. It is therefore reasonable to speculate that we may have reached the ceiling of therapeutic response using broadly immunosuppressive agents in LN, and that a therapy that is more targeted to impact the pathogenic processes driving the progression of ESRD may enable achievement of greater renal response.

The pathogenesis of LN is complex, involving autoantibody deposition in the glomerulus, activation of complement and macrophages, cell proliferation, production of extracellular matrix proteins, pro-inflammatory cytokines, chemokines and MMPs, which then link through multiple mechanisms to tubular damage, tubulointerstitial inflammation and fibrosis [15, 16]. In order to improve patient outcome, targeted therapies for LN should ideally impact the common pathological features of LN that are responsible for progression of ESRD including glomerulosclerosis and tubulointerstitial fibrosis [17-19]. The development of more specific therapeutic approaches should result in increased efficacy, without paying the price in safety, tolerability, and side effects.

The cytokine tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK, TNFSF12) is a member of the TNF superfamily that is prominently featured in normal and pathological remodeling of tissues. TWEAK, expressed primarily as a soluble cytokine by infiltrating leukocytes, mediates multiple activities through its receptor FGF-inducible molecule 14 (Fn14, TNFRSF12) which is upregulated locally on epithelial and mesenchymal cell types in injured and diseased target tissues including the kidney. TWEAK is involved in pro-inflammatory responses, vascular activation and angiogenesis, cell growth, cell death, fibrogenic responses, and progenitor responses.

In this paper, we will review the evidence supporting a role for the TWEAK/Fn14 pathway in driving the development and progression of LN, including the elevated expression of both TWEAK and Fn14 locally in the kidneys in human LN, and the ability of TWEAK acting through Fn14 on various kidney-resident cell types to promote many of the key pathogenic processes underlying LN. In addition, data from multiple animal models of LN and kidney injury in which TWEAK/Fn14 pathway deficiency or pathway blockade by anti-TWEAK monoclonal antibodies (mAbs) has proven efficacious will be presented. Finally, the relevance of TWEAK-driven mechanisms of pathogenesis will be related to human LN. Taken together, targeting the TWEAK/Fn14 pathway may represent an attractive therapeutic option to increase renal response and achieve remission in a greater number of patients due to its tissue protective effect, without an anticipated increase in toxicity and patient morbidity.

II. Introduction to the TWEAK/Fn14 Pathway

TWEAK was cloned and identified as a member of the TNF superfamily in 1997 [20]. Since then, the basic biology of this pathway has been largely elucidated, and considerable evidence has been gathered linking the TWEAK pathway to a variety of inflammatory and autoimmune diseases. In particular, there is a growing appreciation for the pathological role of this pathway in affected tissues in disease, such as the kidney in LN.

A type II transmembrane receptor, TWEAK can be cleaved to generate a soluble cytokine, which is thought to be the predominant form of the ligand. TWEAK mRNA has been detected in a variety of cell types, particularly inflammatory cells such as polymorphonuclear leukocytes, macrophages, DC and NK cells, as well as in B and T cell subsets (Immunological Genome Project Consortium, Datagroup Human Immune Cells Garvan, Gene Symbol TNFSF12, Probe set 205611_at). TWEAK expression has also been reported in some non-hematopoietic cell types, including astrocytes, endothelial and renal tubular cells [21-23]. Consistent with the predominant expression of TWEAK by infiltrating cell types and cleavage of TWEAK from the cell surface, elevated levels of TWEAK have been detected locally in inflamed tissues in a variety of animal models and in human disease. Elevated TWEAK has been observed in the synovial tissues and fluid of rheumatoid arthritis (RA) patients [24], in the inflamed colon of patients with inflammatory bowel disease (IBD) [25], and in brain tissue from active lesions of multiple sclerosis (MS) patients [26]. With respect to expression in the kidney, TWEAK mRNA levels are elevated in murine kidneys in experimental models of kidney injury and disease relevant to LN [27, 28] (and CP, JSM, LB, unpublished) and in human LN kidneys [29], and urinary soluble TWEAK levels are elevated in patients with LN and correlate longitudinally with renal flares [30, 31], as described in more detail below (Section II.ii).

The only known signaling receptor for TWEAK is Fn14, the smallest in the family of TNF receptors. It should be noted that CD163 has been identified as an additional putative binding partner of TWEAK [32]; however, the relevance of this interaction has not been confirmed [33]. Fn14 is expressed on a wide variety of cell types, including epithelial, endothelial and mesenchymal cell lineages, as well as in various progenitor cell populations. In the context of the kidney, Fn14 can be expressed by human mesangial cells, tubular cells and podocytes [34]. Fn14 is also presumably expressed on endothelial cells and interstitial fibroblasts in the kidney since Fn14 expression has generally been reported on vascular cells and fibroblasts [35]. Thus, as discussed further below, the TWEAK/Fn14 pathway may signal in multiple kidney cell types to promote renal pathology in LN. Notably, Fn14 is not expressed on B or T lymphocytes, and consistent with this, the TWEAK/Fn14 pathway does not appear to play a prominent role in the regulation of adaptive immune responses [36].

In normal tissues, in both rodent and man, Fn14 is expressed at relatively low levels. However, Fn14 expression is highly inducible, and can be upregulated in response to not only FGF, as its name implies, but also following stimulation with other growth factors including EGF, PDGF and VEGF, various cytokines including TNF α , IL-1 β , IFN γ , TGF β and IL-13 [25, 35], or TLR ligands [37]. The highly inducible nature of Fn14 expression is evident in animal models of injury and inflammation, and is similarly reflected in human disease tissues. Fn14 expression has been shown to be elevated locally in the tissues of experimental models of injury and inflammation, such as the injured heart [38], vasculature [39, 40], skeletal muscle [41] or denervation [42], intestinal epithelium [37], spinal cord [43-45] and liver [46, 47]. Likewise, upregulated Fn14 expression is detected in human disease tissues including RA synovium [24], MS brain [26], IBD colons [37], inflammatory liver diseases [46], and cancer [48]. In the context of LN, as described in more detail below, Fn14 expression is elevated in the kidneys of lupus prone mice (CP and LB, unpublished) and mouse models of kidney injury [23, 27, 28], as well as in both glomeruli and interstitial kidney mRNA isolated from LN patients [29].

TWEAK signals through its receptor Fn14 to activate multiple downstream signaling pathways, most notably the NF κ B pathway. TWEAK induces both canonical NF κ B signaling via phosphorylation of I κ B and translocation of p50/p65 heterodimers into the nucleus, as well as more sustained non-canonical NF κ B signaling leading to stabilization of NIK, processing of p100 and nuclear translocation of p52/RelB heterodimers [49]. TWEAK signaling through NF κ B has been functionally validated in numerous cellular and in vivo contexts. With regard to signaling in kidney cell types, TWEAK was shown to induce NF κ B signaling in mesangial cells, with chemokine induction being NF κ B-dependent [34, 50]. Similarly, in tubular cells TWEAK can induce NF κ B pathway activation, which is necessary for proliferation and chemokine production [28, 51-53]. Moreover, in vivo administration of TWEAK was shown to induce both canonical and non-canonical NF κ B signaling in the kidney [28, 53] (and JSM, unpublished). Additional signal transduction pathways induced by TWEAK in kidney tubular cells and independent of the NF κ B pathway include MAP kinase signaling via phosphorylation of p38 and ERK1/2, and PI3 kinase/AKT signaling as evidenced by phosphorylation of AKT [52].

II.i. Functional role of TWEAK/Fn14 pathway

The TWEAK/Fn14 axis regulates a variety of cellular activities, and as such TWEAK is considered to be a multifunctional cytokine. Depending on the cellular context, TWEAK may promote inflammation, vascular activation, angiogenesis, cell survival, proliferation or cell death, fibrosis and progenitor cell fate regulation (reviewed in [54]).

The pro-inflammatory effects of TWEAK, namely induction of a wide array of cytokines, chemokines and matrix metalloproteinases, have been described extensively and in a variety of cellular contexts, such as human renal cell types [28, 34, 51, 53], dermal, synovial and gingival fibroblasts [55, 56], endothelial cells [57], macrophages [58], keratinocytes [59], chondrocytes [60], bronchial epithelial cells [61], astrocytes [62], and tumor cells [20]. In the kidney, TWEAK was shown to induce a wide array of chemokines and cytokines and promote the infiltration of immune cells. Significantly, many of the proinflammatory mediators induced by TWEAK are associated with disease in human LN, as described in more detail below (Section IV.iv).

Another well-characterized role of TWEAK is its effects on the vasculature. In accordance with its pro-inflammatory effects, TWEAK can induce endothelial cell activation, with up-regulation of ICAM-1 and E-selectin [57]. It is also well established that TWEAK is pro-angiogenic. TWEAK can promote proliferation of a variety of primary human vascular cells, including aortic endothelial cells, umbilical vein endothelial cells (HUVEC), and dermal and

brain microvasculature endothelial cells [22, 40]. TWEAK can also induce endothelial cell survival and migration [22, 63]. Moreover, in vivo administration of TWEAK was shown to stimulate the formation of new blood vessels comparable in magnitude to that induced by known angiogenic stimuli, such as VEGF [64]. Finally, another interplay between TWEAK and the vasculature is the observation that TWEAK can promote disruption of the neurovascular unit (NVU) and thereby increase blood brain barrier permeability [65]. TWEAK apparently mediates this process by inducing degradation of the extracellular matrix (ECM) via MMP-9 up-regulation, resulting in disruption of astrocytic foot processes which are essential for maintaining the integrity of the NVU. Whether TWEAK can promote vascular permeability in other settings such as the lupus kidney where podocytes wrap around the glomerular capillaries functioning to maintain the integrity of the glomerular filtration barrier has not yet been investigated.

TWEAK can also exert context-dependent effects on cell proliferation, survival and death (reviewed in [66]). These activities have been demonstrated in numerous contexts, including intestinal epithelial cells [37], skeletal muscle [67, 68], endothelial cells [22, 63, 64] and tumor cells (reviewed in [48]). With regard to the kidney, effects of TWEAK on mesangial proliferation both in vitro and in vivo have been reported [34]. TWEAK can alternatively stimulate cell death, as has been shown most notably in the context of tumor cells [48], but also in primary cortical neurons [44, 69] and intestinal epithelial cells [25]. The ability of TWEAK to induce renal cell death has also been demonstrated, particularly in the presence of inflammatory cytokines such as TNF and/or IFN γ [23, 34, 50]. The potential for TWEAK to promote cell proliferation versus cell death in LN kidneys likely depends on the cellular and cytokine context.

Interestingly, growing evidence supports a prominent role for TWEAK in promoting fibrosis, a final common pathway in chronic inflammatory diseases and in particular of ESRD [17-19]. TWEAK overexpression in transgenic mice induces fibrosis in the heart [70] and skeletal muscle [42]. In addition, systemic delivery of an adenoviral-TWEAK but not an adenoviral-control vector induces fibrosis in mouse heart, liver and kidneys (LCB, unpublished). Importantly, a role for endogenous TWEAK/Fn14 in promoting fibrosis was shown by attenuation of fibrosis upon TWEAK/Fn14 pathway blockade or deficiency in several other experimental models: denervation-induced skeletal muscle atrophy [42], atherosclerosis [39], and chronic colitis (T. Dohi and LCB, unpublished), and more recently in models of kidney injury and disease relevant to LN (Section III.vi).

Finally, TWEAK has been shown to regulate progenitor cell biology. Many progenitor cell types express Fn14, including mesenchymal, epithelial, and neural lineage precursors (reviewed in [66]). TWEAK was shown to promote progenitor cell expansion, as demonstrated in vivo in the context of the liver, as well as in cultured liver progenitor cells, namely oval cells [46], as well as osteoblast progenitors [60, 71] and the C2C12 myoblast cell line [41]. Conversely, TWEAK inhibits the differentiation of progenitor cells in vitro, including in systems of myogenesis [41, 68], 3T3L1 adipogenesis [60] and rat neonatal cardiomyocytes [72]. Significantly, the ability of TWEAK to affect progenitor cell fate reflects the capacity of TWEAK to play a role in tissue regeneration as illustrated in contexts of liver and skeletal muscle injury (reviewed in [54]). The intriguing possibility that TWEAK may impact myofibroblast precursor cells has not been explored but could represent a mechanistic role of TWEAK in fibrosis, as discussed further below.

II.ii. TWEAK/Fn14 Pathway expression in LN

With a fundamental understanding of the pathway as described above, a model incorporating the pattern of expression of TWEAK and Fn14 and its relevance in LN emerges. TWEAK is primarily expressed on infiltrating cell types, such as macrophages and other immune cells

as well as renal cells, and is rapidly cleaved from the cell surface to generate the predominantly soluble form of TWEAK. As such, in the context of injury or inflammation, elevated levels of soluble TWEAK can be found locally at the site of diseased tissue. In LN, increased TWEAK expression has been detected in patient glomeruli and tubulointerstitium relative to normal controls [29], and elevated levels of urinary TWEAK are evident in LN patients [30, 31], as described in more detail below. Additionally, PBMCs isolated from LN patients had higher TWEAK mRNA expression compared to SLE patients without renal disease or healthy controls [73], although this data was not reproduced in a separate study [74].

In concert with this appreciation of TWEAK expression, Fn14 receptor expression is tightly controlled, being detected only at low levels in normal healthy tissue but markedly upregulated in LN kidneys. An increase in renal expression of Fn14 relative to healthy controls, both in the glomerular and tubulointerstitial compartments, has been reported in patients with LN [29]. Fn14 may be upregulated on resident cells, including mesangial and tubular cells, podocytes, endothelial cells and interstitial fibroblasts. Thus, this coordination of ligand and receptor expression in the context of disease suggests a role for the TWEAK/Fn14 pathway in driving disease progression in LN.

The most compelling data for a link between TWEAK expression and human LN comes from analysis of urinary TWEAK. In a multi-center cross-sectional study that was subsequently confirmed in an expanded independent study, Schwartz et al [30, 31] reported that lupus patients with active renal disease had higher levels of urinary TWEAK (uTWEAK) compared to lupus patients with non-active renal disease, and that uTWEAK levels correlated significantly with renal disease activity as assessed by the renal SLEDAI scores. Also of note was that elevated uTWEAK levels were not detected in other systemic inflammatory conditions, such as RA, or in other renal diseases. Moreover, uTWEAK levels were higher in patients undergoing a flare as compared to patients with stable disease, and levels of TWEAK were significantly elevated in those patients undergoing renal as compared to non-renal flares. There was also a significant correlation between levels of uTWEAK and uMCP-1 as well as with other traditional biomarkers for LN activity, such as anti-dsDNA antibodies and complement levels. A significant association between uTWEAK levels and renal disease activity over time was also observed in a longitudinal study, with highest uTWEAK levels measured at the time of flare. Finally, serum TWEAK levels were not associated with renal activity, highlighting the elevated local production of TWEAK in the kidney in LN. In an independent study of 73 SLE patients [75], uTWEAK levels were similarly found to be higher in patients with LN, and a positive correlation between uTWEAK and renal SLEDAI was observed.

The accumulating evidence associating urinary TWEAK with human LN disease activity suggests not only that TWEAK may play a role in LN pathogenesis, but also the exciting potential for uTWEAK as a novel biomarker of disease activity in LN. As detailed above, uTWEAK correlates with renal disease, with renal flares, and with other biomarkers of LN activity. In fact, uTWEAK was better at distinguishing between LN and non-LN SLE than standard clinical markers [30]. The prospect of uTWEAK as a biomarker could be envisioned as a component of a biomarker marker panel for the overall clinical management of LN patients.

Taken together, there is an increasing body of evidence linking TWEAK pathway expression, in particular levels of urinary TWEAK, with renal disease in SLE. This highlights the promising opportunity for considering TWEAK as a therapeutic target in LN. In the next section, the pathological activities associated with TWEAK in LN including

compelling data from animal models will be explicated to further establish the case for targeting TWEAK in LN.

III. Pathological mechanisms of TWEAK in LN

The role of TWEAK, as described above, in promoting an inflammatory response, renal cell proliferation and apoptosis, vascular changes, and fibrosis, together with an increasing appreciation for the locally elevated levels of TWEAK in LN patients, suggests that TWEAK may play an active role in the kidney in the context of LN.

Multiple experimental models have been employed to assess the potential contribution of the TWEAK/Fn14 pathway to LN. These include several models in which renal injury is initiated by autoantibody deposition in the glomerulus, as well as other models induced by renal tubular injury, both of which can lead to renal dysfunction and fibrosis. Importantly, all of these models are relevant to LN, especially in light of the mechanisms linking glomerular and tubular injury, as discussed by Lopez-Novoa et al [76] and illustrated in Figure 1. Indeed, evaluation of the TWEAK pathway in many different animal models relevant to LN has validated the significant contribution of this pathway to kidney disease and elucidated its pathological mechanisms. In this section, we will review the animal model data (summarized in Table 1) and accompanying in vitro studies that support a role for TWEAK in several central aspects of the pathogenesis of LN.

III.i. Evaluation of the TWEAK pathway in murine models of lupus and renal disease

The TWEAK pathway has been investigated in multiple animal models relevant to LN. The role of the TWEAK pathway was assessed in the experimental chronic graft-versus-host disease (cGVHD) model of LN, which is induced upon transfer of MHC class II incompatible spleen cells into host mice and is characterized by the development of high titers of autoantibodies against nuclear antigens and autoantibody-mediated glomerulonephritis. Deficiency of Fn14 in this model resulted in decreased renal damage as indicated by proteinuria without affecting autoantibody titers [77]. Decreased immunoglobulin (Ig) deposition, kidney cell proliferation and cytokine expression were also observed in the Fn14 knockout (KO) mice. In a pharmacological study, cGVHD-induced mice were treated with a neutralizing anti-TWEAK monoclonal antibody (mAb), and this similarly resulted in decreased proteinuria and renal inflammation, with no effect on autoantibody levels [77].

A role for the TWEAK pathway was also demonstrated in a nephrotoxic serum transfer nephritis (NTN) model of LN [78], a system which shares many features with human LN and manifests more severe nephritis and renal injury than in cGVHD. In this model, passive transfer of rabbit serum containing pre-formed antibodies against the glomerular basement membrane (GBM) into mice pre-immunized with heterologous rabbit IgG results in a progressive, proliferative glomerulonephritis with significant histopathology. When NTN was induced in Fn14 deficient mice, amelioration of proteinuria and improvement in renal histology was evident. A reduction in inflammatory markers in the kidneys of Fn14 KO mice was also observed. Interestingly, there was a decrease in Ig deposition in the kidneys of Fn14 KO mice despite no difference in circulating titers of mouse anti-rabbit IgG1 and IgG2a subclasses, the latter isotype thought to be highly pathogenic given its Fc effector function; however, a reduction in IgG2b and IgG3 subclasses was evident in the Fn14 deficient mice. In a parallel study, treatment of NTN-induced mice with an anti-TWEAK mAb similarly resulted in reduced proteinuria and improved histopathology without affecting mouse anti-rabbit Ig titers. Mice treated with the anti-TWEAK mAb also exhibited decreased inflammation, renal cell proliferation and fibrosis. Finally, data from a

spontaneous model of lupus in MRL/lpr mice shows reduced proteinuria in Fn14 deficient as compared to wildtype mice [79].

A role for the TWEAK pathway has also been demonstrated in multiple models featuring renal tubular injury. In an experimental model of acute kidney injury (AKI) induced by folic acid overdose and characterized by kidney inflammation, tubular cell death and renal failure, Fn14 expression was shown to be upregulated on the tubular epithelium [28]. When AKI was induced in TWEAK deficient mice, or in WT mice with administration of anti-TWEAK mAb, improvements in renal function as measured by serum creatinine and histologic tubular injury were observed [28, 52]. TWEAK deficiency or neutralization also resulted in decreased inflammatory markers in the kidney as well as a reduced number of infiltrating macrophages and T cells [28, 51-53]. In addition, there was a marked decrease in tubular apoptosis and subsequent proliferation. Similarly, a role for TWEAK was demonstrated in a renal ischemia reperfusion injury (IRI) model [27], where a marked induction of Fn14 expression in the kidney was observed following reperfusion post ischemia. Treatment with an anti-Fn14 mAb resulted in decreased serum creatinine, renal expression of pro-inflammatory cytokines and chemokines, and reduced numbers of interstitial neutrophils and macrophages. In addition, blockade of the TWEAK pathway attenuated development of fibrosis induced by IRI and significantly prolonged survival.

Taken together, there is substantial evidence from multiple animal models supporting a pathological role for TWEAK in the context of LN and other forms of renal injury. In the next sections, we delve into the various processes by which the TWEAK pathway may contribute to disease pathogenesis with supporting data from the animal models described above as well as from in vitro mechanistic studies. Based on these studies, blocking TWEAK is expected to reduce pathological responses of cell types in both the glomeruli and tubules, thereby protecting the kidney from progressive glomerular and tubular damage and dysfunction and progression to ESRD.

III.ii. TWEAK promotes renal inflammation

Inflammation is a hallmark of LN, developing in the glomerulus after autoantibody-mediated glomerular damage and in the tubulointerstitium after tubular injury. Infiltrating leukocytes damage the renal tissue and release proinflammatory mediators, thereby augmenting and sustaining a vicious tissue-damaging cycle. The TWEAK/Fn14 pathway contributes to this key pathogenic process through its ability to induce proinflammatory responses locally in the kidney, including a wide array of cytokines, chemokines, adhesion molecules and MMPs. As discussed above (Section III.i) and shown in Table 1, TWEAK/Fn14 pathway blockade or deficiency reduces disease severity in multiple kidney disease models, and this efficacy is associated with decreased renal inflammatory responses [27, 28, 52, 53, 77, 78].

Notably, TWEAK may promote inflammation in the glomerulus, the site of primary injury in LN, as well as induce tubulointerstitial inflammation, a feature of LN chronicity. TWEAK may thereby promote renal damage and drive disease progression in early as well as later stages of the disease. TWEAK-responsive renal cells comprise the Fn14-expressing glomerular cell types including vascular endothelial cells [57, 64], and mesangial cells and podocytes [34, 50] which produce inflammatory mediators in response to TWEAK stimulation. In addition, Fn14-expressing renal tubular cells [28, 34, 51, 53] and fibroblasts [55, 56] secrete inflammatory mediators in response to TWEAK. It is also possible that TWEAK induces proinflammatory activity in infiltrating macrophages, based on studies in THP-1 cells [58]. Consistent with the ability of TWEAK to act in both the glomerular and tubular compartments, blocking TWEAK reduces macrophage infiltration in glomeruli [78] and macrophage and T cell infiltration in the tubulointerstitium [28, 53] in kidney injury

models. Thus TWEAK may promote inflammation in glomeruli and tubulointerstitial regions, and thereby contribute to histopathological indices of activity as well as chronicity and, consequently, renal dysfunction in LN.

The array of proinflammatory mediators induced by TWEAK in renal cell types is very broad. Specifically, TWEAK was shown to induce expression of MCP-1, IP-10, RANTES, MIP-1 α , ICAM-1 and VCAM-1 in multiple renal cell types in vitro. Importantly, many of these mediators are also associated with disease in human LN and directly implicated in murine LN (discussed further below). TWEAK-induced chemokines were shown to stimulate the migration of human PBMCs, particularly monocytes/macrophages in vitro [34]. This finding may be relevant in human LN, where macrophage infiltrates are strongly correlated with poor outcome [80]. Likewise, when administered in vivo, TWEAK induced chemokine expression in the kidney, including MCP-1 and RANTES. Additional assessment demonstrated induction of the T cell chemoattractant CCL-21, as well as CXCL-16, and infiltration of both macrophages and T cells into the kidney [28, 34, 51, 53]. Thus TWEAK has the potential to promote the infiltration of both innate and adaptive immune cell types in LN.

III.iii. TWEAK stimulates the vasculature

Renal vascular injury and activation are key pathological processes in LN. Vascular injury initially results when glomerular deposits of autoantibody-containing immune complexes engage immune effector mechanisms, consequently resulting in leukocytic infiltrates that mediate vascular activation and further damage. The vascular responses that occur involve the induction of adhesion molecules and endocapillary proliferation [81]. Relevant to this, it is well established that the TWEAK/Fn14 pathway induces these effects on vascular cells. TWEAK promotes endothelial cell survival, endothelial and smooth muscle cell proliferation, and angiogenesis [66]. Thus, TWEAK-mediated Fn14 activation of vascular cells may contribute to pathological vascular responses which are typically observed in LN kidneys.

In addition to vascular activation, the TWEAK/Fn14 pathway may also regulate vascular permeability. Through its promotion of angiogenesis and as a prerequisite for endothelial cell migration, TWEAK induces loss of endothelial cell contacts, and this process causes capillary leakage. Another potential mechanism whereby TWEAK/Fn14 may promote vascular permeability relates to its regulation of ECM turnover. As mentioned above, TWEAK promotes disruption of blood brain barrier vascular permeability by inducing degradation of ECM components of the NVU [65]. Since the glomerular capillary wall consists of fenestrated endothelium, a glomerular basement membrane (GBM), and podocyte foot processes, we speculate that TWEAK might also regulate the turnover of GBM components through its effects on podocytes. Related to this, the TWEAK/Fn14 pathway may regulate GBM composition through its promotion of IL-8 production, as IL-8 was shown to alter the composition of glomerular heparin sulfate glycosaminoglycans, resulting in a decrease of total negative charge, a phenomenon which has been associated with an increased urinary albumin/creatinine ratio [82, 83]. Also of interest, TWEAK has been correlated with an increased angiopoietin-2/1 ratio, and this balance is associated with increased vascular permeability in septic shock patients [84]. Thus, while not yet determined, TWEAK may promote capillary permeability by promoting the production of angiopoietin-2 by endothelial cells and/or suppressing that of angiopoietin-1 by vascular pericytes.

III.iv. TWEAK induces mesangial cell proliferation

Renal mesangial cells are an important cell type that supports the glomerular capillaries. Proliferation of mesangial cells occurs early after immune complex-mediated damage in LN, presumably as a tissue repair mechanism. Mesangial cell hyperplasia, and associated ECM deposition resulting in sclerosis, are histopathological criteria for LN activity and chronicity, respectively. Recently, studies have demonstrated that TWEAK promotes the proliferation of human mesangial cells in vitro and in vivo [34]. In a complementary set of investigations, mesangial cell proliferation was shown to be reduced with Fn14 deficiency in the cGVHD induced nephritis model [77]. Furthermore, both mesangial cell proliferation and glomerular periodic acid Schiff (PAS) staining were reduced with TWEAK blockade in the NTN model (CP, JSM, and LB, unpublished). Thus, TWEAK may contribute to glomerular pathology in LN through its ability to increase the number of Fn14-expressing mesangial cells and perhaps the matrix deposition by this expanded population which may lead to fibrosis (see below).

III.v. TWEAK promotes renal cell death

Tubular injury and resulting atrophy are signs of progressive disease in LN. Tubular injury may result from proteinuria-induced stress on the tubules, proinflammatory mediators and tissue debris resulting from damaged glomeruli, and ischemic damage as renal blood flow is reduced with glomerular scarring. Mediators, including TWEAK, which are present in the renal tissue may also contribute to renal cell death. The ability of TWEAK to stimulate cell death was originally observed when combined with IFN γ on tumor cells [20], and since then TWEAK has been shown to play a role in the cell death of some primary cells, including cortical neurons [44, 69] and intestinal epithelial cells [25]. Notably, it was also shown that TWEAK/Fn14 signaling can induce renal cell death in conjunction with other inflammatory cytokines that are present in the inflammatory milieu in LN. For example, TWEAK can induce death of renal mesangial cells in combination with IFN γ [50]. TWEAK-induced death of podocytes has not been directly assessed in vitro, however Fn14 up-regulation in contexts of podocytopathy in rodents and humans has been observed (A. Ortiz, unpublished). Thus, in line with TWEAK's cell death-inducing potential, TWEAK-induced podocyte death could be a mechanism contributing to loss of the glomerular filtration barrier. TWEAK also potentiated cell death in a renal tubular cell line in combination with TNF and IFN γ , each of which can induce Fn14 expression [23]. These data suggest that TWEAK may contribute to tubular damage and atrophy in LN, and the sequelae of tubular cell death, including tubulointerstitial inflammation, which further promotes tissue damage and replacement of tubules with fibrotic tissue. Complementing these in vitro studies, tubular damage scores and tubulointerstitial inflammation were reduced by TWEAK/Fn14 pathway blockade or deficiency in the NTN (CP, JSM and LB, unpublished), AKI [28] and renal IRI [27] models.

III.vi. TWEAK promotes fibrosis

Amongst the strongest predictors of poor renal outcome in LN are the extent of glomerular scarring and replacement of injured renal tubules by fibrotic tissue [19]. Given recent evidence for a role for the TWEAK/Fn14 pathway in promoting fibrogenic responses, as discussed above, blocking TWEAK as a therapeutic approach may offer substantial benefit in LN. Importantly, support for this hypothesis in models of kidney injury and disease was recently shown by significant reduction in tubulointerstitial renal fibrosis by Fn14 blockade in the renal IRI model [27] and in TWEAK KO mice after unilateral ureteral obstruction (A. Ucero and A. Ortiz, unpublished). In addition, TWEAK blockade reduced tubulointerstitial fibrosis in the NTN model of LN (CP, JSM and LB, unpublished). Thus, blocking TWEAK may be of great benefit in LN by protecting the kidneys from the progression to fibrosis.

There may be multiple mechanisms whereby TWEAK promotes kidney fibrosis. As discussed above, TWEAK/Fn14 may promote fibrosis as a downstream consequence of its proinflammatory activity, effects on cell death and/or tissue damage, and ability to expand mesangial cells which are a source of glomerular ECM production. However, since Fn14 is expressed by many stromal cell types, TWEAK may directly contribute to a fibrogenic response. Fn14 is upregulated by TGF- β on fibroblasts [35] and is also likely expressed on activated fibroblasts, namely myofibroblasts. Thus TWEAK may act on myofibroblasts, or their precursor resident fibroblasts in the kidney, and thereby contribute to a fibrogenic response by regulating proliferation of the fibroblasts, their differentiation into myofibroblasts and ECM production. Indeed, emerging data indicate that TWEAK treatment of fibroblasts induces their proliferation, collagen expression and myofibroblast differentiation (T. S. Zheng, pers. comm). TWEAK treatment of 3T3 fibroblasts also induced the expression of mRNA of several collagen types (T.S. Zheng, pers. comm.). In yet another independent study, it was found that TWEAK promotes fibroblast proliferation and regulates ECM expression (A Ucero and A Ortiz, pers. comm). Future investigations will further inform the mechanisms whereby TWEAK-induced fibrogenic responses may contribute to glomerulosclerosis and tubulointerstitial fibrosis.

In addition, the TWEAK/Fn14 pathway may also regulate fibrosis through its effect on various other putative myofibroblast progenitors, given the precedence for TWEAK's ability to regulate progenitor cell fate, including mesenchymal lineage progenitors [66]. In contexts of renal injury, myofibroblast progenitors appear to include not only the local resident fibroblasts as mentioned above, but also perivascular fibroblasts and pericytes [85, 86]. In addition, there is some, albeit inconsistent, evidence to support the recruitment of myofibroblast precursors from the circulation, namely fibrocytes, which are derived from a hematopoietic stem cell origin, during the inflammatory process. Interestingly, CXCL16 was recently shown to promote the accumulation of bone marrow-derived fibroblast precursors in injured kidneys in a model of renal fibrosis [87]. Given that the TWEAK/Fn14 pathway promotes CXCL16 expression by renal tubular cells [51], it may contribute to the recruitment of these progenitors. The role of the TWEAK and Fn14 pathway in regulating putative myofibroblast precursor cell types warrants future study.

IV. Relevance of TWEAK mechanisms to human LN

In the previous sections, we discussed how modulation of the TWEAK/Fn14 pathway in the context of animal models of lupus and kidney injury can ameliorate renal disease parameters and kidney histopathology via multiple processes underlying this pathology. In this section, we will describe our current understanding of the relevance of these same disease parameters to human LN, with a specific focus on renal function indicators, fibrosis and other histopathological findings, and inflammatory markers.

IV.i. Renal function: Serum creatinine

As described above in models of kidney injury such as AKI or IRI (Section III.i), blockade or deficiency of the TWEAK/Fn14 pathway was shown to impact kidney function, as measured by improvements in serum creatinine levels. Achievement of such an effect in human LN by a therapeutic intervention would be a highly desirable outcome.

Assessment of renal function at baseline, and periodically after that, is a critical aspect in the management of human lupus. Although serum creatinine is not always a very accurate method to assess renal function, measurement of serum creatinine is widely employed for this purpose. Serum creatinine measured at baseline and over time has emerged as an important predictor of critical renal and patient outcomes, which in LN often includes doubling of serum creatinine from baseline, development of ESRD, and death. A lower

baseline serum creatinine was associated with increased probability of achieving a remission [88]. Moreover, a high serum creatinine at baseline was independently associated with a significantly increased risk of achieving a composite index of renal failure, ESRD, and death [89], while increasing serum creatinine predicted treatment resistance and renal relapse [90]. In additional studies as well, serum creatinine was an independent predictor of renal insufficiency and ESRD [88, 91]. Therefore, renal function influences the chance of achieving remission in LN, and serves as an independent predictor of negative outcomes.

IV.ii. Renal activity and/or damage: Proteinuria

In several animal models of LN described above and shown in Table 1, including both spontaneous and induced models of disease, deficiency or blockade of the TWEAK pathway was shown to result in decreased proteinuria. Proteinuria is a sensitive and relatively specific abnormality detected in lupus patients with renal involvement. Indeed, when measured by any one of several available methods, proteinuria is a key measure used clinically for assessment of renal activity and/or damage in LN patients, and has also been shown to predict subsequent development of renal failure. Moreover, the level of proteinuria itself is now recognized as an important, and modifiable, risk factor for progression of renal insufficiency in chronic renal diseases [92, 93]. The contribution of proteinuria to renal injury is multi-factorial, involving podocyte injury, deposition of complement components on tubular cells, induction of tubular hyperplasia, and interstitial fibrosis [93, 94]. Therefore, sustained reduction in proteinuria is a central goal in the current management of kidney disease patients [92-94]. Although for the most part the beneficial effects of renal protective therapies targeting persistent proteinuria have been demonstrated in kidney diseases other than lupus, studies seem to indicate that the proteinuria is toxic/inflammatory in LN as well. In a large cohort of LN patients followed at the Mayo Clinic, increased urine protein was an independent predictor of renal failure [95]. LN patients reaching a composite outcome of renal failure, ESRD, and death had a significantly higher incidence of nephrotic range proteinuria [89]. Finally, the rate of decrease in proteinuria at 6 months indicated which patients would achieve a complete response [96]. Since increases in, and absolute levels of, proteinuria are widely used in the definition of renal flare and remission, the improved renal and patient prognosis associated with fewer disease flares and attaining remission are also strongly supportive of the contribution of proteinuria to progression of the renal injury. Thus, therapies directed at improving renal function, decreasing proteinuria, accelerating achievement of complete remission and/or increasing the number of patients that ultimately reach that outcome, would be expected to have a salutatory effect on the prognosis of patients with lupus associated renal disease.

IV.iii. Fibrosis and other histopathological changes in the kidney

Improvement in renal histopathology was observed in several renal disease models in which TWEAK/Fn14 deficiency or neutralization of TWEAK was evaluated. Specifically, this included decreased glomerular proliferation or tubular damage, less fibrosis, and in some cases reduced renal Ig deposition. Severe proliferative LN often involves histopathologic changes in both glomerular and tubular structures. As we will detail below, in relapsing, chronic, or unresponsive disease, fibrotic changes occur and have particular prognostic significance.

Needle renal biopsy performed percutaneously remains the gold standard for the diagnosis of LN. The 2003 International Society of Nephrology/Renal Pathology Society (ISN/RPS) LN definition is the scoring scheme in widespread use [97]. Many renal histopathologists also utilize the NIH activity and chronicity indices [17], which consider separately acute renal damage and more chronic changes. The need for, and intensity of, immunosuppressive therapy is often primarily determined by the glomerular lesion and indicators of acute

inflammation [18]. Nevertheless, there are an increasing number of studies clearly indicating the presence of chronic tubulointerstitial disease, primarily fibrosis, as one of the most critical prognostic factors in LN [19].

The contribution of interstitial fibrosis to patient outcome emerged from evaluation of the classic, cyclophosphamide-based treatment protocol for LN. Large cohorts of lupus patients receiving different therapies (steroids, azathioprine, cyclophosphamide, alone or in combination) were carefully followed over time. Among 102 patients, the 11 cases of renal failure occurred only among the 72 patients with diffuse proliferative or membranoproliferative disease. Components comprising the chronicity index, including glomerulosclerosis, crescents, and interstitial fibrosis, alone or in a composite index, were best predictive of an outcome of renal failure [98]. African-American ethnicity is associated with a particularly poor renal prognosis in LN [19]; African-Americans who develop renal failure at a significantly higher incidence were more likely to have high risk features including interstitial fibrosis on the pre-treatment biopsy [91]. Similarly, in a single center study from the University of Chicago with 68 patients, a high NIH chronicity index, but not ISN/RPS LN class or the activity index, predicted kidney survival. However, it was only tubulointerstitial chronicity (comprised of fibrosis and tubular atrophy), but not the glomerular components of the chronicity index, which was the determining factor in predicting renal outcome [18].

The predictive value of histopathological features, such as interstitial fibrosis, in pre-treatment biopsies has been further validated in cohorts of patients all treated using the same protocol. Among a cohort of 43 patients with proliferative LN treated with cyclophosphamide of which 50% of patients progressed to renal failure over 2 years, worse renal function and interstitial fibrosis were associated with shorter renal survival [99]. Also using a cyclophosphamide based regimen, interstitial fibrosis was one of the significant features associated with development of ESRD in 5 of 38 patients with severe LN in a Spanish cohort [100]. Finally, the chronicity index and severity of interstitial fibrosis were significantly higher in patients with diffuse proliferative disease that did not enter remission following pulsed methylprednisolone therapy [101].

IV.iv. Markers of inflammation and vascular activation

Across several animal models of both glomerular and tubular disease, it was observed that deficiency or blockade of the TWEAK pathway results in reduced levels of secreted inflammatory markers as well as reduced cellular infiltrates in the kidney. Notably, there has been an increasing body of evidence linking many of the same inflammatory chemokines and cytokines with disease activity in LN. The relevance of several of these inflammatory mediators, namely MCP-1, RANTES, IL-6, IP-10 and VCAM-1, will be highlighted in this section. Of note, these particular mediators have all been shown to be directly inducible by TWEAK and are modulated by disruption of the TWEAK pathway in disease models. Moreover, urinary levels of these mediators are elevated in LN patients, analogous to the elevated urinary TWEAK levels associated with disease. This section will specifically focus on the relevance of these inflammatory mediators to human LN.

a. MCP-1—Monocyte chemoattractant protein (MCP-1; CCL2) is one of most important members of the CC family of chemokines. Many cell types can secrete MCP-1, including monocytes, endothelial cells, fibroblasts, and epithelial cells [102]. MCP-1, through its main receptor CCR2, recruits monocytes, dendritic cells, and T cells to sites of tissue injury and inflammation [102].

Convincing evidence implicates MCP-1 in the pathogenesis of murine LN as well as in human disease. In MRL/lpr lupus-prone mice, MCP-1 blockade using genetically deficient

mice or neutralizing antibodies was shown to be efficacious in ameliorating disease [103-107]. Notably, in the various experimental models of LN in which the TWEAK pathway has been evaluated (Table 1), MCP-1 was consistently found to be down-regulated upon inhibition or deficiency of the TWEAK pathway.

In human lupus, urinary MCP-1 levels are highly associated with disease. In patients followed prospectively in the Ohio SLE study, urinary MCP-1 levels were significantly higher in patients with a renal flare than in SLE patients with a non-renal flare. Urinary MCP-1 levels began to increase several months before the flare event, and were the highest in patients with proliferative disease. There was a slow decline in urinary MCP-1 in patients who responded to therapy, while non-responders remained with persistently high levels [108]. In 87 SLE patients, urinary MCP-1 levels were associated with anti-dsDNA antibodies and renal disease activity [109]. Studies in both adults [110] and children [111] have shown that it is specifically urine, but not serum, MCP-1 that shows significant correlation with renal disease activity. Finally, there is increasing interest in using urinary MCP-1 as part of a composite biomarker score that can non-invasively quantify critical aspects of renal histopathology, including LN activity (e.g. tubulointerstitial inflammation), chronicity and class [112, 113].

b. RANTES—Regulated upon activation, normally T cell expressed and secreted (RANTES; CCL5) is an important chemokine in the attraction of macrophages and T cells through its receptor CCR5. RANTES has been implicated in the pathogenesis of lupus in murine models as well as in human LN. RANTES and CCR5 were shown to be upregulated on infiltrating cells in MRL/lpr kidneys [114, 115]. Overexpression of RANTES in a mouse model was shown to trigger interstitial nephritis [115], whereas RANTES deficient mice were less susceptible to renal injury following the passive transfer of nephritogenic anti-glomerular antibodies [116]. Of note, RANTES levels in the kidney were significantly reduced upon TWEAK pathway inhibition in various models relevant to LN (Table 1).

In human LN, RANTES was evaluated as a predictor of a subsequent renal flare in a large cohort of lupus patients with diffuse proliferative nephritis who had responded to initial therapy with corticosteroids and cyclophosphamide [117]. Of 73 patients, 22 had a renal flare within about 2 years. Eight patients doubled their creatinine levels, and the only predictor for this outcome was the occurrence of renal flares. A persistent, abnormal level of urinary RANTES following induction therapy was an independent predictor of renal flare. Although individual contributions were not reported, elevated expression in PBMCs of RANTES (together with several other chemokines as a composite score) was higher in patients with active nephritis compared to patients with inactive or without nephritis, and correlated with the presence of cumulative organ damage [118]. Finally, at least in some populations, particular genetic polymorphisms in the RANTES promoter region were more common in lupus patients or in defined lupus subgroups [119, 120].

c. IL-6—IL-6, a pleotropic cytokine produced by monocytes (and other antigen presenting cells), fibroblasts, and endothelial cells, has a wide range of immunological effects pertinent to the pathogenesis of lupus [121, 122]. Like TWEAK, IL-6 is a pro-inflammatory cytokine. However, IL-6 can also directly influence lymphocyte populations, a function as yet not associated with TWEAK. Of particular relevance to LN, a B-cell driven autoimmune disease, IL-6 drives the differentiation of B cells into antibody producing plasma cells, and enhances immunoglobulin secretion [122, 123].

IL-6 has been correlated with disease in animal models of lupus as well as in human LN patients. In multiple strains of lupus-prone mice, IL-6 deficiency or IL-6 neutralizing antibodies were shown to be efficacious in ameliorating disease, whereas IL-6 over-

expression was shown to exacerbate disease [124-127]. Likewise, targeting of JAK2, a critical intermediary of IL-6 intracellular signaling, was highly efficacious in experimental models of lupus [128]. Relevant to the TWEAK pathway, IL-6 levels in the kidney were reduced upon TWEAK pathway inhibition in several models relevant to LN (Table I).

In human lupus, IL-6 expression and urinary IL-6 have been correlated with disease. IL-6 levels correlate with increased anti-dsDNA antibodies [122], and IL-6 has been shown to drive secretion of this antibody specificity in vitro. Many biopsies from patients with focal or diffuse proliferative LN demonstrated IL-6 expression in both glomeruli and tubules [129]. High urinary IL-6 levels were present in more than 80% (24/29) of patients with LN, with the highest levels observed in patients with diffuse proliferative GN [130]. Urinary excretion of IL-6 was significantly higher in patients with active as compared to inactive disease, and decreased in 5/6 patients treated with cyclophosphamide [131]. Notably, a promoter polymorphism that influences IL-6 production is independently associated with renal disease in Caucasian lupus patients [132]. Finally, a phase I study of an anti-IL-6 antibody in lupus patients with mild to moderate disease activity demonstrated a decrease in anti-dsDNA antibodies and a significant improvement in SLEDAI scores in 8/15 patients [133].

d. IP-10—Interferon-gamma-inducible protein 10 (IP-10; CXCL10), is produced by interferon-stimulated monocytes, fibroblasts, and endothelial cells, while the receptor CXCR3 is expressed on activated T cells and mesangial cells. Through CXCR3 (which also binds to CXCL9/MIG), IP-10 is important in directing T cells to sites of inflammation and in modulating angiogenesis [121, 134, 135]. Aside from promoting TH1 responses, IP-10 may also contribute to glomerular pathology by inducing proliferation of mesangial cells [135, 136].

In lupus-prone mice, a genome wide mRNA analysis revealed that IP-10 was upregulated in nephritic kidneys [137]. Notably, IP-10 was significantly reduced in murine models of LN when the TWEAK pathway was disrupted (Table 1).

Evidence in human patients has provided a stronger correlation between IP-10 and LN. Avihingason et al [138] measured urinary mRNA levels of various cytokines and growth factor genes in 26 lupus patients to determine whether these would be helpful in non-invasive identification of LN class and predicting response to therapy. In pre-biopsy urine samples, IP-10 performed significantly better than proteinuria in distinguishing between class IV and other LN classes. Moreover, IP-10 decreased over time in patients responding to therapy, but not in those who were treatment resistant. In renal biopsies of patients with LN, CXCR3 positive cells were a major component of tubulointerstitial infiltrates, but were rarely found in glomeruli. Moreover, CXCR3 positivity showed a significant correlation with proteinuria, the percentage of sclerosed glomeruli, and renal function [139]. In a similar study [140], most infiltrating cells were identified as CXCR3+, of which a majority were T cells. In patients with LN, urinary CXCR3+CD4+ cells were enriched as compared to peripheral blood, and correlated with disease activity. Surprisingly, one recent study found lower tubulointerstitial CXCR3 expression in 42 patients with LN as compared to 10 controls [29].

e. VCAM-1—Vascular cell adhesion molecule 1 (VCAM-1), a member of the Ig superfamily, is an adhesion molecule expressed on macrophages, dendritic cells, and endothelial cells. Through binding to the VLA4 integrin, VCAM-1 promotes tethering and adhesion of leukocytes to endothelial cells, and plays an important role in cell recruitment into tissues following inflammatory stimuli [121, 141].

VCAM-1 levels are associated with renal disease in murine lupus and in SLE patients. In several murine lupus prone strains, increased VCAM-1 expression was detected in the kidney and/or urinary VCAM-1 levels were elevated and correlated with proteinuria [141, 142]. Of note, VCAM-1, which is inducible by TWEAK, was decreased upon TWEAK pathway blockade in the NTN model of LN (Table 1).

In human lupus, urinary VCAM-1 is associated with disease. Molad et al [143] studied urinary VCAM-1 and ICAM-1 excretion in 24 patients with lupus. Urinary VCAM-1, but not ICAM-1, were significantly higher in lupus patients as compared to normal controls, and showed a significant correlation with disease activity and damage, decreased C3 and creatinine clearance, and albuminuria. Supporting these findings, urinary VCAM-1 levels were increased in patients with Class III, IV and V nephritis, as compared to patients with milder renal disease (Class I and II) or lupus patients without kidney disease. Moreover, there was a negative correlation between urinary VCAM-1 levels and serum complement levels [144]. Finally, analogous to the data in murine models, Wu et al found the highest levels of urinary VCAM-1 in patients with active renal lupus as compared to normal controls, inactive lupus, or severe lupus but without renal involvement [141].

IV.v. Summary of TWEAK relevance to human LN

It is evident that many prominent clinical features of human LN constitute disease-related parameters that were ameliorated by disruption of the TWEAK pathway in various animal models relevant to LN. The cumulative animal model data suggest that inhibition of TWEAK in human LN may be expected to reduce kidney inflammation and damage and inhibit progression of renal fibrosis, resulting in decreased proteinuria, enhanced renal function, and extended kidney survival. Taken together, TWEAK represents an attractive target for therapeutic targeting in LN.

In light of the importance of early diagnosis and prompt treatment of LN for achieving optimal renal outcomes, identification of novel biomarkers that are helpful in patient management and have prognostic significance has become a major priority in lupus research. Several of these parameters (e.g. proteinuria) are serially measured in lupus patients even without other evidence of active disease or previous renal involvement. Support is increasing for incorporation of some of the other disease indicators discussed above (e.g. MCP-1), into routine clinical practice. Considering the weight of evidence pointing to the role of TWEAK in the pathogenesis of LN, we suggest that monitoring urinary TWEAK levels may potentially be valuable either alone, or more likely in conjunction with a panel of biomarkers, in non-invasive early detection of renal flare and/or early identification of therapeutic responses.

V. Opportunity for targeting the TWEAK pathway in the clinic

BIIB023 is a monoclonal antibody against TWEAK in clinical development for the treatment of lupus nephritis. To date, BIIB023 has completed a phase I double-blind, placebo-controlled, single-dose study in subjects with rheumatoid arthritis (RA) [145]. BIIB023 treatment was well tolerated across all dose groups in this study. No dose-dependent safety finding was observed in any of the dose groups. In addition, there was no increase in infection rate associated with the use of BIIB023 in this study.

Although this was the first use in human study, a trial neither designed nor powered to assess efficacy, a number of potential TWEAK pathway and inflammation-related biomarkers were measured to assess modulation of these markers upon treatment with BIIB023. Analysis of data from a multiplex panel of biomarkers indicated a trend toward down-modulation of some of the inflammatory markers in higher dose cohorts (3 to 20 mg/

kg), indicating dose dependent activity of BIIB023 upon administration of a single dose. These markers include MCP-1, ICAM-1, A-SAA, BAFF, E-Selectin, IL-6, and IP-10.

BIIB023 is currently being developed for the treatment of LN in The Anti-Tweak in Lupus Nephritis Patient Study (ATLAS study - NCT01499355). The ATLAS study is a randomized double-blind, placebo-controlled proof-of-concept study of BIIB023 in subjects with active LN. The primary objective of this study is to evaluate the efficacy of BIIB023 as an add-on after initial therapy with standard of care to induce a complete or partial renal response as assessed by proteinuria and renal function in subjects with ISN/RPS Class III or IV LN. Secondary objectives include assessment of the effect of BIIB023 on renal histopathology, the effect of BIIB023 on extra-renal SLE disease activity/manifestations, and the PK and immunogenicity of BIIB023.

The intended purpose of adding BIIB023 to background therapy for LN is to increase the number of patients achieving remission as well as to achieve a more rapid renal response. The anticipated favorable safety and tolerability profile of BIIB023 enables its use as add-on to current LN standard of care. The unique mechanism of action of BIIB023 in protecting local pathological events in the kidney gives hope that combination with standard of care therapy will indeed result in improved clinical outcome for LN patients. In addition, BIIB023 may ultimately have a beneficial impact on other manifestations of SLE as its tissue protective modality may impact joint, muscle, skin and neurological involvement.

VI. Conclusion

Renal involvement remains a challenge for clinicians as current standard of care therapies do not provide adequate renal response for LN patients. Available therapies are largely immunosuppressive and do not typically target the complex pathologic mechanisms underlying LN or key features of disease, namely glomerulosclerosis and tubulointerstitial fibrosis. The TWEAK/Fn14 axis represents a novel pathway which has been mechanistically linked to multiple pathological processes occurring locally in LN kidneys, including inflammation, vascular activation, mesangial cell proliferation, renal cell death, and fibrosis. Blockade or deficiency of the pathway is ameliorative in multiple experimental models with clinical and histopathological features relevant to LN. Significantly, reductions in both glomerular pathology and tubulointerstitial fibrosis were achieved. In addition, elevated levels of urinary TWEAK correlate with renal disease activity in LN patients. An anti-TWEAK neutralizing antibody is currently being developed in LN as a renal protective agent that has the potential to improve efficacy without increased safety risk when added-on to standard of care therapy. Thus, TWEAK represents a new target exemplifying a novel therapeutic paradigm which may offer renal protection for LN patients. Finally, we are delighted to contribute to the special issue honoring Dr. Pierre Youinou of Brest University in recognition of his long-standing contributions in rheumatology. This is part of the special series of the Journal of Autoimmunity which has honored outstanding immunologists, including Ian Mackay, Noel Rose, Chella David and Harry Moutsopoulos; each of these issues is an attempt to provide state-of-the-art reviews on subjects that are immunologically cutting-edge [146-150].

References

1. Borchers AT, Aoki CA, Naguwa SM, Keen CL, Shoenfeld Y, Gershwin ME. Neuropsychiatric features of systemic lupus erythematosus. *Autoimmunity reviews*. 2005; 4:329–44. [PubMed: 16081024]
2. Cameron JS. Lupus nephritis. *J Am Soc Nephrol*. 1999; 10:413–24. [PubMed: 10215343]

3. Dooley MA, Jayne D, Ginzler EM, Isenberg D, Olsen NJ, Wofsy D, et al. Mycophenolate versus azathioprine as maintenance therapy for lupus nephritis. *The New England journal of medicine*. 2011; 365:1886–95. [PubMed: 22087680]
4. Ioannidis JP, Boki KA, Katsorida ME, Drosos AA, Skopouli FN, Boletis JN, et al. Remission, relapse, and re-remission of proliferative lupus nephritis treated with cyclophosphamide. *Kidney Int*. 2000; 57:258–64. [PubMed: 10620207]
5. Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. *Medicine (Baltimore)*. 2003; 82:299–308. [PubMed: 14530779]
6. Korbet SM, Lewis EJ, Schwartz MM, Reichlin M, Evans J, Rohde RD. Factors predictive of outcome in severe lupus nephritis. *Lupus Nephritis Collaborative Study Group*. *Am J Kidney Dis*. 2000; 35:904–14. [PubMed: 10793026]
7. Chan TM, Tse KC, Tang CS, Lai KN, Li FK. Long-term outcome of patients with diffuse proliferative lupus nephritis treated with prednisolone and oral cyclophosphamide followed by azathioprine. *Lupus*. 2005; 14:265–72. [PubMed: 15864912]
8. Chen YE, Korbet SM, Katz RS, Schwartz MM, Lewis EJ, Collaborative Study G. Value of a complete or partial remission in severe lupus nephritis. *Clinical journal of the American Society of Nephrology : CJASN*. 2008; 3:46–53. [PubMed: 18003764]
9. Houssiau FA, Vasconcelos C, D'Cruz D, Sebastiani GD, Garrido Ed Ede R, Danieli MG, et al. Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. *Arthritis Rheum*. 2002; 46:2121–31. [PubMed: 12209517]
10. Furie R, Petri M, Zamani O, Cervera R, Wallace DJ, Tegzova D, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum*. 2011; 63:3918–30. [PubMed: 22127708]
11. Appel GB, Contreras G, Dooley MA, Ginzler EM, Isenberg D, Jayne D, et al. Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. *J Am Soc Nephrol*. 2009; 20:1103–12. [PubMed: 19369404]
12. Badsha H, Edwards CJ. Intravenous pulses of methylprednisolone for systemic lupus erythematosus. *Seminars in arthritis and rheumatism*. 2003; 32:370–7. [PubMed: 12833245]
13. Sfikakis PP, Boletis JN, Lionaki S, Vigklis V, Fragiadaki KG, Iniotaki A, et al. Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial. *Arthritis Rheum*. 2005; 52:501–13. [PubMed: 15693003]
14. Waldman M, Appel GB. Update on the treatment of lupus nephritis. *Kidney Int*. 2006; 70:1403–12. [PubMed: 16929249]
15. Cook HT, Botto M. Mechanisms of Disease: the complement system and the pathogenesis of systemic lupus erythematosus. *Nature clinical practice Rheumatology*. 2006; 2:330–7.
16. Nangaku M, Couser WG. Mechanisms of immune-deposit formation and the mediation of immune renal injury. *Clin Exp Nephrol*. 2005; 9:183–91. [PubMed: 16189625]
17. Austin HA 3rd, Muenz LR, Joyce KM, Antonovych TA, Kullick ME, Klippel JH, et al. Prognostic factors in lupus nephritis. Contribution of renal histologic data. *The American journal of medicine*. 1983; 75:382–91. [PubMed: 6351607]
18. Hsieh C, Chang A, Brandt D, Guttikonda R, Utset TO, Clark MR. Predicting outcomes of lupus nephritis with tubulointerstitial inflammation and scarring. *Arthritis Care Res (Hoboken)*. 2011; 63:865–74. [PubMed: 21309006]
19. Mok CC. Prognostic factors in lupus nephritis. *Lupus*. 2005; 14:39–44. [PubMed: 15732286]
20. Chicheportiche Y, Bourdon PR, Xu H, Hsu YM, Scott H, Hession C, et al. TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. *JBiolChem*. 1997; 272:32401–10.

21. Desplat-Jego S, Varriale S, Creidy R, Terra R, Bernard D, Khrestchatisky M, et al. TWEAK is expressed by glial cells, induces astrocyte proliferation and increases EAE severity. *JNeuroimmunol.* 2002; 133:116–23. [PubMed: 12446014]
22. Donohue PJ, Richards CM, Brown SA, Hanscom HN, Buschman J, Thangada S, et al. TWEAK Is an Endothelial Cell Growth and Chemotactic Factor That Also Potentiates FGF-2 and VEGF-A Mitogenic Activity. *ArteriosclerThrombVascBiol.* 2003; 23:594–600.
23. Justo P, Sanz AB, Sanchez-Nino MD, Winkles JA, Lorz C, Egido J, et al. Cytokine cooperation in renal tubular cell injury: the role of TWEAK. *Kidney Int.* 2006; 70:1750–8. [PubMed: 17003819]
24. van Kuijk AW, Wijbrandts CA, Vinkenoog M, Zheng TS, Reedquist KA, Tak PP. TWEAK and its receptor Fn14 in the synovium of patients with rheumatoid arthritis compared to psoriatic arthritis and its response to TNF blockade. *Ann Rheum Dis.* 2009
25. Kawashima R, Kawamura YI, Oshio T, Son A, Yamazaki M, Hagiwara T, et al. Interleukin-13 damages intestinal mucosa via TWEAK and Fn14 in mice—a pathway associated with ulcerative colitis. *Gastroenterology.* 2011; 141:2119–29. e8. [PubMed: 21893119]
26. Serafini B, Magliozzi R, Rosicarelli B, Reynolds R, Zheng TS, Aloisi F. Expression of TWEAK and its receptor Fn14 in the multiple sclerosis brain: implications for inflammatory tissue injury. *J Neuropathol Exp Neurol.* 2008; 67:1137–48. [PubMed: 19018248]
27. Hotta K, Sho M, Yamato I, Shimada K, Harada H, Akahori T, et al. Direct targeting of fibroblast growth factor-inducible 14 protein protects against renal ischemia reperfusion injury. *Kidney Int.* 2011; 79:179–88. [PubMed: 20927042]
28. Sanz AB, Justo P, Sanchez-Nino MD, Blanco-Colio LM, Winkles JA, Kreztler M, et al. The cytokine TWEAK modulates renal tubulointerstitial inflammation. *J Am Soc Nephrol.* 2008; 19:695–703. [PubMed: 18235096]
29. Lu J, Kwan BC, Lai FM, Choi PC, Tam LS, Li EK, et al. Gene expression of TWEAK/Fn14 and IP-10/CXCR3 in glomerulus and tubulointerstitium of patients with lupus nephritis. *Nephrology (Carlton).* 2011; 16:426–32. [PubMed: 21303425]
30. Schwartz N, Rubinstein T, Burkly LC, Collins CE, Blanco I, Su L, et al. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. *Arthritis Res Ther.* 2009; 11:R143. [PubMed: 19785730]
31. Schwartz N, Su L, Burkly LC, Mackay M, Aranow C, Kollaros M, et al. Urinary TWEAK and the activity of lupus nephritis. *J Autoimmun.* 2006; 27:242–50. [PubMed: 17257812]
32. Bover LC, Cardo-Vila M, Kuniyasu A, Sun J, Rangel R, Takeya M, et al. A previously unrecognized protein-protein interaction between TWEAK and CD163: potential biological implications. *J Immunol.* 2007; 178:8183–94. [PubMed: 17548657]
33. Fick A, Lang I, Schafer V, Seher A, Trebing J, Weisenberger D, et al. Studies of binding of tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) to fibroblast growth factor inducible 14 (Fn14). *J Biol Chem.* 2012; 287:484–95. [PubMed: 22081603]
34. Gao HX, Campbell SR, Burkly LC, Jakubowski A, Jarchum I, Banas B, et al. TNF-like weak inducer of apoptosis (TWEAK) induces inflammatory and proliferative effects in human kidney cells. *Cytokine.* 2009; 46:24–35. [PubMed: 19233685]
35. Winkles JA. The TWEAK-Fn14 cytokine-receptor axis: discovery, biology and therapeutic targeting. *Nat Rev Drug Discov.* 2008; 7:411–25. [PubMed: 18404150]
36. Burkly LC, Dohi T. The TWEAK/Fn14 pathway in tissue remodeling: for better or for worse. *Adv Exp Med Biol.* 2011; 691:305–22. [PubMed: 21153335]
37. Dohi T, Borodovsky A, Wu P, Shearstone JR, Kawashima R, Runkel L, et al. TWEAK/Fn14 pathway: a nonredundant role in intestinal damage in mice through a TWEAK/intestinal epithelial cell axis. *Gastroenterology.* 2009; 136:912–23. [PubMed: 19109961]
38. Chorianopoulos E, Heger T, Lutz M, Frank D, Bea F, Katus HA, et al. FGF-inducible 14-kDa protein (Fn14) is regulated via the RhoA/ROCK kinase pathway in cardiomyocytes and mediates nuclear factor-kappaB activation by TWEAK. *Basic Res Cardiol.* 2009
39. Munoz-Garcia B, Moreno JA, Lopez-Franco O, Sanz AB, Martin-Ventura JL, Blanco J, et al. Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) enhances vascular and renal damage induced by hyperlipidemic diet in ApoE-knockout mice. *Arterioscler Thromb Vasc Biol.* 2009; 29:2061–8. [PubMed: 19778942]

40. Wiley SR, Cassiano L, Lofton T, Davis-Smith T, Winkles JA, Lindner V, et al. A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. *Immunity*. 2001; 15:837–46. [PubMed: 11728344]
41. Girgenrath M, Weng S, Kostek CA, Browning B, Wang M, Brown SA, et al. TWEAK, via its receptor Fn14, is a novel regulator of mesenchymal progenitor cells and skeletal muscle regeneration. *Embo J*. 2006; 25:5826–39. [PubMed: 17124496]
42. Mittal A, Bhatnagar S, Kumar A, Paul PK, Kuang S. Genetic ablation of TWEAK augments regeneration and post-injury growth of skeletal muscle in mice. *Am J Pathol*. 177:1732–42. [PubMed: 20724600]
43. Desplat-Jego S, Creidy R, Varriale S, Allaire N, Luo Y, Bernard D, et al. Anti-TWEAK monoclonal antibodies reduce immune cell infiltration in the central nervous system and severity of experimental autoimmune encephalomyelitis. *Clinical Immunology*. 2005; 117(1):15–23. [PubMed: 16027043]
44. Potrovita I, Zhang W, Burkly L, Hahm K, Lincecum J, Wang MZ, et al. Tumor necrosis factor-like weak inducer of apoptosis-induced neurodegeneration. *J Neurosci*. 2004; 24:8237–44. [PubMed: 15385607]
45. Yepes M, Brown SA, Moore EG, Smith EP, Lawrence DA, Winkles JA. A soluble Fn14-Fc decoy receptor reduces infarct volume in a murine model of cerebral ischemia. *AmJ Pathol*. 2005; 166:511–20. [PubMed: 15681834]
46. Jakubowski A, Ambrose C, Parr M, Lincecum JM, Wang MZ, Zheng TS, et al. TWEAK induces liver progenitor cell proliferation. *J ClinInvest*. 2005; 115:2330–40.
47. Tirmitz-Parker JE, Viebahn CS, Jakubowski A, Klopchik BR, Olynyk JK, Yeoh GC, et al. Tumor necrosis factor-like weak inducer of apoptosis is a mitogen for liver progenitor cells. *Hepatology*. 2010; 52:291–302. [PubMed: 20578156]
48. Michaelson JS, Burkly LC. Therapeutic Targeting of TWEAK/Fn14 in Cancer: Exploiting the Intrinsic Tumor Cell Killing Capacity of the Pathway. *Results Probl Cell Differ*. 2009
49. Saitoh T, Nakayama M, Nakano H, Yagita H, Yamamoto N, Yamaoka S. TWEAK induces NF- κ B p100 processing and long-lasting NF- κ B activation. *JBiolChem*. 2003
50. Campbell S, Burkly LC, Gao HX, Berman JW, Su L, Browning B, et al. Proinflammatory effects of tweak/fn14 interactions in glomerular mesangial cells. *J Immunol*. 2006; 176:1889–98. [PubMed: 16424220]
51. Izquierdo MC, Sanz AB, Mezzano S, Blanco J, Carrasco S, Sanchez-Nino MD, et al. TWEAK (tumor necrosis factor-like weak inducer of apoptosis) activates CXCL16 expression during renal tubulointerstitial inflammation. *Kidney Int*. 2012
52. Sanz AB, Sanchez-Nino MD, Izquierdo MC, Jakubowski A, Justo P, Blanco-Colio LM, et al. Tweak induces proliferation in renal tubular epithelium: a role in uninephrectomy induced renal hyperplasia. *J Cell Mol Med*. 2009; 13:3329–42. [PubMed: 19426154]
53. Sanz AB, Sanchez-Nino MD, Izquierdo MC, Jakubowski A, Justo P, Blanco-Colio LM, et al. TWEAK activates the non-canonical NF κ B pathway in murine renal tubular cells: modulation of CCL21. *PLoS One*. 2010; 5:e8955. [PubMed: 20126461]
54. Burkly LC, Michaelson JS, Zheng TS. TWEAK/Fn14 pathway: an immunological switch for shaping tissue responses. *Immunological reviews*. 2011; 244:99–114. [PubMed: 22017434]
55. Chicheportiche Y, Chicheportiche R, Sizing I, Thompson J, Benjamin CB, Ambrose C, et al. Proinflammatory activity of TWEAK on human dermal fibroblasts and synoviocytes: blocking and enhancing effects of anti-TWEAK monoclonal antibodies. *Arthritis Res*. 2002; 4:126–33. [PubMed: 11879548]
56. Hosokawa Y, Hosokawa I, Ozaki K, Nakae H, Matsuo T. Proinflammatory effects of tumour necrosis factor-like weak inducer of apoptosis (TWEAK) on human gingival fibroblasts. *Clinical and experimental immunology*. 2006; 146:540–9. [PubMed: 17100776]
57. Harada N, Nakayama M, Nakano H, Fukuchi Y, Yagita H, Okumura K. Pro-inflammatory effect of TWEAK/Fn14 interaction on human umbilical vein endothelial cells. *BiochemBiophysResCommun*. 2002; 299:488–93.

58. Kim SH, Kang YJ, Kim WJ, Woo DK, Lee Y, Kim DI, et al. TWEAK can induce pro-inflammatory cytokines and matrix metalloproteinase-9 in macrophages. *CircJ*. 2004; 68:396–9. [PubMed: 15056843]
59. Jin L, Nakao A, Nakayama M, Yamaguchi N, Kojima Y, Nakano N, et al. Induction of RANTES by TWEAK/Fn14 interaction in human keratinocytes. *J Invest Dermatol*. 2004; 122:1175–9. [PubMed: 15140220]
60. Perper SJ, Browning B, Burkly LC, Weng S, Gao C, Giza K, et al. TWEAK is a novel arthritogenic mediator. *J Immunol*. 2006; 177:2610–20. [PubMed: 16888023]
61. Xu H, Okamoto A, Ichikawa J, Ando T, Tasaka K, Masuyama K, et al. TWEAK/Fn14 interaction stimulates human bronchial epithelial cells to produce IL-8 and GM-CSF. *BiochemBiophysResCommun*. 2004; 318:422–7.
62. Saas P, Boucraut J, Walker PR, Quiquerez AL, Billot M, Desplat-Jego S, et al. TWEAK stimulation of astrocytes and the proinflammatory consequences. *GLIA*. 2000; 32:102–7. [PubMed: 10975915]
63. Jakubowski A, Browning B, Lukashev M, Sizing I, Thompson JS, Benjamin CD, et al. Dual role for TWEAK in angiogenic regulation. *JCell Sci*. 2002; 115:267–74. [PubMed: 11839778]
64. Lynch CN, Wang YC, Lund JK, Chen YW, Leal JA, Wiley SR. TWEAK induces angiogenesis and proliferation of endothelial cells. *JBiolChem*. 1999; 274:8455–9.
65. Polavarapu R, Gongora MC, Winkles JA, Yepes M. Tumor necrosis factor-like weak inducer of apoptosis increases the permeability of the neurovascular unit through nuclear factor-kappa B pathway activation. *J Neurosci*. 2005; 25:10094–100. [PubMed: 16267216]
66. Burkly LC, Michaelson JS, Hahm K, Jakubowski A, Zheng TS. TWEAKing tissue remodeling by a multifunctional cytokine: role of TWEAK/Fn14 pathway in health and disease. *Cytokine*. 2007; 40:1–16. [PubMed: 17981048]
67. Bhatnagar S, Mittal A, Gupta SK, Kumar A. TWEAK causes myotube atrophy through coordinated activation of ubiquitin-proteasome system, autophagy, and caspases. *J Cell Physiol*. 2011
68. Dogra C, Hall SL, Wedhas N, Linkhart TA, Kumar A. Fibroblast growth factor inducible 14 (Fn14) is required for the expression of myogenic regulatory factors and differentiation of myoblasts into myotubes. Evidence for TWEAK-independent functions of Fn14 during myogenesis. *J Biol Chem*. 2007; 282:15000–10. [PubMed: 17383968]
69. Haile WB, Echeverry R, Wu F, Guzman J, An J, Wu J, et al. Tumor necrosis factor-like weak inducer of apoptosis and fibroblast growth factor-inducible 14 mediate cerebral ischemia-induced poly(ADP-ribose) polymerase-1 activation and neuronal death. *Neuroscience*. 171:1256–64. [PubMed: 20955770]
70. Jain M, Jakubowski A, Cui L, Shi J, Su L, Bauer M, et al. A novel role for tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in the development of cardiac dysfunction and failure. *Circulation*. 2009; 119:2058–68. [PubMed: 19349318]
71. Vincent C, Findlay DM, Welldon KJ, Wijenayaka AR, Zheng TS, Haynes DR, et al. Pro-inflammatory cytokines TNF-related weak inducer of apoptosis (TWEAK) and TNFalpha induce the mitogen-activated protein kinase (MAPK)-dependent expression of sclerostin in human osteoblasts. *J Bone Miner Res*. 2009; 24:1434–49. [PubMed: 19292615]
72. Novoyatleva T, Diehl F, van Amerongen MJ, Patra C, Ferrazzi F, Bellazzi R, et al. TWEAK is a positive regulator of cardiomyocyte proliferation. *Cardiovasc Res*. 2010; 85:681–90. [PubMed: 19887380]
73. Zhi-Chun L, Qiao-Ling Z, Zhi-Qin L, Xiao-Zhao L, Xiao-Xia Z, Rong T. Tumor Necrosis Factor-Like Weak Inducer of Apoptosis (TWEAK) Mediates p38 Mitogen-Activated Protein Kinase Activation and Signal Transduction in Peripheral Blood Mononuclear Cells from Patients with Lupus Nephritis. *Inflammation*. 2011
74. Wang C, Chen LL, Pan HF, Leng RX, Qin WZ, Ye DQ. Expression of human tumor necrosis factor-like weak inducer of apoptosis in patients with systemic lupus erythematosus. *Clinical rheumatology*. 2012; 31:335–9. [PubMed: 21968693]
75. El-Shehaby A, Darweesh H, El-Khatib M, Momtaz M, Marzouk S, El-Shaarawy N, et al. Correlations of urinary biomarkers, TNF-like weak inducer of apoptosis (TWEAK),

- osteoprotegerin (OPG), monocyte chemoattractant protein-1 (MCP-1), and IL-8 with lupus nephritis. *Journal of clinical immunology*. 2011; 31:848–56. [PubMed: 21691937]
76. Lopez-Novoa JM, Rodriguez-Pena AB, Ortiz A, Martinez-Salgado C, Lopez Hernandez FJ. Etiopathology of chronic tubular, glomerular and renovascular nephropathies: clinical implications. *J Transl Med*. 2011; 9:13. [PubMed: 21251296]
 77. Zhao Z, Burkly LC, Campbell S, Schwartz N, Molano A, Choudhury A, et al. TWEAK/Fn14 interactions are instrumental in the pathogenesis of nephritis in the chronic graft-versus-host model of systemic lupus erythematosus. *J Immunol*. 2007; 179:7949–58. [PubMed: 18025243]
 78. Xia YCS, Herlitz L, Michaelson JS, Burkly LC, Putterman C. TWEAK/Fn14 pathway blockade attenuates renal disease in autoantibody-induced nephritis. *American College of Rheumatology 75th Natl Meeting, Arthritis Rheum*. 2011; 63:S212.
 79. Xia YWJ, Michaelson JS, Burkly LC, Putterman C. Deficiency of the TWEAK receptor Fn14 is protective in the MRL/lpr mouse model of lupus nephritis. *American College of Rheumatology 75th Natl Meeting, Arthritis Rheum*. 2011; 63:S212.
 80. Hill GS, Delahousse M, Nochy D, Remy P, Mignon F, Mery JP, et al. Predictive power of the second renal biopsy in lupus nephritis: significance of macrophages. *Kidney Int*. 2001; 59:304–16. [PubMed: 11135084]
 81. Appel GB, Pirani CL, D'Agati V. Renal vascular complications of systemic lupus erythematosus. *J Am Soc Nephrol*. 1994; 4:1499–515. [PubMed: 8025223]
 82. Garin EH, West L, Zheng W. Effect of interleukin-8 on glomerular sulfated compounds and albuminuria. *Pediatric nephrology (Berlin, Germany)*. 1997; 11:274–9.
 83. Garin EH, West L, Zheng W. Interleukin-8 alters glomerular heparan sulfate glycosaminoglycan chain size and charge in rats. *Pediatric nephrology (Berlin, Germany)*. 2000; 14:284–7.
 84. Nagai M, Hirayama K, Ebihara I, Higuchi T, Imaizumi M, Maruyama H, et al. Serum TNF-related and weak inducer of apoptosis levels in septic shock patients. *Ther Apher Dial*. 2011; 15:342–8. [PubMed: 21884467]
 85. Duffield JS, Humphreys BD. Origin of new cells in the adult kidney: results from genetic labeling techniques. *Kidney Int*. 2011; 79:494–501. [PubMed: 20861816]
 86. Liu Y. Cellular and molecular mechanisms of renal fibrosis. *Nat Rev Nephrol*. 2011; 7:684–96. [PubMed: 22009250]
 87. Chen G, Lin SC, Chen J, He L, Dong F, Xu J, et al. CXCL16 recruits bone marrow-derived fibroblast precursors in renal fibrosis. *J Am Soc Nephrol*. 2011; 22:1876–86. [PubMed: 21816936]
 88. Korbet SM, Schwartz MM, Evans J, Lewis EJ. Severe lupus nephritis: racial differences in presentation and outcome. *J Am Soc Nephrol*. 2007; 18:244–54. [PubMed: 17167111]
 89. Contreras G, Pardo V, Cely C, Borja E, Hurtado A, De La Cuesta C, et al. Factors associated with poor outcomes in patients with lupus nephritis. *Lupus*. 2005; 14:890–5. [PubMed: 16335581]
 90. Mok CC, Ho CT, Chan KW, Lau CS, Wong RW. Outcome and prognostic indicators of diffuse proliferative lupus glomerulonephritis treated with sequential oral cyclophosphamide and azathioprine. *Arthritis Rheum*. 2002; 46:1003–13. [PubMed: 11953978]
 91. Austin HA 3rd, Boumpas DT, Vaughan EM, Balow JE. High-risk features of lupus nephritis: importance of race and clinical and histological factors in 166 patients. *Nephrol Dial Transplant*. 1995; 10:1620–8. [PubMed: 8559480]
 92. Praga M. Therapeutic measures in proteinuric nephropathy. *Kidney international Supplement*. 2005:S137–41. [PubMed: 16336567]
 93. Praga M, Morales E. Renal damage associated with proteinuria. *Kidney international Supplement*. 2002:S42–6. [PubMed: 12410854]
 94. Hebert LA, Wilmer WA, Falkenhain ME, Ladson-Wofford SE, Nahman NS Jr. Rovin BH. Renoprotection: one or many therapies? *Kidney Int*. 2001; 59:1211–26. [PubMed: 11260381]
 95. Donadio JV Jr, Hart GM, Bergstralh EJ, Holley KE. Prognostic determinants in lupus nephritis: a long-term clinicopathologic study. *Lupus*. 1995; 4:109–15. [PubMed: 7795613]
 96. Korbet SM, Lewis EJ, the Collaborative Study G. Complete remission in severe lupus nephritis: assessing the rate of loss in proteinuria. *Nephrol Dial Transplant*. 2011

97. Markowitz GS, D'Agati VD. Classification of lupus nephritis. Current opinion in nephrology and hypertension. 2009; 18:220–5. [PubMed: 19374008]
98. Austin HA 3rd, Muenz LR, Joyce KM, Antonovych TT, Balow JE. Diffuse proliferative lupus nephritis: identification of specific pathologic features affecting renal outcome. *Kidney Int.* 1984; 25:689–95. [PubMed: 6482173]
99. Conlon PJ, Fischer CA, Levesque MC, Smith SR, St Clair EW, Allen NB, et al. Clinical, biochemical and pathological predictors of poor response to intravenous cyclophosphamide in patients with proliferative lupus nephritis. *Clinical nephrology.* 1996; 46:170–5. [PubMed: 8879851]
100. Cortes-Hernandez J, Ordi-Ros J, Labrador M, Segarra A, Tovar JL, Balada E, et al. Predictors of poor renal outcome in patients with lupus nephritis treated with combined pulses of cyclophosphamide and methylprednisolone. *Lupus.* 2003; 12:287–96. [PubMed: 12729052]
101. Kanno A, Hotta O, Yusa N, Taguma Y. Predictive factors of clinical outcome in patients with diffuse proliferative lupus nephritis treated early by intravenous methylprednisolone pulse therapy. *Renal failure.* 2007; 29:41–7. [PubMed: 17365909]
102. Yadav A, Saini V, Arora S. MCP-1: chemoattractant with a role beyond immunity: a review. *Clinica chimica acta; international journal of clinical chemistry.* 2010; 411:1570–9.
103. Kulkarni O, Eulberg D, Selve N, Zollner S, Allam R, Pawar RD, et al. Anti-Ccl2 Spiegelmer permits 75% dose reduction of cyclophosphamide to control diffuse proliferative lupus nephritis and pneumonitis in MRL-Fas(lpr) mice. *J Pharmacol Exp Ther.* 2009; 328:371–7. [PubMed: 18997060]
104. Kulkarni O, Pawar RD, Purschke W, Eulberg D, Selve N, Buchner K, et al. Spiegelmer inhibition of CCL2/MCP-1 ameliorates lupus nephritis in MRL-(Fas)lpr mice. *J Am Soc Nephrol.* 2007; 18:2350–8. [PubMed: 17625118]
105. Perez, dL; Maier, H.; Nieto, E.; Vielhauer, V.; Luckow, B.; Mampaso, F., et al. Chemokine expression precedes inflammatory cell infiltration and chemokine receptor and cytokine expression during the initiation of murine lupus nephritis. *Journal of the American Society of Nephrology.* 2001; 12:1369–82. [PubMed: 11423566]
106. Shimizu S, Nakashima H, Masutani K, Inoue Y, Miyake K, Akahoshi M, et al. Anti-monocyte chemoattractant protein-1 gene therapy attenuates nephritis in MRL/lpr mice. *Rheumatology(Oxford).* 2004
107. Tesch GH, Schwarting A, Kinoshita K, Lan HY, Rollins BJ, Kelley VR. Monocyte chemoattractant protein-1 promotes macrophage-mediated tubular injury, but not glomerular injury, in nephrotoxic serum nephritis. *Journal of Clinical Investigation.* 1999; 103(1):73–80. [PubMed: 9884336]
108. Rovin BH, Song H, Birmingham DJ, Hebert LA, Yu CY, Nagaraja HN. Urine chemokines as biomarkers of human systemic lupus erythematosus activity. *J AmSocNephrol.* 2005; 16:467–73.
109. Kiani AN, Johnson K, Chen C, Diehl E, Hu H, Vasudevan G, et al. Urine osteoprotegerin and monocyte chemoattractant protein-1 in lupus nephritis. *J Rheumatol.* 2009; 36:2224–30. [PubMed: 19648301]
110. Alzawawy A, Zohary M, Ablordiny M, Eldalie M. Estimation of monocyte-chemoattractantprotein-1 (Mcp-1) level in patients with lupus nephritis. *International journal of rheumatic diseases.* 2009; 12:311–8. [PubMed: 20374368]
111. Marks SD, Shah V, Pilkington C, Tullus K. Urinary monocyte chemoattractant protein-1 correlates with disease activity in lupus nephritis. *Pediatric nephrology (Berlin, Germany).* 2010; 25:2283–8.
112. Brunner HI, Bennett MR, Mina R, Suzuki M, Petri M, Kiani AN, et al. Non-invasive renal protein biomarkers are associated with histological features of lupus nephritis. *Arthritis Rheum.* 2012
113. Zhang X, Nagaraja HN, Nadasdy T, Song H, McKinley A, Prosek J, et al. A composite urine biomarker reflects interstitial inflammation in lupus nephritis kidney biopsies. *Kidney Int.* 2012; 81:401–6. [PubMed: 21993584]
114. Perez de Lema G, Maier H, Franz TJ, Escibese M, Chilla S, Segerer S, et al. Chemokine receptor Ccr2 deficiency reduces renal disease and prolongs survival in MRL/lpr lupus-prone mice. *J Am Soc Nephrol.* 2005; 16:3592–601. [PubMed: 16267157]

115. Moore KJ, Wada T, Barbee SD, Kelley VR. Gene transfer of RANTES elicits autoimmune renal injury in MRL-Fas(1pr) mice. *Kidney International*. 1998; 53:1631–41. [comment]. [PubMed: 9607194]
116. Xie C, Zhou XJ, Liu X, Mohan C. Enhanced susceptibility to end-organ disease in the lupus-facilitating NZW mouse strain. *Arthritis Rheum*. 2003; 48:1080–92. [PubMed: 12687552]
117. Tian S, Li J, Wang L, Liu T, Liu H, Cheng G, et al. Urinary levels of RANTES and M-CSF are predictors of lupus nephritis flare. *Inflammation research : official journal of the European Histamine Research Society [et al]*. 2007; 56:304–10.
118. Fu Q, Chen X, Cui H, Guo Y, Chen J, Shen N, et al. Association of elevated transcript levels of interferon-inducible chemokines with disease activity and organ damage in systemic lupus erythematosus patients. *Arthritis research & therapy*. 2008; 10:R112. [PubMed: 18793417]
119. Liao CH, Yao TC, Chung HT, See LC, Kuo ML, Huang JL. Polymorphisms in the promoter region of RANTES and the regulatory region of monocyte chemoattractant protein-1 among Chinese children with systemic lupus erythematosus. *J Rheumatol*. 2004; 31:2062–7. [PubMed: 15468376]
120. Ye DQ, Yang SG, Li XP, Hu YS, Yin J, Zhang GQ, et al. Polymorphisms in the promoter region of RANTES in Han Chinese and their relationship with systemic lupus erythematosus. *Archives of dermatological research*. 2005; 297:108–13. [PubMed: 16032408]
121. Reyes-Thomas J, Blanco I, Putterman C. Urinary biomarkers in lupus nephritis. *Clinical reviews in allergy & immunology*. 2011; 40:138–50. [PubMed: 20127204]
122. Yap DY, Lai KN. Cytokines and their roles in the pathogenesis of systemic lupus erythematosus: from basics to recent advances. *Journal of biomedicine & biotechnology*. 2010; 2010:365083. [PubMed: 20467470]
123. Tackey E, Lipsky PE, Illei GG. Rationale for interleukin-6 blockade in systemic lupus erythematosus. *Lupus*. 2004; 13:339–43. [PubMed: 15230289]
124. Finck BK, Chan B, Wofsy D. Interleukin 6 promotes murine lupus in NZB/NZW F1 mice. *J Clin Invest*. 1994; 94:585–91. [PubMed: 8040314]
125. Mihara M, Takagi N, Takeda Y, Ohsugi Y. IL-6 receptor blockage inhibits the onset of autoimmune kidney disease in NZB/W F1 mice. *Clinical and experimental immunology*. 1998; 112:397–402. [PubMed: 9649207]
126. Ryffel B, Car BD, Gunn H, Roman D, Hiestand P, Mihatsch MJ. Interleukin-6 exacerbates glomerulonephritis in (NZB × NZW)F1 mice. *Am J Pathol*. 1994; 144:927–37. [PubMed: 8178944]
127. Cash H, Relle M, Menke J, Brochhausen C, Jones SA, Topley N, et al. Interleukin 6 (IL-6) deficiency delays lupus nephritis in MRL-Fas1pr mice: the IL-6 pathway as a new therapeutic target in treatment of autoimmune kidney disease in systemic lupus erythematosus. *J Rheumatol*. 2010; 37:60–70. [PubMed: 19955044]
128. Lu LD, Stump KL, Wallace NH, Dobrzanski P, Serdikoff C, Gingrich DE, et al. Depletion of autoreactive plasma cells and treatment of lupus nephritis in mice using CEP-33779, a novel, orally active, selective inhibitor of JAK2. *J Immunol*. 2011; 187:3840–53. [PubMed: 21880982]
129. Herrera-Esparza R, Barbosa-Cisneros O, Villalobos-Hurtado R, Avalos-Diaz E. Renal expression of IL-6 and TNFalpha genes in lupus nephritis. *Lupus*. 1998; 7:154–8. [PubMed: 9607638]
130. Iwano M, Dohi K, Hirata E, Kurumatani N, Horii Y, Shiiki H, et al. Urinary levels of IL-6 in patients with active lupus nephritis. *Clinical nephrology*. 1993; 40:16–21. [PubMed: 8358870]
131. Tsai CY, Wu TH, Yu CL, Lu JY, Tsai YY. Increased excretions of beta2-microglobulin, IL-6, and IL-8 and decreased excretion of Tamm-Horsfall glycoprotein in urine of patients with active lupus nephritis. *Nephron*. 2000; 85:207–14. [PubMed: 10867535]
132. Santos MJ, Fernandes D, Capela S, da Silva JC, Fonseca JE. Interleukin-6 promoter polymorphism -174 G/C is associated with nephritis in Portuguese Caucasian systemic lupus erythematosus patients. *Clinical rheumatology*. 2011; 30:409–13. [PubMed: 21153673]
133. Illei GG, Shirota Y, Yarboro CH, Daruwalla J, Tackey E, Takada K, et al. Tocilizumab in systemic lupus erythematosus: data on safety, preliminary efficacy, and impact on circulating plasma cells from an open-label phase I dosage-escalation study. *Arthritis Rheum*. 2010; 62:542–52. [PubMed: 20112381]

134. Neville LF, Mathiak G, Bagasra O. The immunobiology of interferon-gamma inducible protein 10 kD (IP-10): a novel, pleiotropic member of the C-X-C chemokine superfamily. *Cytokine Growth Factor Rev.* 1997; 8:207–19. [PubMed: 9462486]
135. Vielhauer V, Anders HJ, Schlondorff D. Chemokines and chemokine receptors as therapeutic targets in lupus nephritis. *Semin Nephrol.* 2007; 27:81–97. [PubMed: 17336691]
136. Romagnani P, Beltrame C, Annunziato F, Lasagni L, Luconi M, Galli G, et al. Role for interactions between IP-10/Mig and CXCR3 in proliferative glomerulonephritis. *J Am Soc Nephrol.* 1999; 10:2518–26. [PubMed: 10589690]
137. Liu J, Karypis G, Hippen KL, Vegoe AL, Ruiz P, Gilkeson GS, et al. Genomic view of systemic autoimmunity in MRL/lpr mice. *Genes Immun.* 2006; 7:156–68. [PubMed: 16508641]
138. Avihingsanon Y, Phumesin P, Benjachat T, Akkasilpa S, Kittikowit V, Praditpornsilpa K, et al. Measurement of urinary chemokine and growth factor messenger RNAs: a noninvasive monitoring in lupus nephritis. *Kidney Int.* 2006; 69:747–53. [PubMed: 16518330]
139. Segerer S, Banas B, Wormle M, Schmid H, Cohen CD, Kretzler M, et al. CXCR3 is involved in tubulointerstitial injury in human glomerulonephritis. *Am J Pathol.* 2004; 164:635–49. [PubMed: 14742268]
140. Enghard P, Humrich JY, Rudolph B, Rosenberger S, Biesen R, Kuhn A, et al. CXCR3+CD4+ T cells are enriched in inflamed kidneys and urine and provide a new biomarker for acute nephritis flares in systemic lupus erythematosus patients. *Arthritis Rheum.* 2009; 60:199–206. [PubMed: 19116922]
141. Wu T, Xie C, Wang HW, Zhou XJ, Schwartz N, Calixto S, et al. Elevated urinary VCAM-1, P-selectin, soluble TNF receptor-1, and CXC chemokine ligand 16 in multiple murine lupus strains and human lupus nephritis. *J Immunol.* 2007; 179:7166–75. [PubMed: 17982109]
142. Nakatani K, Fujii H, Hasegawa H, Terada M, Arita N, Ito MR, et al. Endothelial adhesion molecules in glomerular lesions: association with their severity and diversity in lupus models. *Kidney Int.* 2004; 65:1290–300. [PubMed: 15086468]
143. Molad Y, Miroshnik E, Sulkes J, Pitlik S, Weinberger A, Monselise Y. Urinary soluble VCAM-1 in systemic lupus erythematosus: a clinical marker for monitoring disease activity and damage. *Clin Exp Rheumatol.* 2002; 20:403–6. [PubMed: 12102480]
144. Abd-Elkareem MI, Al Tamimy HM, Khamis OA, Abdellatif SS, Hussein MR. Increased urinary levels of the leukocyte adhesion molecules ICAM-1 and VCAM-1 in human lupus nephritis with advanced renal histological changes: preliminary findings. *Clin Exp Nephrol.* 2010; 14:548–57. [PubMed: 20714774]
145. Wisniacki NC, VL, Coddling CE. Phase I, Randomized, Double-Blind, Placebo-Controlled, Single Dose, Dose Escalation Study to Evaluate the Safety, Tolerability and Pharmacokinetics of BIIB023 (Anti-TWEAK) in Subjects with Rheumatoid Arthritis. *Arthritis & Rheumatism.* 2011; 63:S858.
146. Kapsogeorgou EK, Gourzi VC, Manoussakis MM, Moutsopoulos HM, Tzioufas AG. Cellular micrornAs (miRNAs) and Sjogren's syndrome: Candidate regulators of autoimmune response and autoantigen expression. *J Autoimmun.* 2011; 37:129–35. [PubMed: 21632209]
147. Rose NR. The genetics of autoimmune thyroiditis: The first decade. *J Autoimmun.* 2011; 37:88–94. [PubMed: 21683550]
148. Scheinecker C, Bonelli M, Smolen JS. Pathogenetic aspects of systemic lupus erythematosus with an emphasis on regulatory T cells. *J Autoimmun.* 2010; 35:269–75. [PubMed: 20638240]
149. Youinou P, Haralampos M. Moutsopoulos: A lifetime in autoimmunity. *J Autoimmun.* 2010; 35:171–5. [PubMed: 20673706]
150. Miyagawa F, Gutermuth J, Zhang H, Katz SI. The use of mouse models to better understand mechanisms of autoimmunity and tolerance. *J Autoimmun.* 2010; 35:192–8. [PubMed: 20655706]

Figure 1. Role of TWEAK/Fn14 pathway role in glomerular and tubular pathological processes involved in the development of kidney fibrosis and ESRD (modified from [76])
 Italics indicate parameters that link glomerular and tubular injury/disease. Red text indicates cell types that express Fn14 in response to injury and thus where anti-TWEAK should protect kidney from pathological events occurring in glomeruli or tubules.

TABLE 1
Evaluation of the TWEAK/Fn14 pathway in animal models

The various murine disease models are categorized as primary injury due to antibody-mediated glomerular damage or tubular damage. The role of the TWEAK/Fn14 pathway was evaluated in some models using TWEAK or Fn14 deficient mouse, in other models using anti-TWEAK or anti-Fn14 blocking antibodies, and in some cases the pathway was validated with multiple approaches. Effects on disease activity, inflammatory processes and/or kidney histopathology is indicated. ND indicates that effect on these parameters has not yet been determined.

Primary Injury	Animal Model	TWEAK /Fn14 deficiency or TWEAK /Fn14 blocking antibodies reduce:		
		Disease Activity	Inflammatory Processes	Kidney Histopathology
Antibody-mediated glomerular damage	Chronic graft vs. host disease-induced nephritis ⁷⁷	• Proteinuria	• RANTES, MCP-1, IP-10, IL-6 • Macrophages	• Cell proliferation • Ig deposition
	Nephrotoxic serum transfer nephritis ⁷⁸	• Proteinuria	• RANTES, MCP-1, IP-10, ICAM-1 • VCAM-1 • Macrophages	• Cell proliferation • PAS positivity • Crescent formation • Tubular damage • Tubulointerstitial fibrosis
	MRL/lpr spontaneous model of lupus ⁷⁹	• Proteinuria	ND	ND
Tubular damage	Acute kidney injury (folic acid) ^{28, 51-53}	• Serum creatinine • Serum BUN • Mortality	• RANTES, MCP-1, CCL21, CXCL16, CXCR6 • Macrophages, T cells	• Tubular injury • Tubular apoptosis • Tubular proliferation
	Renal ischemia reperfusion injury ²⁷	• Serum creatinine	• MCP-1, ICAM-1, IL-1 β , MIP-2, E-selectin, TNF α • Macrophages, neutrophils	• Tubular injury • Tubular apoptosis • Tubulointerstitial fibrosis