Pathogenic perspectives for the role of inflammation in diabetic nephropathy

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ABSTRACT

Diabetes and its complications have become a public health problem. One of the most important complications is diabetic nephropathy, which is nowadays the main cause of chronic renal failure. In spite of our greater understanding of this complication, the intimate mechanisms leading to the development and progression of renal injury are not well understood. New perspectives in activated innate immunity and inflammation appear to be relevant factors in the pathogenesis of diabetes. Moreover, different inflammatory molecules, including adipokines, Toll-like receptors, chemokines, adhesion molecules and pro-inflammatory cytokines, may be critical factors in the development of microvascular diabetic complications, including nephropathy. This new pathogenic perspective leads to important therapeutic considerations, with new pathogenic pathways becoming important therapeutic targets that can be translated into clinical treatments for diabetic nephropathy.

INTRODUCTION

DN (diabetic nephropathy) is one of the most important concerns in nephrology, as well as in medicine at large. Rapidly increasing rates of DM (diabetes mellitus) throughout the developed world represents an emerging epidemic with profound consequences. This epidemic is likely to drive previously unforeseen rates of vascular target organ complications [1]. End-stage renal disease in diabetes, particularly Type 2 DM, has been described as a medical catastrophe of worldwide dimensions [2].

Over the past decade, a number of important research developments in the area of DM and its complications have changed the way we think about this disease. DM is not only a metabolic disorder, but at the present time we know that diverse molecules related to inflammation play a significant role in the development of diabetes and diabetic complications [3–5].

NEW CONCEPT OF DM AS AN INFLAMMATORY DISEASE

The fact that activation of the innate immune system and low-grade chronic inflammation are closely involved in the pathogenesis of Type 2 DM has modified our vision of the disease. This new pathogenic perspective leads to important therapeutic considerations, with new pathogenic pathways becoming important therapeutic targets that can be translated into clinical treatments for diabetic nephropathy.
Figure 1  Schematic representation of the participation of inflammation in the pathogenesis of DN

of this disease. This hypothesis suggests that long-term innate immune system activation, resulting in chronic inflammation, elicits disease instead of repair, leading to the development of Type 2 DM [3,6]. In addition, long-term low-grade inflammation may play a key role in the onset of several disturbances leading to DN, including insulin resistance, hyperglycaemia, oxidative stress and endothelial dysfunction, with secondary consequences (increased urinary albumin excretion) playing a critical role in perpetuating renal damage and progression. Probably, the right answer is the result of an interaction of genetic and environmental factors leading to an inflammatory milieu with a continuous perpetuation of injury factors (Figure 1).

Several cross-sectional and longitudinal studies in the general population, as well as in non-diabetic subjects, in individuals with IGT (impaired glucose tolerance) or IFG (impaired fasting glucose), and in newly diagnosed or established patients with Type 2 DM, have shown that acute-phase reactants and pro-inflammatory cytokines are positively correlated with measures of insulin resistance [4–9]. Furthermore, patients with DM have elevated levels of inflammatory parameters compared with non-diabetic control subjects [9–11]. Finally, important prospective studies have clearly confirmed that circulating inflammatory markers, acute-phase reactants and pro-inflammatory cytokines are strongly associated with the risk of developing Type 2 DM [12–15].

On the other hand, it has been reported that endothelial dysfunction, an abnormality closely related to insulin resistance [16,17], is common in subjects with DN [18]. Specifically, it has been shown that diabetic subjects with renal disease often have an impaired release of NO. Moreover, mice genetically deficient in eNOS (endothelial NO synthase) which are made diabetic develop lesions resembling human diabetic renal disease [19–21]. Interestingly, endothelial dysfunction has been shown to lead to an uncoupling of the VEGF (vascular endothelial growth factor)/NO axis, resulting in pro-inflammatory and proliferative effects [20,22].

In recent years, potential new perspectives in the pathogenesis of Type 2 DM and its complications have been matters of great interest. Classical theories state that insulin resistance is the primary abnormality leading to Type 2 DM, as well as accompanying pathological conditions such as hypertension and dyslipidaemia. From this point of view, endothelial dysfunction merely represents the consequence of hyperglycaemia and other related metabolic abnormalities. An alternative concept is that endothelial dysfunction is a central element in the spectrum of metabolic disorders in DM. According to this concept, endothelial dysfunction in large arteries, which represents an early and prominent event in atherothrombotic disease, is paralleled by endothelial dysfunction in resistance vessels and metabolically important capillary beds, which contributes to the development of the
metabolic disease in DM [23]. In addition, in the last decade a new hypothesis has emerged with great interest in the notion that activation of the innate immune system and subsequent development of a low-grade chronic inflammatory reaction (an ongoing cytokine-induced acute-phase response) is closely involved in the pathogenesis of DM [24,25]. This theory has lead to the question of whether innate immune system activation and low-grade inflammation are pathogenic mechanisms of other manifestations of Type 2 DM as well as diabetic complications.

### INFLAMMATORY MOLECULES, OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION IN DN

In spite of the improvement in our knowledge on DN, from a pathophysiological point of view, the precise mechanisms leading from chronic hyperglycaemia to the development of renal injury are complex and not clearly known. Experimental and clinical studies have demonstrated the significant role of inflammatory molecules (including adipokines, chemokines, adhesion molecules and cytokines; Tables 1 and 2) and endothelial dysfunction in the setting of DN. More important, several experimental studies have shown that the beneficial effects of different therapeutic strategies to prevent the development or ameliorate renal injury in diabetes are associated with anti-inflammatory actions [26–29].

### Adipokines: adiponectin, leptin and resistin

### Adiponectin

Adiponectin has been highlighted in the pathogenesis of obesity-related illnesses, including Type 2 DM, because of its role in the regulation of insulin sensitivity as well as vascular endothelial function [30]. In addition, adiponectin signalling mechanisms through its receptors Adipo-R1 and Adipo-R2, including AMPK (AMP-activated protein kinase) and cAMP/PKA (cAMP-dependent protein kinase) pathways, have been implicated in the functions of endothelial and inflammatory cells.

Experimental studies have shown that adiponectin improves insulin sensitivity through a number of different mechanisms [31], including stimulation of glucose utilization and fatty acid oxidation in skeletal muscles and liver, facilitation of glucose uptake by an increase in GLUT-4 (glucose transporter-4) expression and its translocation, and suppression of gluconeogenesis in the liver [32,33]. Interestingly, clinical studies have shown that plasma HMW (high-molecular weight (‘mass’)) adiponectin concentrations have a better relationship with glucose tolerance and insulin sensitivity than the total plasma concentration of adiponectin [34]. Therefore impaired multimerization of adiponectin also appears to participate in the pathogenesis of Type 2 DM [35].

On the other hand, others studies have shown that a loss of endogenous adiponectin is associated with abnormal leucocyte–endothelium interactions. Ouedraogo et al. [36] have shown that adiponectin-deficient mice had a significant increase in leucocyte rolling and leucocyte adhesion in the microcirculation. Adhesion of circulating leucocytes to the vascular endothelium causes endothelial dysfunction by exposing endothelial cells to the damaging action of inflammatory mediators released by activated leucocytes [37]. Concerning endothelial function, adiponectin deficiency drastically reduced levels of endothelial NO, as adiponectin preserves endothelial NO by directly shifting the balance between NO and ROS (reactive oxygen species) generation in a direction favourable to NO availability. A negative relationship between systemic oxidative stress and circulating adiponectin

### Table 1 Effects of adipokines potentially relevant to renal injury in diabetes

<table>
<thead>
<tr>
<th>Adipokine</th>
<th>Effect relevant to renal injury</th>
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<tr>
<td>Monocyte/macrophage</td>
<td>↓ Phagocytic activity; ↓ NF-κB activation; ↓ TNF-α secretion; ↓ scavenger receptor expression</td>
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<tr>
<td>Endothelial cell</td>
<td>↓ Inflammatory signalling; ↑ NO bioavailability; ↓ abnormal leucocyte–endothelium interactions; ↓ ROS generation; ↓ TNF-α-induced expression of cell adhesion molecules; ↓ TNF-α-stimulated phosphorylation of IκBα; ↓ secretion of IL-8</td>
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<tr>
<td>Smooth muscle cell</td>
<td>↓ VSMC signalling response; sequesters VSMC growth factors</td>
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<tr>
<td>Leptin</td>
<td>↑ Matrix metalloproteinase (MMP-2) expression and activity; stimulates proliferation and TGF-β1 expression in renal glomerular endothelial cells; ↑ glucose uptake, mRNA expression of TGF-β1 type 2 receptor, and type 1 collagen production in cultured mesangial cells; ↑ the innate immune system and shift the cognate immune system towards Th1&gt;Th2; indirectly ↑ sympathetic nerve trafficking and renal sodium retention</td>
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<tr>
<td>Resistin</td>
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<tr>
<td>Endothelial cell</td>
<td>↑ ET-1 mRNA expression and secretion; ↑ expression of VCAM-1 and MCP-1; ↓ insulin signalling and eNOS activation</td>
</tr>
<tr>
<td>Smooth muscle cell</td>
<td>↑ VSMC proliferation</td>
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levels has been shown in rodent models of obesity and the metabolic syndrome [38]. In addition, adiponectin suppresses TNF-α (tumour necrosis factor-α)-induced inflammatory changes in endothelial cells by blocking IκB [inhibitor of NF-κB (nuclear factor κB)] phosphorylation and subsequent NF-κB activation without affecting other TNF-α-mediated phosphorylation signals, including JNK (c-Jun N-terminal kinase), p38 MAPK (mitogen-activated protein kinase) SAPK-2 (stress-activated protein kinase-2) and the serine/threonine kinase Akt [39].

Other relevant implications of adiponectin are of interest in the setting of DN. The interactions of adiponectin with PDGF (platelet-derived growth factor)-BB, basic FGF (fibroblast growth factor) and HB-EGF (heparin-binding epidermal growth factor) precluded the binding to their respective membrane receptors and attenuated the DNA synthesis and cell proliferation induced by these growth factors in VSMCs (vascular smooth muscle cells) [40]. Additional adiponectin effects include the suppression of leucocytic colony formation and the reduction in phagocytic activity, foam-cell transition and TNF-α secretion from macrophages [41,42]. Because inflammatory signalling in leucocytes is at least in part mediated by TLRs (Toll-like receptors), it is interesting that gAd (globular C-terminal adiponectin) has been shown to be able to inhibit TLR-mediated activation of the NF-κB cascade in cultured mouse macrophages [43]. Finally, experimental studies have indicated that adiponectin accumulates in the injured kidney, and prevents glomerular and tubulointerstitial injury through modulating inflammation and oxidative stress [44].

In human studies, the presence of hypoadiponectinaemia has been reported in patients with obesity, Type 2 DM and coronary artery disease [45–47]. Adiponectin has anti-inflammatory and antioxidative properties [48–50]. Clinically, hypoadiponectinaemia is closely associated with increased levels of inflammatory markers such as CRP (C-reactive protein) and IL-6 (interleukin-6) [51]. In vitro, recombinant adiponectin suppresses the TNF-α-induced expression of endothelial adhesion molecules and TNF-α in macrophages, and selectively increases the expression of TIMP-1 (tissue inhibitor of metalloproteinases-1), which protects the vascular wall from plaque rupture, in human monocyte-derived macrophages through the induction of IL-10, an anti-inflammatory cytokine [52–56].

Weight reduction in obese subjects by gastric reduction surgery, lifestyle modifications or medical therapy has been accompanied by increasing plasma adiponectin concentrations [57–59]. Similarly, treatment with thiazolidinediones potently increases plasma adiponectin levels [60–63]. It is of interest that these interventions particularly increase the plasma concentration of the insulin-sensitizing HMW adiponectin form [64–66]. Other drugs that increase, to a certain extent, plasma adiponectin concentrations are: ACEIs (angiotensin-converting enzyme inhibitors; captopril and ramipril) and ARBs [AngII (angiotensin II) receptor blockers; losartan, candesartan, valsartan and telmisartan] [67–72], a clonidine-like sympatho-inhibitory antihypertensive agent (rilmenidine) [73], fenofibrate [74] and a cannabinoid-1 receptor blocker (rimonabant) [75].

Table 2  Potential implications of chemokines, adhesion molecules, pro-inflammatory cytokines and inflammatory markers in DN

<table>
<thead>
<tr>
<th>Marker</th>
<th>Implication</th>
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<tr>
<td>MCP-1</td>
<td>Activation and recruitment of monocytes that promote macrophage accumulation and activation</td>
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<tr>
<td>Adhesion molecule</td>
<td>Attachment (sticking) of leucocytes, monocytes and macrophages to the endothelium and subsequent transendothelial transmigration</td>
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<tr>
<td>Pro-inflammatory cytokine</td>
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<tr>
<td>IL-1</td>
<td>Increases vascular endothelial permeability and proliferation of mesangial cells and matrix synthesis; induces intraglomerular haemodynamic abnormalities related to prostaglandin synthesis by mesangial cells</td>
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<tr>
<td>IL-6</td>
<td>Stimulates mesangial cell proliferation, enhances fibronectin expression, affects extracellular matrix dynamics at both mesangial and podocyte levels, and increases endothelial permeability; increases the expression of adhesion molecules on endothelial and VSMCs; activates the local RAS, and enhances TGF-β signalling via modulation of TGF-β receptor trafficking</td>
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<tr>
<td>IL-18</td>
<td>Promotes development of a Th1 lymphocyte response by induction of IFN-γ production; modulates activity of NK cells, increases TNF-γ and IL-1 production by macrophages; up-regulates the expression of adhesion molecules, and induces NO production in the area of inflammation</td>
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<td>TNF-α</td>
<td>Induces haemodynamic misbalance between vasodilator and vasoconstrictive mediators; promotes the local generation of ROS, with alteration of the barrier function of the glomerular capillary wall; significantly contributes to sodium retention and renal hypertrophy</td>
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<tr>
<td>Inflammatory marker</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>Inflammatory and cardiovascular markers; immunoregulatory functions include enhancement of leucocyte reactivity, complement fixation, modulation of platelet activation and clearance of cellular debris from sites of active inflammation</td>
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This might provide a scientific rationale for the use of these drugs in order to increase plasma adiponectin concentrations in high-risk populations.

Leptin

In contrast with adiponectin, leptin exerts several potentially pro-inflammatory effects, such as impairment of endothelial function, stimulation of inflammatory signalling pathways, increase in oxidative stress, reduction in paraxonase activity, and stimulation of platelet aggregation and migration, as well as stimulation of hypertrophy and proliferation of VSMCs. Endothelial cells express leptin receptors, having enhanced endothelial ROS in response to leptin stimulation.

Several clinical studies have demonstrated that high leptin levels predict acute cardiovascular events. Some investigations have suggested that the leptin/adiponectin ratio might be a useful index for cardiovascular risk and may be a suitable cardiovascular marker in patients with Type 2 DM [76–79]. It has been reported that leptin levels are increased in both patients with Type 1 and Type 2 DM with microalbuminuria or macroalbuminuria [80,81]. Likewise, Wilson et al. [82] have reported that the urine leptin concentration in Pima Indians correlated positively with the urinary albumin/creatinine ratio and inversely correlated with the glomerular filtration rate.

Direct and indirect renal effects of leptin could be relevant for DN in Type 2 DM. Leptin is primarily metabolized in the kidney, presumably by binding to megalin, a multi-ligand receptor in the proximal tubule, with subsequent tubular uptake and endocytosis. The kidney expresses abundant concentrations of Ob-Ra (short isoform of the leptin receptor). In cultured renal rat endothelial cells, as well as in mesangial cells obtained from db/db mice, leptin can signal through Ob-Ra. Leptin stimulates the proliferation of glomerular endothelial cells, increases TGF (transforming growth factor)-β1 synthesis and collagen type IV production. In contrast, leptin did not influence TGF-β1 production in mesangial cells, but the peptide stimulated glucose transport in these cells, increased collagen type I synthesis and led to an up-regulation of surface TGF-β type II receptors through signal transduction pathways involving PI3K (phosphoinositide 3-kinase). Leptin also stimulates hypertrophy, but not proliferation, in cultured rat mesangial cells. Infusion of leptin for 3 weeks into normal rats promotes the development of glomerulosclerosis and proteinuria [83].

Resistin

Resistin contributes to dysglycaemia in obesity and promotes endothelial cell impairment. There are several studies where resistin was positively correlated with the levels of inflammatory markers [84,85]. In endothelial cells in vitro, resistin was able to increase ET-1 (endothelin-1) mRNA expression and secretion; resistin-treated cells also had an increased expression of VCAM-1 (vascular cell adhesion molecule-1) and MCP-1 (monocyte chemoattractant protein-1) [86–89]. In addition, in these cells, resistin inhibited insulin signalling and eNOS activation via the up-regulation of PTEN (phosphatase and tensin homologue deleted on chromosome 10) through a mechanism involving p38 MAPK activation of sites in the PTEN promoter [90]. Proliferation of VSMCs has been shown to be stimulated by resistin via both the ERK1/2 (extracellular-signal-regulated kinase 1/2) and Akt signalling pathways [91].

On the basis on these facts, strategies that include an increase in adiponectin or a reduction in leptin and resistin would be a potential therapeutic option in the treatment of complications associated with Type 2 DM.

TLRs

TLRs are a family of pattern recognition receptors that play a critical role in the innate immune system by activating pro-inflammatory signalling pathways in response to microbial pathogens [92]. At sites of inflammation, leucocytes are present in tissues and are exposed to multiple TLR agonists arising from endogenous damage and infections. Combined TLR4/monocyte activation and TLR3/tissue cell activation result in a cooperative response, leading to a further enhancement of cytokine generation. The outcome with respect to the inflammatory response will, therefore, be dependent on the pattern of TLR expression on leucocytes and tissue cells, the interaction between leucocytes and tissue cells, and the nature of the agonists and the TLR with which it interacts [92,93]. TLR4, the best-characterized TLR, binds to LPS (lipopolysaccharide) of Gram-negative bacterial cell walls [92]. Upon binding of LPS to TLR4 and its co-receptors CD14 and MD-2, the adaptor protein MyD88 (myeloid differentiation factor 88) is recruited to the TIR (Toll/IL-1 receptor) domain of the receptor. Interaction of the TIR domain of TLR4 and MyD88 triggers a downstream signalling cascade, leading to activation of the NF-κB pathway, which then activates the transcription of many pro-inflammatory genes that encode inflammatory molecules, including cytokines, chemokines and other effectors of the innate immune response [92].

Intracellular kinases linked to inflammatory signalling, including PKCθ (protein kinase C θ), IKKα (IkB kinase α) and JNK, appear to play a significant role in NEFA [non-esterified (‘free’) fatty acid]-induced insulin resistance [94–97]. In light of findings indicating a pathophysiological role for NEFAs in inflammation and insulin resistance, it is intriguing that the lipid component of LPS is sufficient to trigger TLR4 signalling. In particular, a medium-chain fatty acid component of LPS, lauric acid, has been shown to initiate TLR4 signalling in a macrophage cell line. These results suggest that activation of TLR4 in adipocytes might be implicated in the
onset of insulin resistance in obesity and Type 2 DM [98–100]. The basic mechanism of danger recognition via TLR signalling in response to infectious and non-infectious cellular damage might also be involved in renal disease. Initial studies suggest that TLR2 and TLR4 can induce chemokine expression in tubular epithelial cells, and that TLR9 is expressed on infiltrating antigen-presenting cells during immune injury. TLR-mediated immune activation may occur during any type of renal injury by exposure to an increasing number of exogenous or endogenous molecules, and it has been implicated in diverse renal and vascular disease models, including immune complex glomerulonephritis, allograft rejection, acute renal injury, lupus nephritis etc. [101–105]. These findings suggest that the selective targeting of pattern recognition receptors and the pathways they trigger may be useful clinically. Progress towards therapeutics designed to target TLR signalling is already underway.

Chemokines
There is a growing body of evidence that recruitment of inflammatory cells from the circulation into renal tissue is a critical feature of various renal diseases. In particular, infiltrating activated T-cells and monocytes are suggested to initiate renal tissue damage, eventually leading to a progressive loss of renal function [106,107]. There is evidence from in vivo and in vitro studies that differential expression of chemokines and their receptors provides the molecular mechanisms that lead to the precise co-ordination of inflammatory cell migration in renal inflammatory diseases [108]. However, the diabetic kidney comprises a large and complex pathophysiological network of interrelated actions not yet fully known.

MCP-1
Experimental studies have demonstrated that MCP-1-mediated macrophage accumulation and activation is a critical mechanism in the development of early DN [109]. In addition, up-regulation of kidney MCP-1 has been shown as a feature of human diabetic renal injury associated with macrophage recruitment, urinary albumin excretion, tubulointerstitial injury and disease progression [110–113]. On the basis of these findings, neutralizing MCP-1 activity could be an important therapeutic goal in the treatment of DN.

From this perspective, a recent experimental study has shown that blockade of the MCP-1/CCR2 (CC chemokine receptor 2) pathway ameliorated glomerulosclerosis, indicating a crucial role for this pathway in the progression of DN [114]. Other authors have shown that blockade of the RAS (renin–angiotensin system) in patients with Type 2 DM and DN is associated with a reduction in urinary MCP-1 levels as well as an improvement in renal function [115]. A new treatment strategy for DN based on aldosterone blockade by spironolactone may offer beneficial renoprotective effects through anti-inflammatory mechanisms via the modulation of MCP-1 [116,117]. In addition, recent in vitro and in vivo studies have demonstrated that the potential protective role of vitamin D in DN may be related to MCP-1, since vitamin D suppresses high-glucose-induced MCP-1 up-regulation in mesangial cells by blocking the activation of NF-κB [118]. 1,25 Dihydroxyvitamin D3 effectively attenuates the MCP-1 induction at both the mRNA and transcriptional levels by blocking p65/p50 binding to the NF-κB sites in the gene promoter. Thus administration of vitamin D analogues can inhibit the synthesis and activity of MCP-1 and ameliorate the glomerular injury in diabetes [118].

Adhesion molecules
ICAM-1 (intercellular adhesion molecule-1)
The multi-step paradigm of leucocyte trafficking involves sequential and overlapping interactions of signal molecules initiated by selectin-mediated rolling interactions, followed by the firm integrin-dependent arrest on Ig ligands and transendothelial migration towards a chemokine gradient. The detailed molecular mechanisms that direct macrophage migration are not fully characterized, but chemokines/chemokine receptors as well as integrins are involved in this process. Increased expression of ICAM-1, which serves as a ligand for LFA-1 (lymphocyte function-associated antigen-1), was detected in models of DN, with a direct association with renal injury and progression [119,120]. Therefore it is likely that ICAM-1 interacts with T-cells and stimulates migration into the kidney. Homing of CD4+ cells into the glomeruli of diabetic kidneys was decreased in ICAM-1-deficient db/db mice compared with ICAM-1-intact db/db mice [121,122].

Concerning clinical studies, it has been shown that patients with both Type 1 and Type 2 DM and DN have elevated concentrations of ICAM-1 compared with subjects without renal injury, suggesting that this molecule can be of pathogenic importance for the development of renal damage [123,124]. From a therapeutic perspective, all of these findings suggest that the modulation of ICAM-1 activity may be an important goal for treating DN. Therefore blockade or interference with receptor activation, or reduction in ICAM-1 expression in endothelial and other renal cells, is a potential target for therapeutic interventions [125], especially taking into account that antagonists of ICAM-1 are currently under development [126].

VCAM-1
Experimental studies have shown an increased expression of VCAM-1 by endothelial cells as well as by infiltrating cells in the interstitium of kidneys from diabetic mice [127]. In addition, cross-sectional clinical studies have shown an elevation of circulating VCAM-1 and E-selectin levels in patients with DN, which may result from underlying systemic endothelial dysfunction,
increased VCAM-1 production in damaged renal tubular or glomerular epithelial cells and/or decreased renal clearance of this molecule, depending on the stage of nephropathy [128–130]. More importantly, clinical prospective investigations in individuals with Type 2 DM have shown that patients with increased albuminuria and high plasma concentrations of soluble VCAM-1 and CRP had an increased risk of death. In addition, markers of endothelial dysfunction and inflammatory activity were strongly associated with increases in urinary albumin excretion during the 10-year follow-up. These associations were independent of major risk factors for developing elevated urinary albumin excretion, such as high blood pressure and poor glycaemic control [130].

Pro-inflammatory cytokines and inflammatory biomarkers

IL-1, IL-6 and IL-18

Macrophages incubated with GBMs (glomerular basement membranes) from diabetic rats produced significantly greater levels of IL-1 and TNF-α than macrophages incubated with membranes from non-diabetic animals. This finding suggested for the first time that pro-inflammatory cytokines could participate in the development of DN [132,133]. Later studies have demonstrated that intrinsic renal cells (endothelial, mesangial, glomerular and tubular epithelial cells) are able to synthesize pro-inflammatory cytokines [134,135].

Diverse investigations have shown that IL-1 is up-regulated in the diabetic kidney [136,137]. Moreover, it has been demonstrated that IL-1 increases vascular endothelial permeability, and it is involved in the proliferation of mesangial cells and matrix synthesis, as well as in the development of intraglomerular haemodynamic abnormalities related to prostaglandin synthesis [138,139]. Suzuki et al. [140] studied IL-6 mRNA in kidney biopsies of Japanese patients with DN. Using in situ hybridization, they found a relationship between the severity of diabetic glomerulopathy and the expression of IL-6 mRNA in glomerular cells (mesangial cells and podocytes). Thus it is plausible that IL-6 affects extracellular matrix dynamics at the mesangial cell and podocyte levels, contributing to both mesangial expansion and GBM thickening [141]. Renal IL-6 expression has been related to mesangial proliferation, tubular atrophy and the intensity of interstitial infiltrates in diverse models of renal disease, suggesting a contributing role in the progression of renal disease [142–144]. Finally, regarding IL-18, Moriwaki et al. [145] have reported that patients with Type 2 DM had significantly higher serum levels of this cytokine than healthy controls. In addition, IL-18 levels were increased in diabetic patients with the development of urinary albumin excretion. In 2005, Nakamura et al. [146] showed that urinary albumin excretion rates in patients with Type 2 DM were independently associated with both serum and urinary IL-18 levels and, moreover, serum and urinary IL-18 correlated positively with changes in albuminuria during the follow-up. A recent observational study has shown that elevated IL-18 may be a predictor of early renal dysfunction in Type 2 DM [147].

TNF-α

Although there has been great progress in our understanding of the multiple functions of TNF-α and of the respective underlying mechanisms at a cellular and molecular level, much still needs to be learned about TNF-α biology. For example, does TNF-α contribute to or protect from tissue damage in acute or chronic diseases? The accumulating findings suggest a very differential action of TNF-α and indicate that tissue type, precise cellular context and composition, timing and duration of TNF-α action are important parameters determining the net effect of this cytokine in vivo.

Over the past few years, in addition to the implications of several ILs, most attention has been paid to the role of TNF-α in the setting of DN. Monocytes and macrophages are the primary source of TNF-α, although intrinsic renal cells are also able to synthesize this cytokine [134,135]. Our group has shown previously in an experimental model of DN that renal mRNA expression of TNF-α is significantly increased approx. 2.5-fold in diabetic rats compared with normal rats [137,148]. This cytokine and its receptor family have a variety of bioactivities that may promote the development of inflammatory actions related to the development and progression of renal injury in DM [149,150]. Experimental studies have demonstrated that urinary albumin excretion significantly correlates with renal cortical mRNA levels and urinary TNF-α excretion in animal models of DN [137,148]. Moreover, it has been shown that increased urinary TNF-α excretion, as well as increased TNF-α levels in renal interstitial fluid, precede the significant increase in albuminuria [151]. TNF-α significantly contributes to sodium retention and renal hypertrophy, characteristic alterations during the early stages of DN [152], whereas exposure of tubular epithelial cells to TNF-α significantly increases the synthesis and secretion of lymphocyte and neutrophil chemoattractant factors, as well as the cell-surface expression of ICAM-1 [153]. Finally, TNF-α, independent from haemodynamic factors or effects of recruited inflammatory cells, promoted the local generation of ROS, with alterations in the barrier function of the glomerular capillary wall resulting in enhanced albumin permeability [154].

Regarding clinical studies, it has been reported that a direct and significant association occurs between serum TNF-α and urinary protein excretion in diabetic patients with normal renal function and microalbuminuria, as well as in subjects with overt nephropathy and renal insufficiency [10,155]. Likewise, urinary TNF-α levels
were also elevated in diabetic patients with increased urinary albumin excretion and, furthermore, there was a significant rise in urinary TNF-α excretion as DN progressed. Moreover, multivariate analysis shows a significant and independent relationship between urinary TNF-α and urinary albumin excretion. Interestingly, in these studies a significant correlation between serum and urinary concentrations of TNF-α was not found, suggesting an intrarenal production of this cytokine [10].

Renal TNF-α production appears to be related to hyperglycaemia and AGEs (advanced glycation end-products) [135]. Likewise, it might also be related to AngII, which has been demonstrated to regulate the synthesis of inflammatory cytokines in the kidney [156]. The exact mechanisms by which AngII can stimulate TNF-α production are not completely known, although activation of the transcription factor NF-κB, release of prostaglandin E2 and reduction in intracellular cAMP may play central roles [156–158].

Importantly, modulation of TNF-α has been suggested as a potential therapeutic strategy in DN. Pentoxifylline is a methylxanthine-derived phosphodiesterase inhibitor with beneficial haemorrhheological actions widely used in the treatment of peripheral circulatory disorders. In addition, pentoxifylline is able to inhibit the accumulation of TNF-α mRNA and the transcription of the TNF-α gene, suppressing the synthesis of this cytokine [159,160]. In different experimental models of renal disease where TNF-α plays a significant pathogenic role, such as lupus nephritis [161], crescentic glomerulonephritis [162], mesangial proliferative glomerulonephritis [163] and remnant kidney model [164], pentoxifylline has shown to have beneficial effects in preventing or attenuating renal injury. Regarding DN, DiPetrillo and Gesek [165], as well as our group [137], have observed that pentoxifylline administration was able to prevent the increase in renal TNF-α expression, synthesis and excretion during experimental DM. In addition, pentoxifylline therapy ameliorated renal sodium retention and renal hypertrophy, the initial pathological changes associated with DN. Together with these experimental studies, clinical results support the efficacy of pentoxifylline as a therapeutic agent for DN. It had been shown that pentoxifylline reduces urinary protein excretion in diabetic subjects, both with normal renal function [166,167] and renal insufficiency [155]. Furthermore, prospective trials have demonstrated that the addition of pentoxifylline to blockers of the RAS (ACEIs [168] and ARBs) [169] is associated with a significant reduction in urinary albumin excretion. Finally, in a previous study in patients with proteinuric primary glomerular diseases, in addition to the previously described effects on TNF-α, pentoxifylline significantly reduced urinary protein excretion, which was closely associated with a decrease in urinary MCP-1 excretion [170].

CRP

CRP is thought to have several characteristics that imply a fundamental role in natural host defence. Specifically, CRP is a member of the pentraxin family of oligomeric proteins involved with pattern recognition in innate immunity [171–173]. Reported immunoregulatory functions of CRP include enhancement of leucocyte reactivity, complement fixation, modulation of platelet activation and clearance of cellular debris from sites of active inflammation [171,174,175].

It is well known that CRP, a sensitive circulating marker of inflammation, is independently associated with an increased risk of CVD (cardiovascular disease) [176–180]. In addition, there is now substantial experimental evidence and more recent findings from clinical studies suggesting that CRP, as well as IL-6, are sensitive physiological markers of subclinical systemic inflammation, which are associated with insulin resistance, the metabolic syndrome, hyperglycaemia and overt Type 2 DM [181–184]. Regarding DN, it has been reported that inflammatory parameters (including CRP) in patients with Type 2 DM at early stages of nephropathy are independently associated with clinical biomarkers of glomerular and tubulointerstitial damage [185–187].

Oxidative stress and endothelial dysfunction

Hyperglycaemia is the major causal factor in the development of endothelial dysfunction in patients with DM. There is experimental and clinical evidence linking endothelial dysfunction to increased production of ROS. Previous studies have identified vascular NADPH oxidase and an uncoupled eNOS (NOS3), as well as mitochondrial enzymes, as important superoxide sources in the setting of hyperglycaemia [188]. High glucose induces intracellular ROS directly via glucose metabolism and auto-oxidation and indirectly through the formation of AGEs and their receptor binding. ROS mimic the stimulatory effects of elevated glucose and up-regulate TGF-β1, PAI-1 (plasminogen activator inhibitor-1) and ECM (extracellular matrix) proteins by glomerular mesangial cells, thus leading to mesangial expansion. ROS activate other signalling molecules, such as PKC and MAPKs, and transcription factors, such as NF-κB, AP-1 (activator protein-1), and Sp-1 (specificity protein-1), leading to transcription of genes encoding cytokines, growth factors and ECM proteins [189].

CONCLUSIONS

Over the past decade, multiple studies have helped to elucidate the associations between DM, inflammation and DN. It is now clear that inflammation is a cardinal process in the appearance of DM, and that the inflammatory milieu in DM contributes significantly to the development
of many of the complications of this disease, including DN. The relationships between this inflammatory state and the development and progression of DN involve very complex network processes. Diverse inflammatory molecules play significant roles in this scenario, including adipokines, TLRs, chemoattractant cytokines (chemokines), adhesion molecules and pro-inflammatory cytokines. At the present time, current treatment of DN is still suboptimal, both in preventing its development as well as in halting its progression. A better understanding of the role of inflammatory molecules and processes in the context of DN will facilitate the development of new and improved therapeutic targets and strategies that can be translated successfully into clinical applications.

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