Clinical Science (2013) **124**, 139–152 (Printed in Great Britain) doi: 10.1042/CS20120198

Review Article

Inflammation and the pathogenesis of diabetic nephropathy

Jun WADA and Hirofumi MAKINO

Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan

Abstract

The most problematic issue in clinical nephrology is the relentless and progressive increase in patients with ESRD (end-stage renal disease) worldwide. The impact of diabetic nephropathy on the increasing population with CKD (chronic kidney disease) and ESRD is enormous. Three major pathways showing abnormality of intracellular metabolism have been identified in the development of diabetic nephropathy: (i) the activation of polyol and PKC (protein kinase C) pathways; (ii) the formation of advanced glycation end-products; and (iii) intraglomerular hypertension induced by glomerular hyperfiltration. Upstream of these three major pathways, hyperglycaemia is the major driving force of the progression to ESRD from diabetic nephropathy. Downstream of the three pathways, microinflammation and subsequent extracellular matrix expansion are common pathways for the progression of diabetic nephropathy. In recent years, many researchers have been convinced that the inflammation pathways play central roles in the progression of diabetic nephropathy, and the identification of new inflammatory molecules may link to the development of new therapeutic strategies. Various molecules related to the inflammation pathways in diabetic nephropathy include transcription factors, pro-inflammatory cytokines, chemokines, adhesion molecules, Toll-like receptors, adipokines and nuclear receptors, which are candidates for the new molecular targets for the treatment of diabetic nephropathy. Understanding of these molecular pathways of inflammation would translate into the development of anti-inflammation therapeutic strategies.

Key words: adhesion molecule, adipokine, chemokine, cytokine, diabetic nephropathy, nuclear receptor, transcription factor.

INTRODUCTION

The incidence of patients with ESRD (end-stage renal disease) is relentlessly and rapidly increasing worldwide. The incidence of reported ESRD is greatest in Taiwan, at 404 per million in 2005, followed by the U.S., Jalisco (Mexico), Shanghai and Japan (in 2004), with reported rates of 351, 302, 275 and 267 per million respectively. Many countries with high rates of ESRD also treat a large proportion of patients with diabetes. In Jalisco, for example, 60% of new ESRD patients have a primary diagnosis of diabetes, compared with the 11–13% reported by Russia and Norway and the 5% reported by Iceland (http://www.usrds.org/adr.htm). The impact of diabetic nephropathy on the increasing population with CKD (chronic kidney disease) and ESRD is enormous. The intensified multifactorial intervention in patients with Type 2 diabetes resulted in reduced risk of microangiopathy, cardiovascular events and mortality in Steno type 2 randomized studies [1–3]; however, the incidence of ESRD is progressively increasing worldwide. In such circumstances, the identification of inflammation-related molecules and pathways which are critically involved in the progression of diabetic nephropathy, would strongly enhance and promote the development of new therapeutic strategies (Table 1) [4,5]. In the present review, we focus on the current status of research related to microinflammation in the pathogenesis of diabetic nephropathy and describe future perspectives for the translational research of diabetic nephropathy.

Abbreviations: 1,25(OH)2D3, 1-α,25-dihydroxyvitamin D_3; AGE, advanced glycation end-product; AMPK, AMP-activated protein kinase; CCL2, CC chemokine ligand 2; CCR2, CC chemokine receptor 2; CXCL, CXC chemokine ligand; CX3CL1, CX3C chemokine ligand 1; CX3CR1, CX3C chemokine receptor 1; DAMP, damage-associated molecular pattern; ECM, extracellular matrix; ESRD, end-stage renal disease; FXR, farnesoid X receptor; HMGB1, high-mobility group box 1; ICAM1, intercellular adhesion molecule 1; IFNγ, interferon γ; IκB, inhibitory κB; IL, interleukin; JAK2, Janus kinase 2; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor κB; NR1H4, nuclear receptor subfamily 1, group H, member 4; PKC, protein kinase C; PPAR, peroxisome-proliferator-activated receptor; RAGE, receptor for AGE; RAS, renin–angiotensin system; REL, *v-rel* avian reticuloendotheliosis viral oncogene homologue; SELE, selectin E; siRNA, small interfering RNA; STAT, signal transducer and activator of transcription; STZ, streptozotocin; TG, triacylglycerol; TGF-β, transforming growth factor-β; TLR, Toll-like receptor; TNF, tumour necrosis factor; TZD, thiazolidinedione; VCAM1, vascular cell adhesion molecule 1; VDR, vitamin D receptor.

Correspondence: Professor Hirofumi Makino (email junwada@md.okayama-u.ac.jp).

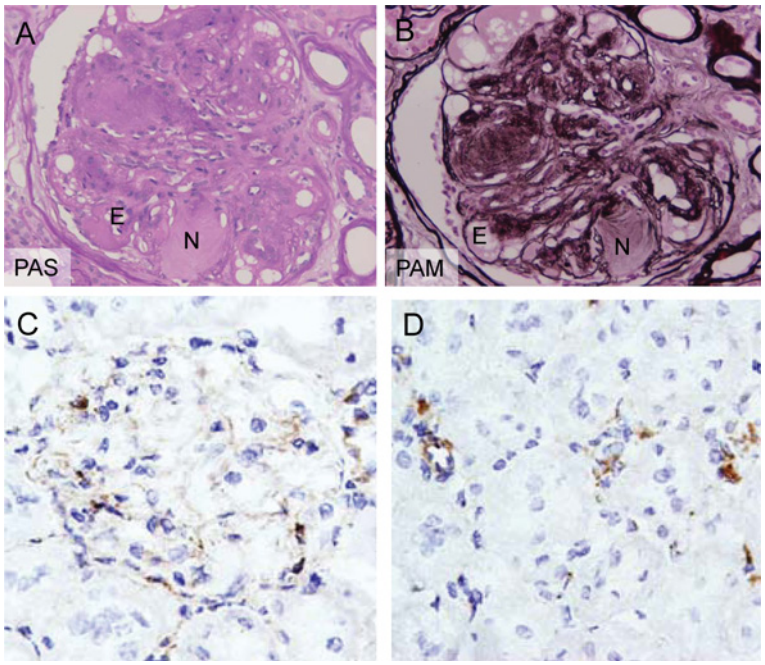

Figure 1 Pathological changes in diabetic nephropathy
(**A** and **B**) Renal biopsy specimens from patients with diabetic nephropathy stained with periodic acid/Schiff (PAS) (**A**) and periodic acid/methenamine silver stain (PAM) (**B**). E, exudative lesion; N, nodular lesion. (**C** and **D**) Immunostaining of ED1-positive macrophages in glomeruli (**C**) and interstitial area (**D**) obtained from rat models of diabetic nephropathy. This Figure was reproduced from Diabetes: a journal of the American Diabetes Association by American Diabetes Association; Stanford University © 2006 Reproduced with permission of AMERICAN DIABETES ASSOCIATION; [151].

Table 1 Inflammatory molecules in diabetic nephropathy

Category	Molecule
Transcription factors	NF-κB
Pro-inflammatory cytokines and signalling molecules	IL-6
	IL-18
	IL-1
	TNF
	JAK2 and STAT-1, -3 and -5
Chemokines	CCL2 (MCP-1) and CCR2
	CXCL12 (stromal-cell-derived factor-1)
	CX3CL1 (fractalkine) and CX3CR1
Adhesion molecules	Intercellular adhesion molecule 1 (ICAM1)
	Vascular cell adhesion protein 1 (VCAM1)
	E-selectin (SELE)
TLRs	TLR2
	TLR4
Adipokines	Adiponectin
	Leptin
Nuclear receptors	VDR
	NR1H4 (FXR)
	PPARα
	PPARγ
	PPARδ

OVERVIEW FOR THE PROGRESSION OF DIABETIC NEPHROPATHY

Diabetic nephropathy is well-characterized, with the accumulation of ECM (Figure 1), and the progression of diabetic nephropathy consists of three steps: (i) glomerular hypertrophy and hyperfiltration; (ii) inflammation of glomeruli and tubulointerstitial regions; and (iii) reduction of cell number by apoptosis and accumulation of ECM [6]. The natural history of diabetic nephropathy shows an analogy with the progression of pancreatic islet β-cell failure in Type 2 diabetes, i.e. hypertrophy of pancreatic islets, proliferation of β-cells associated with inflammatory responses, and subsequent loss of β-cells by apoptosis and fibrosis of the pancreatic islets (Figure 2) [7]. Diabetic nephropathy is a complex disease, since the functional impairment and structural remodelling of the kidney is tightly linked to the changes in specific cell types in the kidney. For example, hyperglycaemia induced phenotypic changes in mesangial cells expressing the embryonic form of myosin heavy chains (SMemb), which links to the subsequent enhanced production of ECM and glomerulosclerosis [8]. Hyperglycaemia induces cell cycle arrest and cellular hypertrophy of podocytes, and the expression of nephrin is reduced. As a result, the permselectivity of glomerular capillaries is impaired and this induces proteinuria and subsequent focal and global sclerosis. In addition to the resident cells of the kidney, the differential roles of macrophages have been demonstrated. The tissue remodelling in diabetic nephropathy reveals analogy with a wound healing response upon tissue damage. Pro-inflammatory

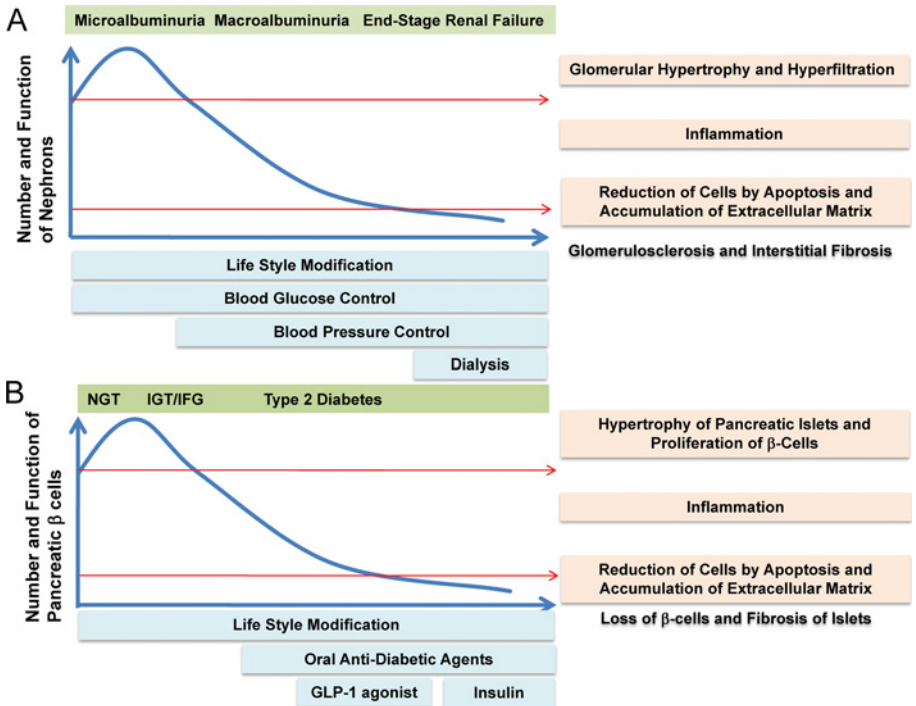

Figure 2 **Similarity of the natural history of Type 2 diabetes and diabetic nephropathy**
In both glomeruli and pancreatic islets, hypertrophy and proliferation was followed by the reduction in cell numbers due to inflammation and apoptosis associated with accumulation of the ECM, i.e. fibrosis. (**A**) Course of diabetic nephropathy. (**B**) Couse of Type 2 diabetes.

macrophages, such as M1 macrophages, exacerbate the renal cell damage, but there are anti-inflammatory M2 macrophages which promote epithelial and vascular repair [9].

In the last 20 years, the three major pathways in the development of diabetic nephropathy were identified. Upstream of the three pathways, hyperglycaemia is the major driving force of the progression to the end-stage renal diseases from diabetic nephropathy. Downstream of the three major pathways, microinflammation and subsequent ECM expansion is a common pathway for the progression of diabetic nephropathy (Figure 3). The first major pathway is abnormality of intracellular metabolism, such as the activation of polyol and PKC (protein kinase C) pathways. The amelioration of diabetic nephropathy in animal models was reported in STZ (streptozotocin)-induced diabetic rats treated with aldose reductase inhibitor [10] and db/db mice treated with PKCβ inhibitor [11]. These agents are at preclinical stages of development and their efficacy for the treatment of diabetic nephropathy will be demonstrated in the near future. The second pathway is the formation of AGEs (advanced glycation end-products) by hyperglycaemia, which induce dysfunction of glomerular cells and activation of macrophages [12,13]. The RAGE (receptor for AGE) pathway is critically involved in the transduction of the subsequent cell signalling related to inflammation and oxidative stress [14]. Several AGE-formation inhibitors, such as pyridoxamine [15], LR-90 [16] and KIOM-79 [17], were tested in animal models of diabetic nephropathy, suggesting that the AGE–RAGE system is a new therapeutic target for the treatment of diabetic nephropathy. In addition, oxidative stress also induces dysfunction of various glomerular cells and contributes to the progression of diabetic nephropathy. For the source of oxidative stress, NADPH oxidase and mitochondria were two major candidates. The application of NADPH oxidase inhibitors ameliorated the progression of the diabetic nephropathy of animal models [18,19]. The third pathway is intraglomerular hypertension induced by glomerular hyperfiltration. The amelioration of glomerular hyperfiltration by RAS (renin–angiotensin system) inhibitors reveals apparent beneficial effects on reduction of proteinuria and progression of diabetic nephropathy both in animal experiments and clinical studies [20,21].

These three major pathways induce glomerular endothelial cell injuries accompanied by expression of adhesion molecules and chemokines, which result in macrophage infiltration into renal tissues [4,5]. Prolonged microinflammation is the common pathway for progression of diabetic nephropathy, and anti-inflammatory agents would be beneficial for the amelioration of diabetic nephropathy [22,23]. TGF-β1 (transforming growth factor-β1) is a well-characterized key molecule for the accumulation of ECM glycoproteins, and treatment with monoclonal TGF-β1 antibody prevents renal insufficiency in db/db mice [24].

TRANSCRIPTION FACTORS

Several transcription factors such as USF (upstream stimulatory factor) 1 and 2, AP1 (activator protein 1), NF-κB (nuclear factor κB), CREB (cAMP-response-element-binding protein),

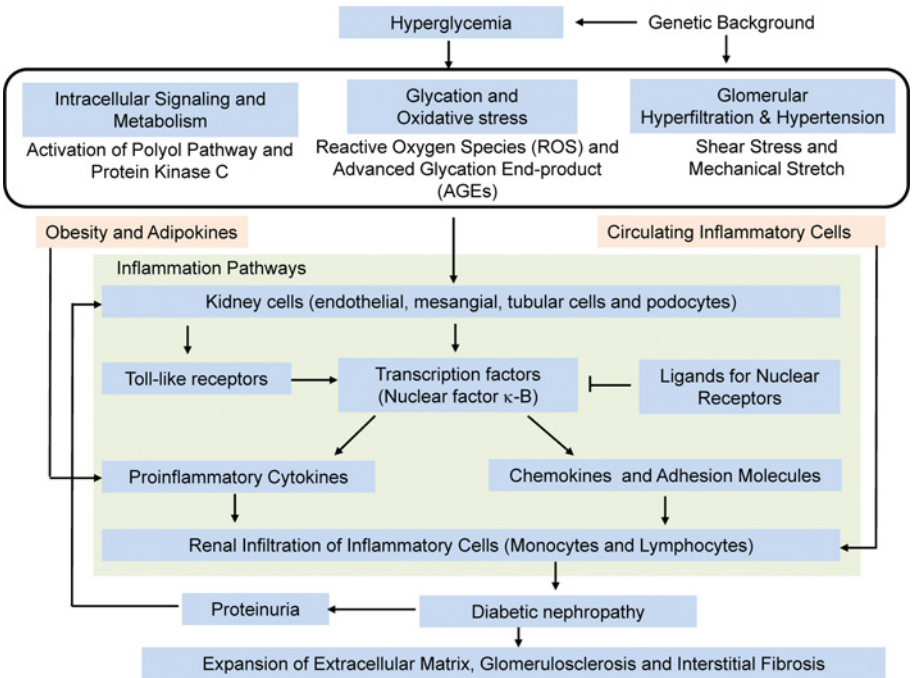

Figure 3 Inflammatory pathways in the pathogenesis of diabetic nephropathy

NFAT (nuclear factor of activated T-cells) and Sp1 (stimulating protein 1) were activated in hyperglycaemic environments. These transcription factors regulate the genes related to inflammation and ECM turnover [25]. Among the transcription factors, NF-κB is the most important in the pathogenesis of diabetic nephropathy. NF-κB is activated by a wide variety of stimuli such as cytokines, oxygen radicals, inhaled particles, ultraviolet irradiation and bacterial or viral products. In diabetic kidney disease, proteinuria itself is the important activator for NF-κB and is an important pro-inflammatory stimulus for tubular cells [26]. NF-κB1 or NF-κB2 is bound to REL (*v-rel* avian reticuloendotheliosis viral oncogene homologue), RELA and RELB to form the NF-κB complex and it is inhibited by IκB (inhibitory κB) proteins (NF-κBIA or NF-κBIB), which inactivate NF-κB by trapping it in the cytoplasm. Phosphorylation of serine residues on IκB proteins leads to destruction via the ubiquitination pathway, thereby allowing activation of the NF-κB complex. The activated NF-κB complex translocates into the nucleus and binds DNA at κB-binding motifs such as 5′-GGGRNNYYCC-3′ or 5′-HGGARNYYCC-3′ (where H is A, C or T; R is an A or G purine; and Y is a C or T pyrimidine). NF-κB is induced by high glucose and is activated in peripheral blood mononuclear cells [27,28] and also in kidney biopsy specimens [26]. NF-κB binds to the promoter regions of several genes that play a pivotal role in the pathogenesis of diabetic nephropathy, such as those encoding TGF-β1, AKR1B1 (aldo-keto reductase family 1, member B1), CCL2 [CC chemokine ligand 2; also known as MCP-1 (monocyte chemoattractant protein-1)] and ICAM1 (intercellular adhesion molecule 1) [27,29–32]. NF-κB is also integrated in various biological pathways that are functionally involved in the pathogenesis of diabetic nephropathy, such as PKCβ [33], RAS [34], AGE accumulation [35] and oxidative stress [36]. Suppression of NF-κB activation by various agents, such as thiazolidinediones [37], 1,25-dihydroxyvitamin D_3 [38], cilostazol [39] and curcumin [40], could lead to amelioration of diabetic nephropathy, suggesting the importance of NF-κB as a therapeutic target of diabetic nephropathy.

PRO-INFLAMMATORY CYTOKINES

Several pro-inflammatory cytokines are involved in the pathogenesis of various diseases such as rheumatic diseases and metabolic disorders such as insulin resistance in Type 2 diabetes and obesity. In addition, their potential as therapeutic targets is now at clinical stages and many antibodies or decoy receptors are clinically used in the therapy of various autoimmune diseases. In diabetic nephropathy, inflammatory cytokines such as IL (interleukin)-1, IL-6, IL-18 and TNF (tumour necrosis factor) are critically involved in pathogenesis [5]. IL-1A and IL-1B are structurally distinct forms of IL-1 and they are synthesized by a variety of cell types, including macrophages, B-cells and fibroblasts, and are potent mediators of inflammation and immunity. Increased expression of IL-1 in animal models of diabetes have been demonstrated [32,41], and they are associated with increased expression of ICAM1, VCAM1 (vascular cell adhesion molecule 1) and SELE (selectin E; also known as E-selectin). IL-1RN (IL-1 receptor antagonist) is a protein that binds to IL-1 receptors and inhibits the binding of IL-1A and IL-1B. As a consequence, the biological activities of these cytokines are neutralized in physiological and pathophysiological inflammatory responses. Tarlow et al. [42] showed that the polymorphism in intron 2 of the *IL1RN* gene is caused by a variable copy number of an 86-bp sequence. *IL1RN* has 5 alleles, comprising between two and six repeats

of the 86-bp sequence. Interestingly, significant associations between two-repeat alleles (IL1RN∗2) and diabetic nephropathy were found [43].

IL-6 is another important immunoregulatory cytokine that activates cell surface signalling assembly involving IL-6, IL-6RA (IL-6 receptor α) and the shared signalling receptor gp130 [IL-6ST (IL-6 signalling transducer)]. *IL6* mRNA was first demonstrated in glomeruli and the interstitium in renal biopsy specimens derived from patients with diabetic nephropathy [44] and elevation of IL-6 was demonstrated in serum and urine [45–47]. Although serum and urinary IL-6 is a candidate marker for diabetic nephropathy [45], IL-6 is tightly correlated with body fat mass and insulin resistance both with and without diabetic nephropathy [48]. Concomitant measurement of kidney tubular markers and urinary excretion of cytokines are more relevant to depict a potential role in the kidney, which directly reflects intrarenal inflammation in diabetic nephropathy [49].

TNF is a multifunctional pro-inflammatory cytokine secreted predominantly from monocytes and macrophages that is functional in lipid metabolism, coagulation, insulin resistance and endothelial biology. TNF plays a pivotal role in the pathogenesis of rheumatoid arthritis, and the inhibition of TNF by anti-TNF therapy suppresses pro-inflammatory cytokine production by $CD4^+CD25^-$ T-cells with significant therapeutic effects. In addition, TNF is also produced in adipocytes and macrophages and it is closely related to inflammation and insulin resistance in obesity. TNF expression was increased both in urine and renal tissues [50], and the inhibition of TNF by infliximab reduced the excretion of albuminuria in the STZ-induced rat diabetic model [51]. In humans, the elevation of serum TNF levels and structural changes in kidney tissues have been demonstrated [52–54]. However, it is still uncertain whether clinically available anti-TNF therapies exert significant efficacy to prevent the progression of diabetic nephropathy.

IL-18 was originally cloned as an IFNγ (interferon γ)-inducing factor that augments natural killer cell activity in spleen cells. IFNγ induced the expression of IL-18 and other cytokines, such as TNF and IL-1β, and increased the expression of adhesion molecules, such as VCAM1. Elevated urinary excretion levels of IL-18 have been reported in patients with diabetic nephropathy and they are closely related to the progression of diabetic nephropathy [55–61].

Although macrophage is recognized as a major player in the progression of diabetic nephropathy, Th1, Th2 and Th17 cells are also involved in the tissue damage that occurs in diabetic nephropathy [62]. These subsets of T-cells secrete a specific combination of cytokines and they may be involved in the inflammation of kidney tissues during the progression of diabetic nephropathy. Type 1 diabetes is a prototypic Th1 disease, and the Th1 response is enhanced. Thus it is possible that Th1 cells are involved in the progression of kidney disease in patients with Type 1 diabetes. Elevated levels of ICAM1 and SELP (selectin P) and increased levels of IFNγ were closely related to the infiltration of Th1 cells in the glomeruli [63]. However, in Type 2 diabetes, little is known about the mechanism of Th1 activation, although increased serum levels of IFNγ and IL-2R (IL-2 receptor) have been reported in Type 2 diabetes [64]. Th2 cells, producing IL-4 and IL-10, can contribute to suppress Th1 cell activation. IL-10 exerts anti-inflammatory and immunosuppressive effects, and a low production capacity of IL-10 was associated with metabolic syndrome and Type 2 diabetes [65]. In contrast, elevated IL-10 levels were observed in the sera of the patients with diabetic nephropathy, and a positive correlation of IL-10 and albuminuria was found, suggesting a possible pathogenic role of IL-10 in the pathogenesis of diabetic nephropathy [66]. Th17 is the third distinct subset of helper T-cells and is critically involved in the pathogenesis of autoimmune diseases such as rheumatoid arthritis. Studies have suggested that Th17 cells in human Type 1 [67,68] and Type 2 [69] diabetes promote inflammation through elevated IFNγ and IL-17A. The roles of subsets of helper T-cells and secreted cytokines in diabetic nephropathy need to be characterized further in future investigations.

Finally, the JAK2 (Janus kinase 2) and STAT (signal transducer and activator of transcription)-1, -3 and -5 pathway is enhanced by various stimuli, such as high glucose concentration, AGEs and angiotensin II, and various chemokines, growth factors and ECM proteins are STAT-dependent genes and are closely related to cell proliferation [70,71]. The inhibition of JAK/STAT pathways by AG-490, a specific JAK2 inhibitor [72], statins [73,74] and SOCS1 (suppressor of cytokine signalling 1) ameliorated the progression of diabetic neuropathy. These studies suggested that JAK2 inhibitors or SOCS1 enhancers are beneficial in the treatment of diabetic nephropathy by improving inflammatory responses by suppressing CCL2 and TGF-β.

CHEMOTAXIS AND CHEMOKINES

The accumulation of macrophages in various tissues is a characteristic feature of both Type 2 diabetes and diabetic nephropathy. The infiltration of macrophages plays a central role in the progression of both atherosclerosis and diabetic nephropathy through various mechanisms including the production of ROS (reactive oxygen species), cytokines and various proteases. In the process of chemotaxis, chemokines and their receptors play key roles in the migration of immune cells into the kidney tissues. CCL2 is a member of the small inducible gene family and plays a role in the recruitment of monocytes to the site of tissue injury and inflammation. In mouse mesangial cell culture, high-glucose-induced ECM protein expression and TGF-β levels were significantly attenuated by mutant CCL2, chemokine, CCR2 (CC chemokine receptor 2) siRNA (small interfering RNA) and the CCR2 inhibitor RS102895 [75]. Blocking the CCL2/CCR2 pathway in diabetic mice ameliorated glomerulosclerosis, indicating that the CCL2/CCR2 pathway plays a crucial role in the progression of diabetic nephropathy [76]. It has been reported that specific antagonists, such as small molecules [77–80] and an RNA aptamer [81], which disrupt the CCL2/CCR2 pathway, improve diabetic nephropathy in db/db mice. Furthermore, in human urinary samples, CCL2 levels are elevated and they are prognostic factors to predict the progression of diabetic nephropathy [82–85]. CCL2 may be the therapeutic target for diabetic nephropathy, since the inhibition of RAS [86,87] and anti-inflammatory therapy using clarithromycin reduced the production of CCL2 [88].

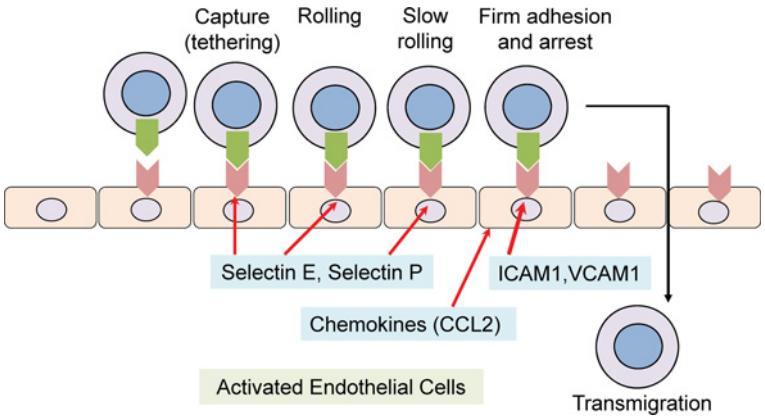

Figure 4 Leucocyte infiltration from blood vessels into tissues

CXCL (CXC chemokine ligand) 12, also known as stromal-cell-derived factor-1) is a homoeostatic chemokine with multiple functions including cell homing, tumour metastasis, angiogenesis and tissue regeneration after acute injuries. CXCL12 is produced by podocytes, contributing to podocyte loss, and specific inhibitors ameliorated proteinuria and glomerulosclerosis in db/db mice [89]. In addition, dual blockade of both CCL2 and CXCL12 by specific RNA aptamers exerted additive effects on the progression of diabetic kidney disease and maintained the highest rates of glomerular filtration in db/db mice [90].

CX3CL1 (CX3C chemokine ligand 1; also known as fractalkine) exists in two forms as a membrane-anchored or as a shed 95-kDa glycoprotein. The soluble CX3CL1 has potent chemoattractant activity for T-cells and monocytes and induces adhesion between activated endothelial cells, which express its receptor CX3CR1 (CX3C chemokine receptor 1). Up-regulation of CX3CL1 and CX3CR1 was reported in the glomeruli of STZ-induced diabetic rat kidneys [91], and AGEs induced the up-regulation of mRNA expression of CX3CL1 [92]; however, data for human diabetic nephropathy are rather scarce.

ADHESION MOLECULES

The infiltration of immune cells into tissues consists of several steps: tethering, rolling, firm adhesion and arrest, and transmigration from the vasculatures (Figure 4). Various adhesion molecules are involved in the process and they initiate the immune responses in the local tissues. ICAM1 is a ligand for the integrin LFA-1 (lymphocyte function-associated antigen 1) and is induced by the activation of NF-κB and by pro-inflammatory cytokines, such as IL-1, IL-6 and TNF, which promote the adhesion of leucocytes to vascular endothelial cells. Up-regulated expression of ICAM1 has been reported in various animal models, such as Wistar fatty rats [93] and STZ-induced diabetic rats [10]. The up-regulation of ICAM1 is mediated by the activation of the PKC/NF-κB pathway in rat mesangial cells [32]. In the patients with diabetic nephropathy, soluble forms of VCAM1 and ICAM are elevated during the progression of diabetic nephropathy from microalbuminuria to overt nephropathy [94]. The urinary excretion of CCL2 and ICAM1 was significantly reduced by the treatment with intensive insulin therapy in incipient diabetic nephropathy [95]. ICAM1-deficient B6 mice are resistant to the development of diabetic nephropathy in STZ-induced diabetic models [96], and furthermore, in *Lepr*$^{db/db}$ mice, the deficiency of ICAM1 is protective against nephropathy [97]. In both animal models, the infiltration of intraglomerular macrophages was significantly reduced by ICAM1 deficiency associated with the reduction of albuminuria. In addition to ICAM1, the roles of VCAM1 and SELE were also investigated, since they are also involved in the adhesion process of leucocytes and endothelial cells. Although VCAM1 [98] and SELE [99] were elevated in the glomeruli of animal diabetes models and soluble forms in the serum increased [100–102], there is no direct evidence that the blockade of VCAM1 and SELE by antibodies or deletion of the genes ameliorates the progression of diabetic nephropathy in animal models.

INNATE IMMUNITY AND TLRs (TOLL-LIKE RECEPTORS)

In *Drosophila*, the Toll transmembrane receptor plays a central role in the signalling pathways that control dorsal–ventral axis formation and the innate non-specific immune response. In mammalians, TLRs recognize various pathogens through PAMPs (pathogen-associated molecular patterns) consisting of proteins, carbohydrates, lipids and nucleic acids and initiate the immune response. Upon tissue damage and inflammation, various DAMPs (damage-associated molecular patterns), such as heat-shock proteins, fatty acids, modified LDLs (low-density lipoproteins), HMGB1 (high-mobility group box 1) and AGEs, are released and these endogenous ligands are recognized by TLR2 and TLR4 (Figure 5). TLRs have been implicated in diabetes-induced inflammation and vascular complications [103]. TLR2 was up-regulated in the glomeruli in a rat model of diabetes, and it is associated with the up-regulation of molecules in inflammatory pathways, such as MyD88 (myeloid differentiation factor 88), NF-κB and CCL2 [104]. In TLR2-knockout mice with STZ-induced diabetes, MyD88-dependent signalling, expression of TGF-β, infiltration of M1-dominant macrophages, podocyte loss

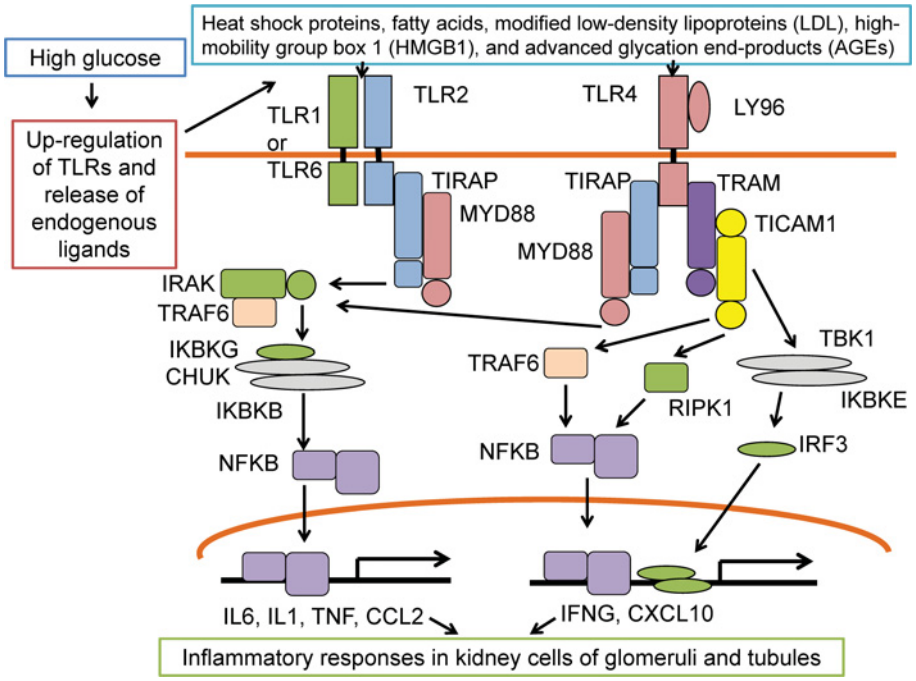

Figure 5 **Schematic diagram of TLR2 and TLR4 signalling in diabetic nephropathy**

CHUK, conserved helix–loop–helix ubiquitous kinase; IFNG, IFNγ; IKBKB, inhibitor of κ light polypeptide gene enhancer in B-cells, kinase β; IKBKE, inhibitor of κ light polypeptide gene enhancer in B-cells, kinase ε; IKBKG, inhibitor of κ light polypeptide gene enhancer in B-cells, kinase γ; IRAK, IL-1-receptor-associated kinase; IRF3, interferon regulatory factor 3; LY96, lymphocyte antigen 96; RIPK1, receptor [TNFRSF (TNF receptor superfamily)]-interacting serine/threonine kinase 1; TBK1, TANK [TRAF (tumour-necrosis-factor-receptor-associated factor)-associated NF-κB activator]-binding kinase 1; TICAM1, TLR adaptor molecule 1; TIRAP, TIR (Toll/IL-1 receptor)-domain-containing adaptor protein; TRAF6, TNF-receptor-associated factor 6; TRAM1, translocation-associated membrane protein 1.

and albuminuria were significantly ameliorated compared with the diabetic wild-type mice [105]. In contrast with TLR2, TLR4 has been implicated in the process of tubulointerstitial inflammation [106]. TLR4, but not TLR2, is up-regulated in the renal tubules of human kidneys with diabetic nephropathy, and the up-regulation of TLR4 is associated with the endogenous TLR4 ligand HMGB1 in diabetic nephropathy. Silencing of TLR4 with siRNA or anti-TLR4 antibodies attenuated high-glucose-induced IκB/NF-κB activation, inhibited the downstream synthesis of IL-6 and CCL2, and impaired the ability of conditioned medium from high-glucose-treated proximal tubule cells to induce transmigration of mononuclear cells [106].

ADIPOKINES

Adipocytes secret various factors, adipokines, and they are dysregulated in obesity and Type 2 diabetes. Various adipokines such as adiponectin, leptin, resistin, visfatin, chemerin and vaspin have been identified and they may link the metabolic abnormalities and microinflammation in Type 2 diabetes. In addition, their roles in the pathogenesis of diabetic nephropathy are now under investigation [4]. ADPOQ (adiponectin) is a hormone secreted by adipocytes and has anti-inflammatory effects. Adiponectin suppressed the activation of NF-κB, TNF-induced monocyte adhesion to aortic endothelial cells and the expression of ICAM1, VCAM1 and SELE. Serum concentrations of adiponectin were significantly lower in patients with metabolic syndrome, obesity, Type 2 diabetes and coronary artery disease. In addition, the identification of ADIPORs (adiponectin receptors) 1 and 2 and intracellular signalling pathways including AMPK (AMP-activated protein kinase) and cAMP/PKA (cAMP-dependent protein kinase) have been implicated in the function of endothelial cells and inflammatory cells. Adiponectin is also implicated in the biology of podocytes and adiponectin-knockout ($Ad^{-/-}$) mice exhibited increased albuminuria and fusion of podocyte foot processes, and infusion of adiponectin increased the activity of AMPK, reduced oxidative stress and reversed the albuminuria. However, the beneficial roles of adiponectin may not be completely applied to the entire course of the natural history of diabetic nephropathy. In early diabetic nephropathy, ultrasonographic FMD (flow-mediated dilation) and adiponectin were significantly lower compared with control subjects [107]. However, in overt diabetic nephropathy with macroalbuminuria or renal insufficiency, serum adiponectin levels increased [108–111] and positively correlated with insulin resistance [109] and negatively correlated with FMD [108]. In addition, the elevation of adiponectin predicts all-cause mortality and end-stage renal disease in patients with Type 1 diabetes and overt diabetic nephropathy [112]. These data suggest that there are differential roles of adiponectin in the various stages of diabetic nephropathy.

Leptin is a 16-kDa protein that plays a critical role in the regulation of body mass by inhibiting food intake and

stimulating energy expenditure. Defects in leptin production cause severe hereditary obesity in rodents and humans. In contrast with adiponectin, leptin exerts several pro-inflammatory effects, such as impairment of endothelial cell functions, by stimulating inflammatory signalling pathways. Patients with generalized lipodystrophy show an association with proteinuria, and renal biopsy revealed focal glomerulosclerosis and membranoproliferative glomerulonephritis, and therapy with recombinant leptin demonstrated a reduction in proteinuria and hyperfiltration [113]. In addition, a genetic model of lipoatrophic diabetes (A-ZIP/F-1 mice) demonstrates a similar histological appearance to that seen in diabetic nephropathy, and leptin completely prevents the development of hyperglycaemia and nephropathy [114], suggesting physiological roles of leptin in the kidney. In contrast, infusion of leptin for 3 weeks into normal rats fosters the development of glomerulosclerosis and proteinuria. In addition, transgenic mice with leptin overexpression demonstrated an increase in collagen type IV and fibronectin mRNA in the kidney [115]. Thus overexpression of leptin may impede the progression of diabetic nephropathy. In human settings, several reports demonstrated that serum leptin levels correlated with proteinuria in Type 2 diabetes [116–119]; however, others reported no association of serum leptin levels and the presence of diabetic nephropathy [120–123]. Taken together, leptin is required in the physiology of the kidney and also impairs the development of diabetic nephropathy by promoting inflammatory responses in kidney tissue.

NUCLEAR RECEPTORS

Nuclear hormone receptors and adopted orphan receptors are molecular targets for Type 2 diabetes and dyslipidemia, since nuclear receptor modulators demonstrate benefits in lipid and glucose metabolism. In addition to metabolism, nuclear receptors seem to be negative regulators of inflammation, oxidative stress and fibrosis and they are also good therapeutic targets for the treatment of diabetic nephropathy [124]. VDR (vitamin D receptor) is an intracellular hormone receptor that specifically binds to 1,25(OH)2D3 (1-α,25-dihydroxyvitamin D_3) and mediates its effects. Vitamin D is known to exert anti-inflammatory effects by acting on macrophages and mesangial cells by suppressing high-glucose-induced up-regulation of NF-κB and CCL2 [38,125,126]. In addition, vitamin D negatively regulates RAS, which plays a critical role in the development of diabetic nephropathy. A renoprotective role of the VDR was demonstrated in VDR-knockout mice with STZ-induced diabetes, which developed severe albuminuria and glomerulosclerosis [127]. 1,25(OH)2D3 suppressed high-glucose-induced activation of RAS and TGF-β in mesangial cells [127]. The combination of the vitamin D analogue and RAS inhibitors synergistically ameliorated diabetic nephropathy in animal models of this condition [128-130]. Beyond RAS inhibitors, vitamin D analogues with anti-inflammatory and optimal reno-protective actions will be screened and tested in both experimental and clinical settings [131,132].

NR1H4 [nuclear receptor subfamily 1, group H, member 4; also known as FXR (farnesoid X receptor)] is an adopted orphan receptor and is highly expressed in the liver, intestine, adrenal gland and kidney. The primary bile acids are the highest-affinity endogenous ligands for FXR and play a critical role in the regulation of bile acid, TG [triacylglycerol (triglyceride)] and cholesterol homoeostasis. The FXR agonist GW4064 decreased glomerulosclerosis, tubulointerstitial fibrosis and proteinuria in C57BL/6J mice subjected to a high-fat diet. In renal mesangial cells, overexpression of FXR or treatment with GW4064 also inhibited SREBP-1c (sterol-regulatory-element-binding protein-1c) and other lipogenic genes, TGF-β and IL-6, suggesting a direct role of FXR in modulating renal lipid metabolism and modulation of fibrosis and inflammation [133]. In STZ-induced diabetes models, accelerated renal injury was observed in FXR-knockout mice. In contrast, treatment with the FXR agonist INT-747 improved renal injury by decreasing proteinuria, glomerulosclerosis and tubulointerstitial fibrosis, and modulating renal lipid metabolism, macrophage infiltration and renal expression of SREBPs, profibrotic growth factors and oxidative stress enzymes in the diabetic DBA/2J strain [134].

PPAR (peroxisome-proliferator-activated receptor) α is expressed in the proximal tubules and in the medullary thick ascending limb of the loop of Henle. Renal PPARα has an important role in modulating energy utilization in the kidney through the regulation of fatty acid β-oxidation [124]. In PPARα-deficient mice with STZ-induced diabetes, albuminuria and glomerulosclerosis were reduced in association with increased levels of serum free fatty acids and TGs. The number of leucocytes adherent to cultured mesangial cells derived from PPARα-deficient mice was increased and the adherence of leucocytes was inhibited by the PPARα agonist fenofibrate [135]. In db/db mice, fenofibrate treatment significantly reduced urinary albumin excretion accompanied by dramatically reduced glomerular hypertrophy and mesangial matrix expansion [136]. The fibrates reduced renal lipotoxicity, inflammation [137–139], fibrosis and oxidative stress, and subsequently prevented the symptoms of diabetic nephropathy. Actually, in the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study, fenofibrate administration was associated with a significant reduction in the rate of progression of albuminuria in patients with Type 2 diabetes [140]. However, it should be noted that fenofibrate has been shown to cause renal dysfunction in established renal disorders [141].

As members of the nuclear receptor superfamily, PPARs act by controlling networks of target genes. PPARs can be activated by both dietary fatty acids and their metabolic derivatives in the body, and thus serve as lipid sensors that, when activated, can markedly redirect metabolism. PPARγ is predominantly expressed in adipose tissues and is involved in adipocyte differentiation. PPARγ is also expressed in kidney tissues, such as mesangial cells and tubular cells, including IMCD (inner medullary collecting duct) and renal medullary interstitial cells [142–144]. In numerous animal models of Type 1 and Type 2 diabetes, TZDs (thiazolidinediones) ameliorated the albuminuria by relieving oxidative stress [145] and inflammatory responses, such as high-glucose-induced up-regulation of CCL2 [146], ICAM1 [147],

TGF-β [148,149] and NF-κB [37,150]. Besides blood-glucose-lowering and anti-hypertensive effects, TZDs may directly protect the kidneys from the progression of diabetic nephropathy by acting on multiple molecular targets [151]. In clinical studies, The TDZ drug pioglitazone reduced albuminuria and CCL2 urinary excretion in patients with Type 2 diabetes [152,153]. Fibrates have the potential to reduce body mass and may compensate for the body mass gain induced by TZDs, thus future clinical studies are required to determine whether concomitant use of PPARα and PPARγ agonists or dual agonists demonstrate synergistic clinical benefits with fewer side effects [154,155].

PPARδ is abundantly expressed throughout the body, but at only low levels in the liver. PPARδ is a powerful regulator of fatty acid catabolism and energy homoeostasis, with a broad role in fat burning. Activation of PPARδ has been shown to improve insulin resistance, adiposity and plasma HDL (high-density lipoprotein) levels. GW0742, a PPARδ agonist, decreased urinary albumin excretion without altering blood glucose levels in STZ-induced diabetic rats. Macrophage infiltration, mesangial matrix accumulation and type IV collagen deposition were substantially attenuated by GW0742. *In vitro* studies demonstrated that PPARδ activation increased the expression of the anti-inflammatory co-repressor B-cell lymphoma-6, which subsequently suppressed CCL2 and osteopontin expression [156].

CONCLUSIONS

Several lines of evidence have convinced us that inflammation-related molecules and pathways are critically involved in the progression of diabetic nephropathy [4,5]. These molecules and pathways are candidates for the development of novel treatment modalities for diabetic nephropathy. In the case of inflammation observed in rheumatoid arthritis, TNF and IL-6 are located at the top of the hierarchies of cytokine cascade, and anti-TNF and anti-IL-6 therapies reveal potent therapeutic efficacy in the treatment of rheumatoid arthritis. However, it is still unknown whether such hierarchies exist in the pathobiology of diabetic nephropathy. In addition, it still remains elusive whether the long-term suppression of subclinical and mild inflammation has significant benefits over glycaemic control by various oral anti-diabetics and insulin in the progression of diabetic nephropathy. Advances in the basic science and clinical investigations should provide an answer to these questions and we expect the advent of effective treatment modalities for the prevention of diabetic nephropathy.

FUNDING

This work was supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan through a Grant-in-Aid for Scientific Research (B) [grant number 23390241], Challenging Exploratory Research [grant number 23659470], and a Grant-in-Aid for Scientific Research on Innovative Areas ('Molecular Basis and Disorders of Control of Appetite and Fat Accumulation') [grant number 23126516] (all to J.W.), and a Grant-in-Aid for Scientific Research (A) [grant number 21249053] (to H.M.).

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Received 23 April 2012/31 July 2012; accepted 10 August 2012
Published on the Internet 2 October 2012, doi: 10.1042/CS20120198

