Microinflammation in the pathogenesis of diabetic nephropathy

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ABSTRACT

Diabetic nephropathy is the leading cause of end-stage renal failure in developed countries. Furthermore, diabetic nephropathy is related to the risk of cardiovascular diseases and an increase in mortality of diabetic patients. Several factors are involved in the development of nephropathy, including glomerular hyperfiltration, oxidative stress, accumulation of advanced glycation end-products, activation of protein kinase C, acceleration of the polyol pathway and over-expression of transforming growth factor-β. Recently, accumulated data have emphasized the critical roles of chronic low-grade inflammation, 'microinflammation', in the pathogenesis of diabetic nephropathy, suggesting that microinflammation is a common mechanism in the development of diabetic vascular complications. Expression of cell adhesion molecules, chemokines and pro-inflammatory cytokines are increased in the renal tissues of diabetic patients and animals. Deficiency of pro-inflammatory molecules results in amelioration of renal injuries after induction of diabetes in mice. Plasma and urinary levels of cytokines, chemokines and cell adhesion molecules, are elevated and correlated with albuminuria. Several kinds of drugs that have anti-inflammatory actions as their pleiotropic effects showed renoprotective effects on diabetic animals. Modulation of the inflammatory process prevents renal insufficiency in diabetic animal models, suggesting that microinflammation is one of the promising therapeutic targets for diabetic nephropathy, as well as for cardiovascular diseases. (J Diabetes Invest, doi: 10.1111/jdi.12050, 2013)

KEY WORDS: Diabetic nephropathy, Inflammation, Microinflammation

INTRODUCTION

Diabetic nephropathy (DN) is the leading cause of end-stage renal failure in developed countries. Moreover, DN is related to the risk of cardiovascular diseases and an increase in mortality of diabetic patients. However, the established therapeutic strategies based on strict control of blood glucose level and blood pressure, and blockade of the renin–angiotensin system cannot prevent the progression of DN completely.

Several factors are involved in the development of DN, including genetic factors, glomerular hyperfiltration[1], oxidative stress[2], accumulation of advanced glycation end-products (AGEs)[3], acceleration of the polyol pathway, activation of protein kinase C[4], overexpression of transforming growth factor-β (TGF β), followed by increase of extracellular matrices[5]. Recently, accumulated data have emphasized the critical role of the inflammatory process in the pathogenesis of DN. Many kinds of pro-inflammatory molecules, including adhesion molecules, chemokines and cytokines, have been known to play roles in the development of diabetic nephropathy[6]. These pro-inflammatory molecules might be new therapeutic targets for DN, as well as for other inflammatory diseases.

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The present review will focus on the role of microinflammation in the pathogenesis of DN as a common pathway of development of diabetic vascular complications.

MICROINFLAMMATION

It is well known that the inflammatory process is involved in the pathogenesis of atherosclerosis[7]. Activated macrophages play critical roles for the migration and proliferation of smooth muscle cells in the intima, and the rupture of plaque resulting in an acute coronary event. Inflammatory cells mainly composed of macrophages are also seen in the glomeruli and interstitium of patients with DN, suggesting that the inflammatory process is also involved in the development of DN[8,9]. Inflammation is characterized by infiltration of inflammatory cells, increased expression of adhesion molecules, chemokines and pro-inflammatory cytokines, and elevation of serum C-reactive protein (CRP) level. These features are also seen in DN and atherosclerosis although they are quite mild as compared with classic inflammatory diseases, such as rheumatoid arthritis. Therefore, the low-grade inflammation that occurs in atherosclerosis and DN is termed 'microinflammation' to distinguish it from classic inflammation.

ADHESION MOLECULES

Infiltration of leukocytes into inflammatory lesions is mediated by adhesion to endothelial cells and transmigration from vascular lumen to inflammatory sites. Adhesion molecules are

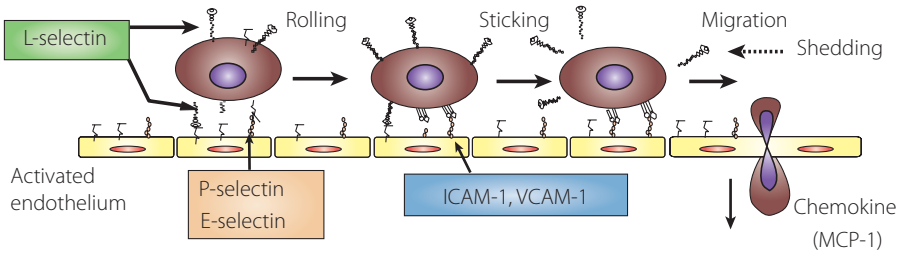

Figure 1 | Mechanism of macrophage infiltration into the inflammatory lesion. Leukocyte infiltration is mediated by the adhesion molecules and chemokines. Selectin molecules mediate the leukocyte rolling on the vascular endothelial cells. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) promote firm attachment of leukocytes and endothelial cells. Monocyte chemoattractant protein-1 (MCP-1) induces the migration of leukocytes from vascular lumen into subendothelium.

expressed on the cell surface, and mediate cell–cell binding and cell-matrix attachment. Leukocyte adhesion to vascular endothelial cells is promoted by adhesion molecules expressed on leukocytes and endothelial cells (Figure 1). Selectin molecules mediate the leukocyte rolling along with endothelial cells at the first step of leukocyte infiltration into inflammatory lesions. At the second step, tight adhesion of leukocytes to the endothelium is mediated by intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)[10].

ICAM-1 AND VCAM-1

ICAM-1 is an adhesion molecule of the immunoglobulin-superfamily and binds to β2 integrins, such as lymphocyte function-associated antigen-1 (LFA-1) and macrophage-1 antigen (Mac-1). There are several studies that have shown the increased expression of adhesion molecules in patients with diabetic nephropathy. Upregulation of ICAM-1 occurs in response to several kinds of stimuli. including pro-inflammatory cytokines[10,11], shear stress[12], oxidative stress, protein kinase C activation and AGEs[13].

We have shown that ICAM-1 is upregulated in the glomeruli and intersitium of diabetic kidney[14]. Increased expression of ICAM-1 has been shown in several models of DN[15] (Figure 2). Furthermore, we showed that the blockade of macrophage infiltration using anti-ICAM-1 antibody ameliorated renal injury and infiltration of macrophage in the glomeruli in streptozotocin-induced diabetic rats[14]. Furthermore, urinary albumin excretion (UAE), renal tissue injuries and inflammation are prevented in ICAM-1 knockout (KO) mice after induction of diabetes by streptozotocin[16]. Interestingly, UAE was not changed between ICAM-1 KO mice and wild-type mice at 4 weeks after induction of diabetes, but significantly decreased in ICAM-1 KO mice rather than in wild-type mice at 12 and 24 weeks. Similar findings are noted in ICAM-1 deficient *db*/*db* mice[17]. Plasma levels of ICAM-1 are increased in patients with DN[18]. Interestingly, Lin *et al.*[19] reported that an elevated baseline plasma level of ICAM-1 is associated with a increasing rate of UAE and the onset of microalbuminuria in the patients with type 1 diabetes who participated in the Diabetes Control and Complications Trial. These findings suggest that the inflammatory axis of ICAM-1 activation to macrophage infiltration plays a pivotal role in the development of diabetic nephropathy (Figure 3).

VCAM-1 is also expressed on endothelial cells, and promotes the adhesion between leukocytes and endothelial cells. VCAM-1 is shown to be increased on endothelial cells and infiltrating cells in the renal interstitium in the diabetic animal model[20]. Circulating VCAM-1 level is increased and is correlated with albuminuria in patients with type 2 diabetes[21]. In addition, it has been shown that high plasma concentrations of soluble VCAM-1is a risk factor for death[22].

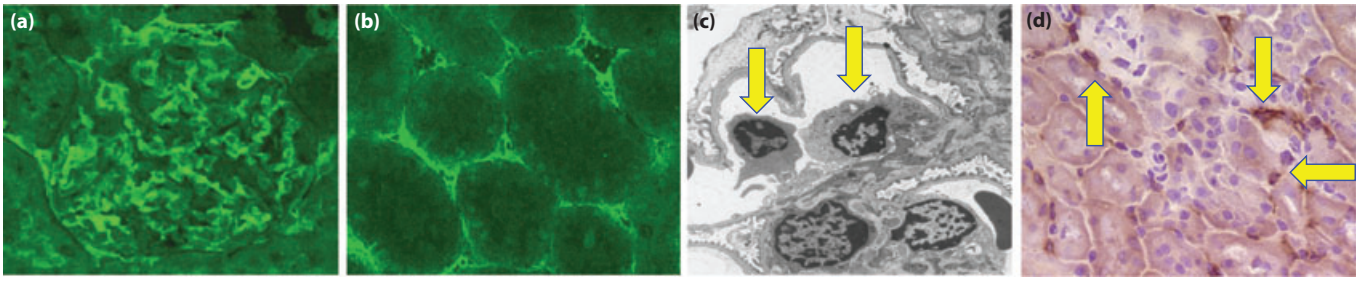

Figure 2 | Expression of intercellular adhesion molecule-1 (ICAM-1) in the (a) glomerulus and (b) interstitium. ICAM-1 is expressed along endothelial cells in the glomerulus and interstitium. Macrophages are infiltrated in the (c) glomerulus and (d) interstitium.

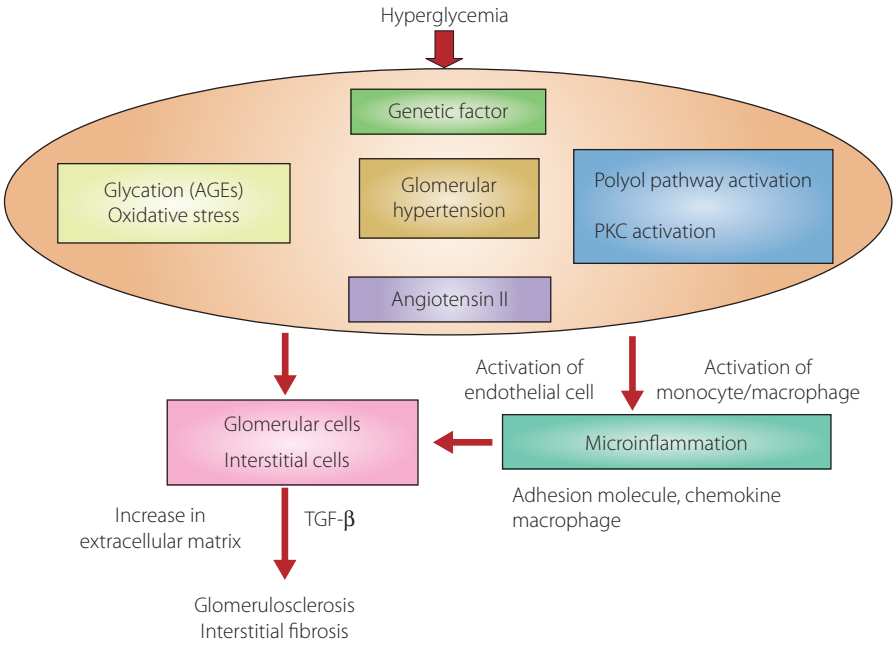

Figure 3 | Pathogenesis of diabetic nephropathy. AGEs, advanced glycation end-products; PKC, protein kinase C; TGF-β, transforming growth factor-β.

Selectins and Selectin Ligands

The selectin family is composed of L-, E- and P-selectin, which promote leukocyte rolling along with vascular endothelial cells in the inflammatory sites[23]. E-selectin is expressed on activated endothelial cells and mediates leukocyte rolling on endothelial cells. Expression of E-selectin is induced by pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α). Expression of E-selectin is upregulated in the peritubular capillaries and is correlated with the number of infiltrating macrophages in the interstitium of patients with diabetic nephropathy[24]. It was also reported that plasma levels of E-selectin are positively correlated with albuminuria and cardiovascular disease in patients with type 1 diabetes[25]. L-selectin is constitutively expressed on leukocytes, and interacts with its ligands distributed on endothelial cells. We previously reported that sulfatide is a major L-selectin-binding molecule in the kidney, and that the interaction between L-selectin and sulfatide plays a critical role in monocyte infiltration into the kidney interstitium; however, it is unknown whether this binding pathway is involved in pathogenesis of DN[26].

Macrophage Scavenger Receptor-A

Macrophage scavenger receptor-A (SR-A) is a multifunctional receptor expressed on macrophages. A number of studies have established the important roles of SR-A in the pathogenesis of atherosclerosis. SR-A is involved in foam cell formation, activation of macrophages, and adhesion of macrophages to atherosclerotic lesions[27,28]. We induced diabetes in SR-A KO and wild-type mice by streptozotocin and found that UAE, and renal tissue injuries, were markedly diminished in diabetic SR-A KO mice[29]. Interestingly, macrophage infiltration and gene expression of pro-inflammatory molecules in the kidneys was dramatically decreased in diabetic SR-A KO mice compared with diabetic wild-type mice. Furthermore, anti-SR-A antibody blocked the attachment of monocytes to type IV collagen substratum, but not to endothelial cells, showing that SR-A promotes macrophage migration into diabetic kidneys by enhancement of the attachment to renal extracellular matrices.

CHEMOKINES

Interaction of chemokines and their receptors promotes the recruitment of inflammatory cells into inflammatory sites. Recent studies showed that several kinds of chemokines, including C-C motif chemokine 2 (CCL2, monocyte chemoattractant protein-1), C-X3-C motif chemokine 1 (CX3CL1, fractalkine) and C-C motif chemokine 5 (CCL5, RANTES), play important roles in the pathogenesis of DN[30].

CCL2 plays a key role in the migration of monocytes into the kidney[31]. Increased expression of CCL2 occurs in the tubulointerstitial lesions of patients with diabetic nephropathy[32]. Urinary excretion of CCL2 is increased in type 2 diabetic patients, and the urinary level of CCL2 correlates with the clinical stage of DN[32]. CCL2 is produced by renal resident cells, as well as from inflammatory cells[33]. It has been reported that high glucose concentrations, AGEs, protein kinase C, oxidative stress and angiotensin II might contribute to the upregulation of CCL2 in the diabetic kidney[34]. CCL2 deficiency reduced renal macrophage infiltration and the progression of diabetic

renal injury in a streptozotocin-induced diabetes model[35]. In addition, C-C chemokine receptor type 2 (CCR2) KO mice also showed a reduction of macrophage infiltration and fibrosis in the kidney in diabetic model[36].

CX3CL1 also promotes the migration of mononuclear cells and also induces adhesion between cells that express its receptor, CX3CR1[37]. CX3CL1 and CX3CR1 are upregulated in the kidneys of diabetic animals[38]. High glucose levels, AGEs and cytokine activation have been reported to upregulate CX3CR1 in diabetic kidneys[39]. Interaction between CX3CL1 and CX3CR1 might enhance the infiltration of monocytes into the interstitium and interstitial injury.

CCL5 (RANTES) is expressed in mesangial cells and tubular epithelial cells[40]. CCL5 is also upregulated by angiotensin II, and pro-inflammatory cytokine. Expression of CCL5 is increased in the interstitium of the kidney in patients with DN[41].

PRO-INFLAMMATORY CYTOKINES

Pro-inflammatory cytokine are increased in inflammatory lesions, and contribute to accelerating and maintaining chronic inflammation in various kinds of inflammatory diseases. These pro-inflammatory cytokines, such as TNF-α and IL-1, have been reported to participate in the pathogenesis of DN[42].

TNF-α induces expression of a variety of effector molecules, such as cytokines and adhesion molecules, apoptosis and necrosis, through the receptors. TNF-α is considered to play a pivotal role in the pathogenesis of diabetic nephropathy through cytotoxicity, apoptosis, necrosis and increased endothelial cell permeability[43–47]. Expression of TNF-α is increased in glomerular and proximal tubular epithelial cells, and is correlated with UAE[48–51]. Serum and urinary concentrations of TNF-α are elevated and related to disease progression in patients with DN[52]. We also reported that serum levels of TNF-α were independently associated with UAE in type 2 diabetic patients[53].

IL-1 is a major pro-inflammatory cytokine that plays a central role in the mechanism of acute and chronic inflammation. IL-1 is known to be increased in the kidney of animal models of DN[54]. In addition, IL-1 stimulates synthesis of expression of prostaglandin E2, suggesting that IL-1 might be related to the change of glomerular hemodynamics[55].

IL-18, which belongs to the IL-1 superfamily, is a pro-inflammatory cytokine secreted from mononuclear cells. Serum concentration of IL-18 is known to be a strong predictor of death in patients with cardiovascular diseases. IL-18 induces expression of pro-inflammatory cytokines, and adhesion molecules and apoptosis[56,57]. In our study, serum and urinary IL-18 levels were significantly elevated in patients with type 2 diabetes as compared with control subjects[58]. We found significant positive correlations between serum and urinary levels of IL-18 and UAE. Serum IL-18 levels were also correlated positively with carotid intima media thickness (IMT) and brachial-ankle pulse wave velocity (baPWV). Furthermore, serum and urinary IL-18 levels correlated positively with AER after 6 months and changes in UAE during the follow-up period of 6 months, suggesting that serum levels of IL-18 might be a predictor of progression of diabetic nephropathy, as well as cardiovascular diseases[58]. Araki *et al.*[59] also showed that elevated levels of IL-18 are a determinant of early renal dysfunction in patients with type 2 diabetes.

Suzuki *et al.*[60] showed that mesangial cells, tubular cells and infiltrating cells expressed IL-6 messenger ribonucleic acid in the renal tissues of patients with DN by *in situ* hybridization. Serum levels of IL-6 were substantially higher in patients with DN than in control patients without DN[61].

THERAPEUTIC IMPLICATIONS

Microinflammation is a potential therapeutic target for DN, because recent growing evidence has shown that the inflammatory process underlies the pathogenesis of DN. To date, there has been no established drug that ameliorates diabetic nephropathy through anti-inflammatory actions in humans, although an increasing number of studies *in vitro* and *in vivo* have suggested the efficacy of anti-inflammatory agents on DN (Table 1).

The potential beneficial anti-inflammatory effects on DN by using immunosuppressive agents in both type 1 and type 2 diabetic animal models has been reported. Utimura *et al.*[62] reported that treatment with mycophenolate mofetil, an immunosuppressive agent, had no effect on blood pressure, glomerular dynamics or blood glucose levels, but prevents albuminuria, glomerular macrophage infiltration and glomerulosclerosis in streptozotocin-induced diabetic rats. Similar findings have been reported in Zucker fatty rats[63]. In contrast, we also examined the effects of methotrexate on streptozotocin-induced diabetic rats[64]. The results showed that methotrexate decreased UAE, renal tissue injuries and inflammation in the kidney. These findings clearly support the fundamental concept that anti-inflammatory effects are beneficial for the treatment of DN,

Table 1 | Renoprotective and anti-inflammatory agents shown in the animal diabetic models

Statin	Ota *et al. Diabetologia* 2003[68]
	Usui *et al. Nephrol Dial Transplant* 2003[67]
Thiazolidinedione	Ohga *et al. Am J Physiol, Renal Physiol*, 2007[69]
Angiotensin II receptor antagonist	Lee *et al. J Am Soc Nephrol* 2004[65]
Spironolactone	Han *et al. J Am Soc Nephrol* 2006[66]
Immunosuppressant	Utimura *et al. Kidney Int* 2003[62]
	Yozai *et al. J Am Soc Nephrol* 2005[64]
Pentoxifyline	DiPetrillo *et al. Am J Nephrol* 2004[71]
Macrolide (erythromycin)	Tone *et al. Diabetologia* 2005[80]
GLP-1 receptor agonist	Park *et al. J Am Soc Nephrol* 2007[77]
	Kodera *et al. Diabetologia* 2011[78]
	Hendarto *et al. Metabolism* 2012[79]
Cholecystokinin	Miyamoto *et al. Diabetes* 2012[74]

GLP-1, glucagon-like peptide-1.

although the adverse effects caused by immunosuppression have not been evaluated.

In contrast, several kinds of drugs that are used for diabetic patients, such as angiotensin converting enzyme (ACE) inhibitor, angiotensin II receptor antagonist (ARB), thiazolidinediones and statins, are known to have anti-inflammatory effects as their pleiotropic effects. The renoprotective effects of ARB and aldosterone receptor blocker are considered to be at least partly related to anti-inflammatory actions through inhibition of nuclear factor-κB (NF-κB)-dependent pathways[65,66].

Statin is known to exert anti-inflammatory effects through inhibition of small-G proteins and NF-κB-dependent inflammatory pathway independent of cholesterol-lowering effects. We examined the renoprotective effects of statin using streptozotocin-induced diabetic rats. Cerivastatin showed the amelioration of UAE, glomerular infiltration of macrophage and activation of NF-κB in the kidney without any change of serum cholesterol level[67]. Okada also showed the preventive effects of cerivastatin on renal injuries in diabetic rats through an anti-inflammatory effect[68].

Thiazolidinediones are also known to have anti-inflammatory actions by stimulation of the peroxisome proliferator-activated receptor (PPAR-γ) in addition to the effects of improvement of insulin resistance. We administered pioglitazone to streptozotocin-induced diabetic rats and found that pioglitazone reduced albuminuria, intraglomerular infiltration of macrophages and activation of NF-κB, suggesting that pioglitazone exerts renoprotective effects through anti-inflammatory effects independent of blood glucose-lowering effects[69].

TNF-α is a major pro-inflammatory cytokine, and is considered to play important roles in the development of diabetic nephropathy. Thus, TNF-α might be a promising therapeutic target for the treatment of DN. Pentoxifylline, which is a xanthine derivative, modulates the expression of TNF-α and other pro-inflammatory cytokines, and attenuates cellular processes involved in the inflammatory response[70]. Treatment with pentoxifylline reduces the expression of TNF-α, IL-1, and IL-6 in the kidney and UAE in diabetic animals[71]. This drug reduced proteinuria and serum levels of TNF-α in patients with diabetes mellitus[72,73].

Recently, we have found that cholecystokinin (CCK) is expressed in the kidney and exerts renoprotective effects through its anti-inflammatory actions. Furthermore, administration of sulfated cholecystokinin octapeptide (CCK-8S) ameliorated albuminuria, podocyte loss, expression of pro-inflammatory genes and infiltration of macrophages in the kidneys of diabetic rats[74].

ANTI-INFLAMMATORY EFFECTS OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONIST

Glucagon-like peptide-1 (GLP-1) is a gut incretin hormone that enhances glucose-dependent insulin secretion of pancreatic β-cells. Today, dipeptidylpeptidase-IV resistant long acting GLP-1 receptor agonists, exendin-4 and liraglutide, are available for treatment of type 2 diabetes. Previous reports have shown that GLP-1 receptor is expressed not only in the pancreas, but also in many organs including the kidney[75–77]. Park *et al.*[77] reported that long-term treatment of exendin-4 ameliorates diabetic nephropathy through improving metabolic anomalies in *db/db* mice. We have recently found that GLP-1 receptor was expressed on glomerular endothelial cells, and showed that exendin-4 directly acted on GLP-1 receptor and attenuated

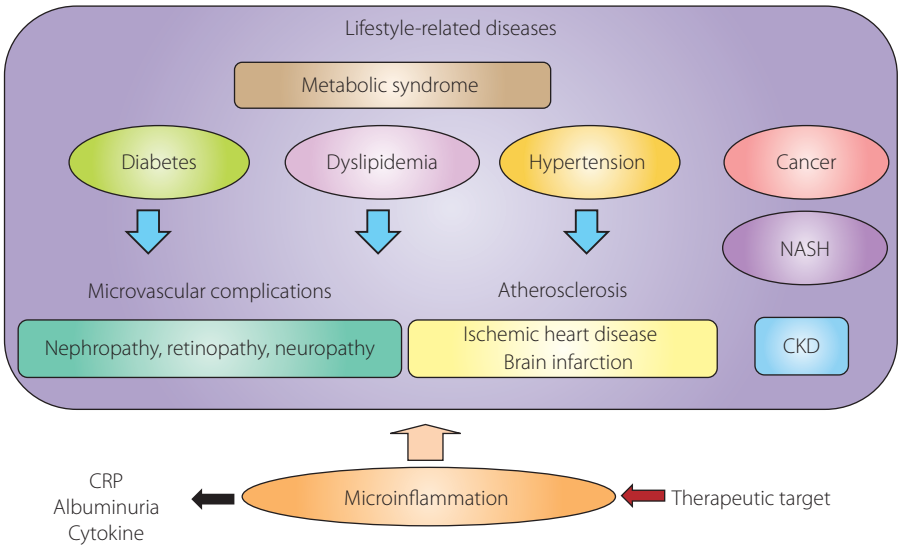

Figure 4 | Microinflammation and lifestyle-related diseases. Microinflammation is a common mechanism of the development of lifestyle-related diseases, including obesity-related insulin resistance, diabetic vascular complications, cardiovascular diseases, chronic kidney disease (CKD), non-alcoholic steatohepatitis (NASH) and some kinds of cancer. Albuminuria, C-reactive protein (CRP) and plasma levels of cytokines might be surrogate clinical markers for the microinflammation.

ICAM-1 expression on glomerular endothelial cells *in vitro*[78]. Furthermore, exendin-4 ameliorated albuminuria, glomerular hyperfiltration, glomerular hypertrophy and mesangial matrix expansion in type 1 diabetic rats without changing blood pressure and bodyweight. Exendin-4 also prevented macrophage infiltration, expressions of ICAM-1 and type IV collagen, oxidative stress, and NF-κB activation in kidney tissue[78]. Furthermore, Hendarto *et al.*[79] recently reported that the GLP-1 analog, liraglutide, protects against oxidative stress and albuminuria in streptozotocin-induced diabetic rats through protein kinase A-mediated inhibition of renal nicotinamide adenine dinucleotide phosphate oxidases. These results show that GLP-1 receptor agonists might be beneficial for DN through anti-inflammatory and anti-oxidative actions independent of blood glucose-lowering effect.

CONCLUSION

There has been accumulating evidence showing that microinflammation is one of the key factors in the pathogenesis of diabetic nephropathy. Abnormalities of blood glucose, blood pressure or dyslipidemia trigger the activation of inflammatory pathways in the diabetic kidney followed by functional and structural renal injury. Microinflammation is a common pathological condition not only in diabetic vascular complications, but also in metabolic syndrome, non-alcoholic steatohepatitis and some kinds of cancer (Figure 4). These findings strongly suggest that microinflammation is a promising new therapeutic target for lifestyle-related diseases.

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We declare no conflicts of interest related to the manuscript.

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