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Immunoinflammation in Diabetic Nephropathy: Molecular Mechanisms and Therapeutic Options

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1. Introduction

The prevalence of diabetes mellitus, predominantly of type 2, has dramatically increased worldwide (Ritz et al., 2011). Diabetic nephropathy (DN) affects approximately one third of people with type 1 or type 2 diabetes mellitus (Reutens et al., 2011). In developed countries, the proportion of patients with diabetic kidney disease has stabilized despite increased use of glucose-lowering medications and renin-angiotensin-aldosterone system (RAAS) inhibitors (de Boer et al., 2011).

DN typically develops after many years of diabetes, and is usually manifested clinically by gradually worsening albuminuria, followed by a decline in glomerular filtration rate, which over years or decades leads to end-stage renal disease. DN is characterized by specific renal morphological and functional alterations. Features of early diabetic renal changes are glomerular hyperfiltration, glomerular and renal hypertrophy, increased urinary albumin excretion, increased basement membrane thickness, and mesangial expansion with the accumulation of extracellular matrix proteins such as collagens, fibronectin, and laminin. Pathologic alterations of the tubulointerstitium such as fibrosis and tubular atrophy are also closely linked to the deterioration of renal function in patients with diabetes of both types 1 and 2 (Wolf, 2000; Schrijvers et al., 2004).

Despite the modern therapies like antidiabetic, antihypertensive, and antioxidant drugs available to treat DN, most of patients continue to show progressive renal damage. It suggests that the key pathogenic mechanisms involved in the induction and progression of DN are still remaining active and unmodified by the present therapies (Balakumar et al., 2009). Many studies have attempted to elucidate the molecular signaling mechanisms that lead to DN so that effective therapies and preventive strategies might be developed. Through these efforts the general understanding of the pathogenic signaling factors that lead to progressive DN has expanded considerably during the past decade (Balakumar et al., 2009; Brosius et al., 2010).

In recent years, extensive research has elucidated several pathways involved in the development and progression of diabetic kidney disease beyond the relevant role of high blood glucose (Schrijvers et al., 2004). Our knowledge of the pathophysiological processes in

DN has notably improved on a genetic and molecular level. Thus, the classic view of metabolic and hemodynamic alterations as the main causes of renal injury in diabetes has been transformed significantly, with clear evidence indicating that these traditional factors are only a partial aspect of a much more complex picture. One of the most important changes is related to the participation of immune-mediated inflammatory processes in the pathophysiology of diabetes mellitus and its complications (Navarro-Gonzalez & Mora-Fernandez, 2008; Galkina & Ley, 2006; Shikata & Makino, 2001; Chow et al., 2004).

Although DN is traditionally considered a non-immune disease, accumulating evidence now indicates that immunologic and inflammatory mechanisms play a significant role in its development and progression. DN also includes a variety of inflammatory responses induced by hyperglycemic conditions. Furthermore, microinflammation is a common major mechanism for the progression of DN. This process is mediated by elements of the immune system, including lymphocytes and monocytes/macrophages, as well as cytokines, growth factors, chemokines, adhesion molecules, enzymes, and nuclear factors. In this review we summarized cell processes, mediators and intracellular pathways participating in the immune and inflammatory response during the development of diabetic renal damage.

2. Mechanisms of immune cell infiltration in the diabetic kidney

2.1 Immune cells

Yet, the molecular and cellular mechanisms of intrarenal inflammation in DN remain poorly characterized. Macrophages are the major inflammatory cells found in diabetic kidneys and their accumulation is a recognized feature in renal biopsies from diabetic patients (Xiao et al., 2009). In different experimental models of DN, renal macrophage accumulation correlates with the severity of glomerular and tubulointerstitial injury (Chow et al., 2004; Chow et al., 2005). However, it remains to be established whether macrophages are a major effector cell of diabetic renal damage, or merely recruited as a response to injury. Previous studies in diabetic animals reported the protective effect of mycophenolate or irradiation via reduction of renal macrophage infiltration (Wu et al., 2006). However, these treatments have additional effects on the kidney and immune system, and cannot be used for determining the long-term effects of macrophages on the progression of DN.

Macrophages mediate immunopathology and tissue remodeling in both non-renal and renal diseases, and blocking macrophage recruitment prevents the progression of many types of kidney disease models (Ricardo et al., 2008). There are at least two subtypes of resident macrophages in tissues: the M1 macrophages, classically activated by Th1 stimuli, that express proinflammatory cytokines and enhances tissue inflammatory response; and the M2 macrophages, alternatively activated by Th2 stimuli, that express antiinflammatory cytokines, and participate in the promotion of tissue repair, remodeling and vasculogenesis (Gordon, 2003). Obviously, modulating the macrophage phenotype is as important as reducing their overall number to prevent glomerular damage. The relative abundance of M1 and M2 macrophages in the injured kidney changes dynamically by recruitment of polarized monocytes or through the effects of local cytokines on macrophages. Different chemokines are able to recruit circulating monocytes to extravascular compartments such as the glomerulus. Classically activated M1 macrophages represent one end of the spectrum as they produce upon stimulation high amounts of reactive oxygen species (ROS), and

proinflammatory cytokines, such as interleukins (ILs), tumor necrosis factor (TNF-α), and interferon-γ (IFN-γ), all of which exacerbate inflammation and tissue injury *in vivo* (Mantovani et al., 2004). Alternatively activated M2 macrophages represent the other end of the spectrum and they participate in the resolution of inflammation through the secretion of antiinflammatory factors such as IL-10 and transforming growth factor-β (TGF-β), as well as by inhibiting the production of proinflammatory cytokines, chemokines, and superoxide anion (Martinez et al., 2008; Ricardo et al., 2008). Recent studies demonstrated a reduction in the severity of glomerular inflammation with adoptive transfer of cytokine-programmed M2 macrophages (Ricardo et al., 2008). Furthermore, the antiinflammatory effects of statins (Fujita et al., 2010) and the angiotensin II type 1 receptor blocker olmesartan (Aki et al., 2010) in experimental glomerulonephritis are mediated through downregulation of M1 macrophage infiltration as well as augmentation of antiinflammatory M2 macrophages and cytokines. Based on this, strategies to control the dynamic balance of macrophage polarization could have therapeutic interests in DN, and future studies will determine the place of these novel approaches in diabetic patients.

T lymphocytes are known to play a significant role in renal injury induced by non-immune insults including ischaemia or toxins (e.g. adriamycin) (Lim et al., 2010). In patients with type 1 diabetes, the presence of nephropathy and proteinuria has been associated with increased activated peripheral blood T cells and also infiltration of T cells into the kidney (Xiao et al., 2009; Moriya et al., 2004; Ichinose et al., 2007), thus suggesting that lymphocyte activation may play a role in early DN. Activated T cells can cause injury directly through cytotoxic effects and indirectly by recruiting and activating macrophages. In addition, kidney autoantigens may develop during chronic diabetic renal injury and, if this occurs, B cells could present these antigens to T cells to promote their activation. Furthermore, diabetic patients have increased levels of serum immunoglobulins, which include antibodies (Abs) against proteins modified by glycoxidation or lipoxidation. These circulating Abs can form immune complexes (Atchley et al., 2002), which may deposit in glomeruli and promote activation of complement or macrophages via receptor interactions. Elements of the diabetic milieu can directly or indirectly activate T cells in diabetic kidneys. CD4+ T cells express the receptor for advanced glycation end products (AGEs) and can respond to AGEs by producing IFN-γ, which could exacerbate inflammation in the diabetic kidney. In addition, hyperglycemia induces macrophage production of IL-12, which can also stimulate CD4 cell production of IFN-γ (Wen et al., 2006; Lim et al., 2010). T lymphocyte-directed immunotherapies with anti-CD3 and anti-CD4 monoclonal Abs also induce disease remission in non-obese diabetic mice (Mehta et al., 2010). Some of them are currently in Phase III clinical trials for prevention of type 1 diabetes (Miller & St, 2011), although their renoprotective effects are not documented.

2.2 Inflammatory mediators

The infiltration of leukocytes into sites of inflammation is mediated by sequential binding to specific cell adhesion molecules and chemokine and cytokine release that together promote rolling, arrest, firm adhesion, transmigration, and activation (Hogg & Berlin, 1995). A large array of cell adhesion molecules, chemokines, and cytokines have been shown to be important in leukocyte accumulation and renal injury in models of non-diabetic and diabetic kidney damage, and some of these mediators are also found elevated in renal biopsies from diabetic patients (Navarro-Gonzalez & Mora-Fernandez, 2008; Galkina & Ley, 2006; Shikata

& Makino, 2001; Chow et al., 2004). The most representative members for each family are discussed below.

2.2.1 Adhesion molecules: ICAM-1

Intercellular adhesion molecule (ICAM)-1 is a 90-kD cell surface glycoprotein of the Ig superfamily involved in the firm attachment of leukocytes to endothelium (Staunton et al., 1988), which interacts with lymphocyte function-associated antigen (LFA)-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) that are present on most leukocytes. ICAM-1 expression is upregulated and associated with leukocyte infiltration and disease progression in experimental models of type 1 and type 2 DN (Sugimoto et al., 1997; Coimbra et al., 2000) and also in diabetic patients (Rubio-Guerra et al., 2009). ICAM-1 is induced by factors common to both types of diabetes, such as hyperglycemia, AGEs, hyperfiltration, and oxidative stress, but it can also be increased by additional elements such as hyperlipidemia, hyperinsulinemia, and elevated levels of circulating TNF-α (Chow et al., 2006). Previous evidence from genetically deficient mice demonstrated that ICAM-1 is a critical mediator of macrophage accumulation in diabetic kidneys both in early and late stages of diabetes (Okada et al., 2003; Chow et al., 2005), while treatment with anti-ICAM-1 monoclonal Ab prevents mononuclear cell infiltration into diabetic glomeruli (Sugimoto et al., 1997). Furthermore, the reduced ICAM-1 overexpression is one of the renoprotective effecs of taurine treatment in streptozotocin-induced diabetic rats (Wang et al., 2008).

2.2.2 Chemokines: MCP-1

Monocyte chemotactic protein-1 (MCP-1) is a small cytokine belonging to the CC chemokine family that is also known as chemokine (C-C motif) ligand 2 (CCL2). MCP-1 recruits monocytes, T cells, macrophages and dendritic cells to sites of tissue injury, infection, and inflammation, and is gaining interest as a mediator of DN. MCP-1 is induced by high glucose concentrations, AGEs and cytokines in cultured renal cells (Chow et al., 2006), and its expression increases progressively in diabetic kidneys from different animal models. In diabetic patients, MCP-1 urinary levels correlate with albuminuria, therefore being considered a marker of renal function decline (Sayyed et al., 2011; Camilla et al., 2011).

MCP-1 is a potent chemokine involved in the accumulation and function of macrophages (Chow et al., 2006), thus playing a role in the inflammatory phase of DN. Renal cells like podocytes and mesangial cells are also able to produce MCP-1 in response to metabolic mediators, such as high glucose, and AGEs, and also by activation of RAAS and TGF-β (Yamagishi & Matsui, 2010). Renal cells are susceptible to paracrine and autocrine activation by MCP-1, through the interaction with CCR2, the main receptor of MCP-1 (Sayyed et al., 2011; Camilla et al., 2011). In fact, MCP-1/CCR2 system is involved in podocyte cytoskeleton reorganization and motility, and also in mesangial expression of fibronectin and type IV collagen (Lee et al., 2009; Park et al., 2008). Diabetic mice with gene deficiency in MCP-1 are protected from glomerular macrophage infiltration, renal injury, and development of albuminuria (Chow et al., 2006).

As upregulation of kidney MCP-1 is a feature of human diabetic renal injury associated with macrophage recruitment and disease progression, neutralizing MCP-1 activity should be viewed as an important therapeutic goal in the treatment of DN. Preclinical studies have demonstrated that blockade of MCP-1/CCR2 system with RO5234444, an orally active

small-molecule CCR2 antagonist, reduced glomerular macrophage content, glomerulosclerosis, and albuminuria in diabetic mice, and also improved glomerular filtration rate (Sayyed et al., 2011; Camilla et al., 2011). Furthermore, the renoprotective effect of several compounds, including pioglitazone (Hu et al., 2010), clarithromycin (Tone et al., 2011), and exenatide (Wu et al., 2011) has been related to the local reduction of MCP-1 activity within the kidney. Furthermore, the indazolic derivative bindarit (AF-2838) is a potent antiinflammatory agent that inhibits chemokine synthesis, particularly MCP-1. Phase II trials in rheumatoid arthritis and lupus nephritis have shown that bindarit significantly reduced urinary MCP-1 and albumin excretion rate. A clinical trial aimed to reduce albuminuria and renal disease progression with bindarit added onto RAAS blockade therapy is ongoing in type 2 diabetic patients with micro- or macroalbuminuria (Cortinovis et al., 2008).

2.2.3 Cytokines: IL-1, IL-6, and TNF-α

There is growing support for the notion that circulating proinflammatory cytokines, such as ILs and TNF-α, are strongly associated with the risk of developing diabetic complications (Shikata & Makino, 2001). ILs comprise a large group of cytokines secreted by leukocytes and other body cells that can be classified as proinflammatory and antiinflammatory. In particular, the proinflammatory IL-1 increases the expression of chemotactic factors and adhesion molecules, enhances vascular endothelial permeability, and stimulates the proliferation of mesangial cells and matrix synthesis (Rivero et al., 2009). Renal IL-1 expression is found increased in diabetic animals and correlates with albuminuria and macrophage content (Hasegawa et al., 1991; Sassy-Prigent et al., 2000; Navarro et al., 2006). Specific blockade of IL-1 activity by the IL-1 receptor antagonist anakinra reduced the release of inflammatory cytokines and chemokines in pancreatic islet from diabetic rats, and also decreased hyperglycemia and improved insulin sensitivity (Ehses et al., 2009). In type 2 diabetic patients, anakinra improved glycemia and beta-cell secretory function and reduced markers of systemic inflammation (Larsen et al., 2007). Further studies are needed to demonstrate the biological effects of this compound on diabetic kidneys.

IL-6 is a pleiotropic cytokine secreted by renal cells in response to a diabetic milieu (Min et al., 2009; Tang et al., 2010a) that stimulates mesangial cell proliferation, affects extracellular matrix dynamics in renal cells, and enhances endothelial permeability (Navarro-Gonzalez & Mora-Fernandez, 2008; Galkina & Ley, 2006; Shikata & Makino, 2001; Chow et al., 2004). Serum IL-6 levels are significantly increased in patients with type 2 DN compared to levels observed in diabetic patients without nephropathy (Navarro-Gonzalez & Mora-Fernandez, 2008; Galkina & Ley, 2006; Dalla et al., 2005), and studies in renal biopsies revealed a significant association between the severity of diabetic glomerulopathy and the expression levels of IL-6 in glomerular cells (Suzuki et al., 1995), thus suggesting a role for IL-6 in the pathogenesis of DN. There are no direct data of treatment against elevated IL-6 levels in DN, however there are indirect evidences. In a recent study in patients with incipient and established DN, the treatment with pentoxyfylline, a methylxanthine derivate and nonselective phosphodiesterase inhibitor, caused a decrease in the urinary albumin excretion, and this renoprotective effect was attributable in part to reduced levels of IL-6 among other proinflammatory mediators (Hasegawa et al., 1991; Sassy-Prigent et al., 2000; Navarro et al., 2006).

TNF-α is a pleiotropic cytokine produced mainly by monocytes/macrophages that is involved in systemic inflammation (Sugimoto et al., 1999). TNF-α exerts cytotoxic effects on renal cells (McCarthy et al., 1998; Min et al., 2009), and it has been shown to participate in

renal damage development in experimental models of renal disease including lupus nephritis, glomerulonephritis, nephropathy, hypertension, and diabetes (McCarthy et al., 1998; Elmarakby & Sullivan, 2010). A role for TNF-α in DN is supported by the finding that urinary albumin excretion significantly correlates with renal TNF-α levels and urinary TNF-α excretion in streptozotocin-induced diabetic rats (Navarro et al., 2005). TNF-α also contributes to sodium retention and renal hypertrophy, which are early characteristic signs of streptozotocin-induced DN (DiPetrillo et al., 2003). Renal TNF-α expression, particularly in the glomerulus and tubulointerestitium, is increased in streptozotocin diabetic rat kidneys, and serum TNF-α is increased in type 2 diabetic patients (Navarro et al., 2005). Therefore, TNF-α plays an important role in the incidence and progression of DN and renal TNF-α levels correlate with markers of DN.

Strategies to inhibit TNF-α have been successfully used in experimental diabetes. DiPetrillo et al. (DiPetrillo et al., 2003) reported that treatment of diabetic rats with the anti-TNF-α agent TNFR:Fc, a soluble TNF-α receptor fusion protein, reduced urinary TNF-α excretion and prevented sodium retention and renal hypertrophy. Similarly, TNF-α inhibition with infliximab, a chimeric monoclonal Ab directed against TNF-α, significantly reduced both albuminuria and urinary TNF-α in streptozotocin-induced diabetic rats (Moriwaki et al., 2007). Unfortunately, no other parameters such as structural changes or hemodynamics were studied. A recent retrospective study evaluated the effects of anti–TNF-α agents on control of type 2 diabetes in patients with rheumatoid arthritis and Crohn's disease. Anti-TNF treatment improved glucose tolerance and control, although future prospective studies are needed to solidify these results (Gupta-Ganguli et al., 2011).

3. Molecules involved in the progression of diabetic renal disease

3.1 Growth factors: TGF-β, CTGF, and VEGF

Hyperglycemia stimulates resident and non-resident renal cells to produce cytokines and growth factors that contribute to the development of renal injury. In particular, the expression of the profibrotic factor TGF-β is increased in both type 1 and type 2 diabetes (Cortinovis et al., 2008). Other growth factors are also implicated, including vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF), among others acting through several intracellular signaling pathways comprising protein kinases and transcription factors (Goh et al., 2008). Recently, specific Abs against different growth factors that might be useful for the treatment of chronic nephropathies, including DN, have been developed (Cortinovis et al., 2008).

3.1.1 TGF-β

TGF-β is a well-known profibrogenic factor which controls synthesis and degradation of extracellular matrix proteins by stimulating transcription of extracellular matrix genes in renal cells and reducing collagenase production, eventually inhibiting matrix turnover. Furthermore TGF-β is involved in tubuloglomerular sclerosis and podocyte apoptosis in diabetes. TGF-β gene and protein levels are significantly increased in glomeruli and tubulointerstitium of type 1 and 2 diabetic patients and animals (Yamagishi & Matsui, 2010; Goh et al., 2008). Factors that regulate TGF-β expression in renal cells include hyperglycemia, AGEs, endothelin, lipids and products of oxidative stress. TGF-β is also modulated by the RAAS (Goh et al., 2008). In fact, angiotensin II stimulates TGF-β and its

receptors in the kidney by various mechanisms, and angiotensin converting enzyme (ACE) inhibition reduces serum and urinary levels of TGF-β (Wolf, 2006). In diabetic patients, treatment with the ACE inhibitor perindopril reduced the intrarenal TGF-β expression and activity (Langham et al., 2006). Furthermore, the antifibrotic agent N-acetyl-seryl-aspartyl-lysyl-proline, which reduced TGF-β-induced extracellular matrix production and prevented renal fibrosis and albuminuria in diabetic db/db mice, conferred an additional renoprotective effect when combined with the angiotensin II receptor antagonist losartan (Sugaru et al., 2006).

A range of novel compounds has been recently examined to inhibit TGF-β and TGF-β-dependent pathways in diabetes. Several blocking Abs against TGF-β effectively reduce mesangial matrix accumulation and glomerulosclerosis in diabetic mouse models (Ziyadeh et al., 2000; Goh et al., 2008), and particularly the TGF-AY1 Ab is in clinical development for the treatment of chronic kidney disease, with focus on DN (Cortinovis et al., 2008). In addition, the soluble human TGF-β type II receptor (sT β RII-Fc), a high-affinity TGF-β1 binding molecule, has been proposed as a potential new agent for the treatment of fibrosis and albuminuria in DN (Russo et al., 2007).

A potential therapeutic approach is the use of micro RNA (miRNA)-based strategies. The miRNAs are short noncoding nucleotides that regulate target messenger RNAs at the post-transcriptional level and are involved in many biological processes (Lorenzen et al., 2011). Recent studies have identified miRNA-mediated circuits controlling auto-upregulation of TGF-β1 and amplification of TGF-β1 signaling that accelerate chronic fibrotic kidney diseases including DN (Kato et al., 2011). In particular, miRNA-92c and miRNA-192 are induced in renal cells by high glucose and TGF-β, and mediate cell apoptosis and extracellular matrix accumulation (Kato et al., 2011; Long et al., 2011). Renal expression of these miRNAs increased in type 1 and type 2 diabetic animals, and *in vivo* knockdown prevented progression of DN. Their widespread and distinct expression patterns under normal and disease states make miRNAs attractive molecular therapeutic targets for human diseases. In fact, different miRNA modulators (such as antagomirs and locked nucleic acid antimiRs) have been developed for specific targeting of miRNAs and respective downstream gene networks (Lorenzen et al., 2011). The therapeutic potential of miRNA-based treatment in DN requires further study.

3.1.2 CTGF

Several reports have described an increased expression of CTGF in diabetic kidneys, that is therefore being considered a marker and a mediator of disease. Synthesis of CTGF is stimulated by TGF-β, hyperglycemia and AGEs, as well as CTGF itself. CTGF induces mesangial cell hypertrophy and cytoskeletal disassembling, upregulates cell production of fibronectin and collagens, and is also involved in epithelial-to-mesenchymal transition of tubular cells (Twigg, 2010; Connolly et al., 2003).

CTGF is also an important downstream mediator of the profibrotic activity of TGF-β. But in contrast to TGF-β, CTGF is not centrally involved in the modulation of inflammation or immune reactions (Goldschmeding et al., 2000) and thus this profibrotic factor may be a more attractive target for new renoprotective therapies. In fact, decreased CTGF expression in the kidney has been suggested as the mechanism involved in the inhibition of diabetic renal damage by different agents including the AGE inhibitors aminoguanidine (Twigg &

Cooper, 2004) and XLF-III-43 (Li et al., 2010), the aldosterone receptor blocker spironolactone (Han et al., 2006), and the flavonoid compound astilbin (Li et al., 2009). More specific therapies include FG-3019, a humanized monoclonal Ab that neutralizes the effects of CTGF in diabetic animals (Cortinovis et al., 2008). FG-3019 is currently under development for idiopathic pulmonary fibrosis, pancreatic cancer and diabetes. In an open-label, dose-escalation Phase Ib trial in type 1 or 2 diabetic patients with incipient nephropathy, FG-3019 effectively decreased albuminuria (Adler et al., 2010), although further validation in a prospective, randomized, blinded study is required.

3.1.3 VEGF

VEGF is a potent inducer of vasopermeability and angiogenesis that plays a major pathophysiological role in DN, despite VEGF exhibiting protective roles in non-diabetic renal disease. Serum levels of VEGF correlate with albuminuria and increase with DN stage in patients with type 1 and 2 diabetes (Hovind et al., 2000). Several experimental model studies have demonstrated that VEGF may contribute to some of the hemodynamic changes in DN, including hyperfiltration and albuminuria. Expression of VEGF and its receptors are modulated by high glucose, endothelin 1, AGEs, angiotensin II, stretch and TGF-β, and their renal expression is increased in diabetic kidneys (Chen & Ziyadeh, 2008; Cooper et al., 1999). Furthermore, VEGF is a trophic factor for glomerular endothelial cells, affects podocyte function, and is also involved in macrophage influx during early DN (Chen & Ziyadeh, 2008).

Since VEGF levels are elevated in patients and animal models of DN, a number of studies have examined the effect of inhibition of VEGF receptor binding or activation. Antagonism of VEGF using a variety of different strategies has been reported to improve the outcome in experimental nephropathies of various origins, including DN. Neutralizing VEGF Abs ameliorate early and long-term renal changes in diabetic animals (De Vriese et al., 2001; Schrijvers et al., 2006), while treatment with the pan-VEGF receptor tyrosine kinase inhibitor SU5416 ameliorates diabetic albuminuria in mice (Sung et al., 2006). Several clinical studies have evaluated the beneficial effect of these anti-VEGF agents in the treatment of diabetic retinopathy, but more studies are needed to determine the viability of such strategy in diabetic renal disease.

3.2 Oxidative stress

Oxidative stress defined as an excessive production of ROS surpassing existing antioxidative defense mechanisms plays a critical role in the pathogenesis of diabetes, and more importantly in the development of diabetic complications, including DN. Free radicals are capable of disturb physiological cell function both directly, by damaging cellular macromolecules such as DNA, proteins, and lipids, and indirectly through the stimulation of multiple pathways, such as protein kinases, polyol and hexosamine pathways and AGEs formation. In addition, low antioxidant bioavailability promotes cellular oxidative stress leading to additional cellular damage (Forbes et al., 2008; Elmarakby & Sullivan, 2010; Noh & Ha, 2011).

Overproduction of ROS in the diabetic milieu is both a direct consequence of hyperglycemia and an indirect consequence through AGEs or mediators of glucotoxicity such as cytokines and growth factors (Noh & Ha, 2011; Forbes et al., 2008). The effects of ROS in renal cells comprise mesangial cell proliferation, expression of growth factors, extracellular matrix accumulation, RAAS activation and induction of epithelial-mesenchymal transition (Noh &

Ha, 2011; Elmarakby & Sullivan, 2010). Among many pathways, nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase and mitochondrial dysfunction have been recognized as two major sources of ROS generation in diabetic kidneys, and NADPH oxidase-derived ROS has been shown to facilitate renal mitochondrial superoxide production in hyperglycemia (Tojo et al., 2007).

Inhibition of ROS production and their activity has been demonstrated to be effective in preventing the development and progression of experimental diabetes. Different approaches including oral administration of resveratrol (Palsamy & Subramanian, 2011) and dietary antioxidant supplementation with N-acetylcysteine, vitamins C and E (Park et al., 2011) and curcuminoids (Sharma et al., 2006) have been shown to reduce oxidative stress and renal inflammation in diabetic animals. Clinical observations have also revealed a positive effect on oxidative stress in diabetic patients (Gupta et al., 2011). Alternative strategies based on upregulation of antioxidant proteins, such as superoxide dismutase, heme oxygenase-1, and catalase, have also been proven to diminish high glucose-induced ROS in cell cultures and animal models (Li et al., 2011). A recent study in diabetic animals reported the renoprotective effects of luteolin (Wang et al., 2011), a plant-derived flavonoid with antiinflammatory and antitumorigenic properties (Lopez-Lazaro, 2009). The improvement of renal function in luteolin treated animals was associated with changes in superoxide dismutase activity, malondialdehyde content and expression of heme oxygenase-1 expression (Wang et al., 2011). New strategies targeting NF-E2-related factor 2, the transcription factor that controls antioxidant protein expression, mitochondrial dysfunction, and NADPH oxidase might provide a potential approach for the prevention and treatment of DN (Noh & Ha, 2011). Presently, designing new antioxidant therapies focus on effective, cell compartment-specific agents that would improve renoprotection in combination with current therapies (Forbes et al., 2008). Reports on their efficacy in clinical trials for inflammatory-associated pathologies, including diabetes, have yet to be published.

4. Intracellular pathways activated in diabetic kidney

Intense investigation revealed that numerous inflammatory signaling pathways such as phospholipases, protein kinase cascades and transcription factors, are implicated in the pathogenesis of DN, from early phase to the progression and final complications. Among them, nuclear factor-κB (NF-κB) and janus kinase/signal transducers and activators of transcription (JAK/STAT) have a relevant role in the control of immunoinflammatory responses in the diabetic kidney. Mechanisms of action and therapeutic opportunities are discussed.

4.1 NF-κB

The transcription factor NF-κB is induced by various cell stress-associated stimuli including growth factors, vasoactive agents, cytokines, and oxidative stress. NF-κB in turn controls the regulation of numerous genes activated during inflammation, such as cytokines, chemokines, growth factors, cellular ligands, and adhesion molecules (Karin & Greten, 2005). The activation and nuclear translocation of NF-κB has been demonstrated in diabetic kidneys from human and rodents (Mezzano et al., 2004; Liu et al., 2010), and also in proximal tubular cells in the urinary sediment of patients with type 2 diabetes (Brosius et al., 2010). Furthermore, a study integrating gene-expression profiling in human renal biopsies

with promoter modeling has identified the specific set of target genes, especially chemokines, containing a specific NF-κB promoter module (NFKB_IRFF_01) with a NF-κB binding site, that were activated in progressive DN (Schmid et al., 2006). These findings emphasize the NF-κB proinflammatory pathway as potentially a major upstream target for developing new renoprotective agents in diabetes. There is a raising number of reported inhibitors of NF-κB pathway with potential benefits for future therapies in humans. Inhibitors can be divided into basic categories according to the step at which NF-κB is blocked: 1) upstream of IκB kinase complex; 2) IκB phosphorylation/degradation; 3) nuclear translocation; 4) DNA binding; and 5) gene transactivation (Gomez-Guerrero et al., 2011). Some of these compounds are in clinical development against various inflammatory diseases, but studies in diabetic patients are scarce.

It has been suggested that the preventive effects of thiazolidinedione, a ligand for peroxisome proliferator-activated receptor-γ, are mediated by its antiinflammatory actions, including inhibition of NF-κB. Thiazolidinedione caused a reduction in intranuclear NF-κB binding activity in type 2 diabetic patients with obesity (Aljada et al., 2001), and ameliorated renal injury in experimental diabetic rats through NF-κB inhibition (Ohga et al., 2007). Other studies demonstrated that 1,25-dihydroxyvitamin D3 suppresses hyperglycemia-induced gene expression by blocking NF-κB activation in mesangial cells (Zhang et al., 2007). Statins and fenofibrate also exhibit a downregulating effect on NF-κB pathway in kidneys from diabetic rats (Usui et al., 2003; Chen et al., 2008). In the same way, a recent report demonstrates that the ameliorative effects of the plant alkaloid berberine on renal dysfunction in diabetic rats is associated with its inhibitory function on NF-κB signal pathway in the kidney (Liu et al., 2010).

NF-κB is modulated by upstream enzymes like poly(ADP-ribose) polymerase (PARP). Increased PARP activity has been shown to participate in the pathogenesis of diabetic complications. Pharmacological inhibition of PARP by two different inhibitors (PJ-34 and INO-1001) decreased kidney hypertrophy in type 1 diabetic mice (Drel et al., 2011) and this effect was associated with a decrease in NF-κB p50 nuclear translocation (Goh et al., 2008). This provides rationale reasons for development and further studies of PARP inhibitors as promising approaches to DN. In fact, PARP inhibitors are currently being tested in clinical trials for cancer.

4.2 JAK/STAT

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is an essential intracellular mechanism of cytokines and other stimuli that regulates gene expression and cellular activation, proliferation, and differentiation. Members of the JAK/STAT pathway have been claimed as new molecular targets of antiinflammatory treatment in acute and chronic inflammatory diseases (de Prati et al., 2005; Marrero et al., 2006), and their activation is involved in the development of the diabetes complications. A recent report using a transcriptomic approach documented enhanced expression of a large number of JAK/STAT mRNAs and JAK2 protein in glomerular and tubulointerstitial regions from patients with both early and progressive DN (Berthier et al., 2009).

JAK/STAT pathway, especially the JAK2/STAT1/STAT3-dependent axis, contributes to high glucose mediated renal cell responses, including enhanced expression of genes involved in leukocyte infiltration, cell growth, and fibrosis (Brosius et al., 2010; Brosius, III,

2008; Amiri et al., 2002; de Prati et al., 2005; Lu et al., 2009). JAK/STAT also mediates the mitogenic and fibrotic actions of cytokines and angiotensin II in the kidney (Marrero et al., 2006), suggesting that modulation of this pathway may prevent diabetic renal disease. Importantly, studies in experimental models of diabetes suggest that the renal protective effects of current drugs like captopril, statins and rosiglitazone (Banes et al., 2004; Shi et al., 2007; Tang et al., 2010b) could be partially attributed to a modulation in JAK/STAT phosphorylation. More selective therapies, such as inhibitors of JAK2 and JAK3, and STAT1 antisense oligonucleotides have been proven to counteract the harmful effects of JAK/STAT activation in cultured renal cells and in experimental models of DN (Wang et al., 2002; Shi et al., 2007; de Prati et al., 2005).

The JAK/STAT pathway is controlled through different mechanisms: 1) receptor internalization; 2) protein tyrosine phosphatases; 3) protein inhibitors of activated STAT; and 4) suppressors of cytokine signaling (SOCS). In particular, SOCS family of intracellular proteins has emerged as a potential target to modulate the magnitude and duration of JAK/STAT signaling (Yoshimura et al., 2007). SOCS are induced by many pathologic stimuli (*e.g.*, cytokines, angiotensin II, chemokines, insulin, immunoglobulins, and lipoproteins) thus indicating their involvement in many biologic processes (Ortiz-Munoz et al., 2009; Yoshimura et al., 2007; Hernandez-Vargas et al., 2005; Gomez-Guerrero et al., 2004). Our group has recently reported an increased expression of SOCS proteins in renal samples from patients with progressive DN and diabetic animals (Ortiz-Munoz et al., 2010). *In vitro*, SOCS induction prevented the expression of STAT-dependent genes including adhesion molecules, chemokines, and cytokines. *In vivo* gene therapy with SOCS-expressing adenovirus reduced JAK/STAT activation and ameliorated the early renal changes in diabetic rats. Further research into inducers of SOCS expression or SOCS mimetics could have therapeutic value to prevent or retard the progression of diabetic complications.

5. Conclusion

The current knowledge of the cellular and molecular processes involved in the initiation and progression of diabetic renal injury continues to expand (Figure 1). Inflammation is now recognized as an important player in the pathogenesis of DN, and a number of studies have been designed to address whether blockade or modulation of specific inflammatory molecules can be beneficial for this disease. Furthermore, the increased understanding of the functionality of signal transduction pathways will lead to identification of therapeutic targets able to specifically downregulate proinflammatory responses and mediators, potentially even harnessing some of the sophisticated regulatory systems designed to normally limit the inflammatory response. Nevertheless, because of multiple molecular links between inflammation, immune response and diabetes complications, it seems unlikely that suppressing one single specific effector molecule could be sufficient to produce clinically relevant benefit. Effective treatment and/or prevention of diabetic renal disease will therefore require an integrated approach combining multiple strategies to target the underlying inflammatory processes. In addition, as diabetes complications require chronic treatment, long-term antiinflammatory therapies could potentially have other side effects despite improving renal function. Future research will provide answers to these uncertainties regarding multiple and long-term interventional therapies based on modulation of immunoinflammatory responses. In fact, progress towards therapeutics

designed to target specific cytokines, chemokines, growth factors and even transcription factors is already well underway. Besides the assessment of pharmacological safety, bioavailability, and efficacy *in vivo*, more clinical studies will further support the potential of such strategies to be used in diabetes therapy.
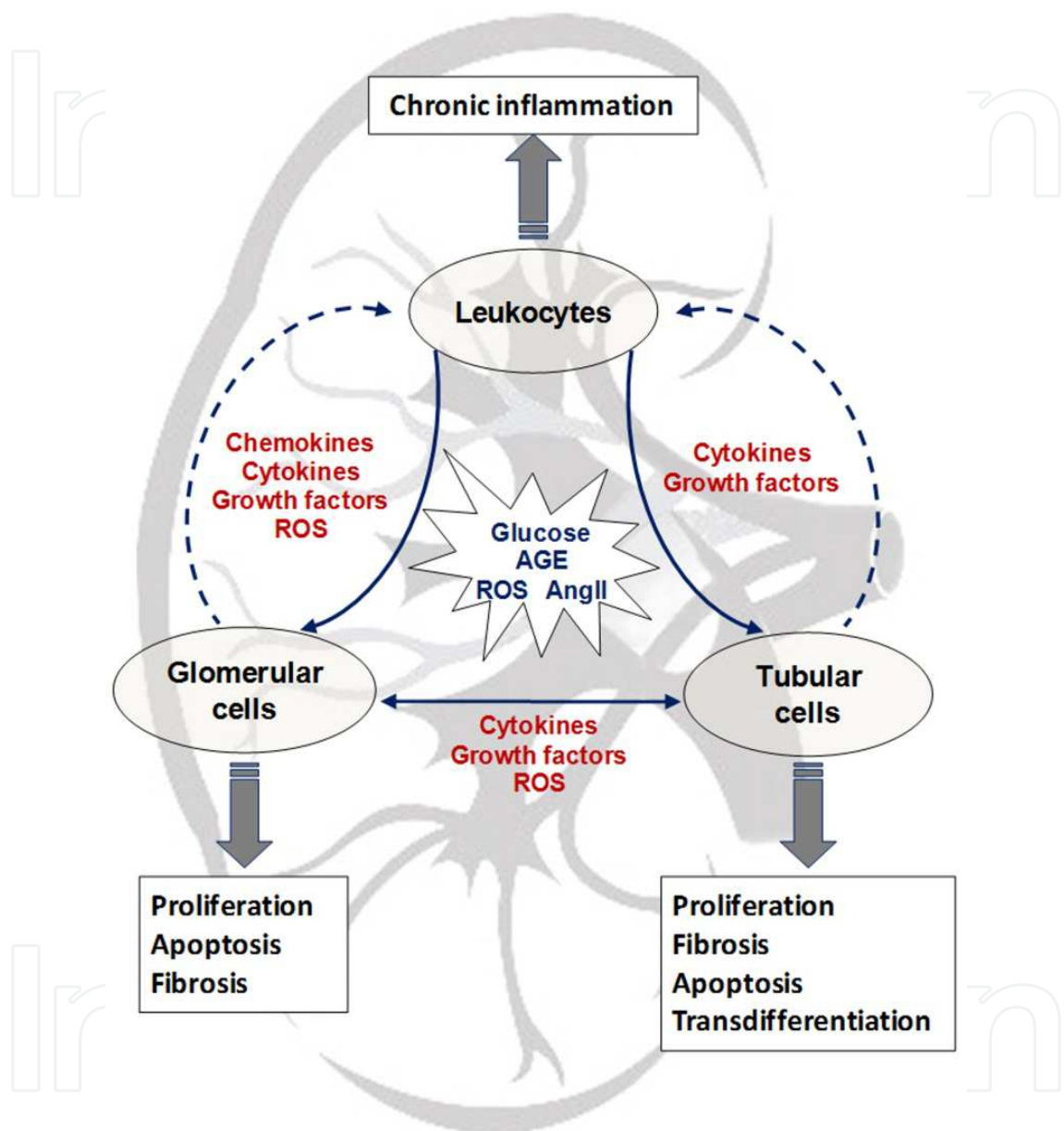

Fig. 1. Immunoinflammatory mechanisms in DN. Elements of the diabetic milieu (high glucose, AGEs, angiotensin, and oxidative stress) induce the expression of chemokines and adhesion molecules by renal cells, which favours leukocyte infiltration into the kidney. Further exposure of kidney macrophages and resident cells to diabetic milieu promotes cell activation, with the subsequent release of proinflammatory cytokines (e.g. IL-1, IL-6, TNF-α), ROS, and profibrotic growth factors (e.g. TGF-β). The initial inflammatory response is self-amplified then causing renal injury and cell death. Furthermore, the fibrotic response induces proliferation and extracellular matrix accumulation in mesangial and tubular cells. Diabetic renal injury then progresses to glomerulosclerosis and tubulointerstitial fibrosis.

6. Acknowledgment

7. References

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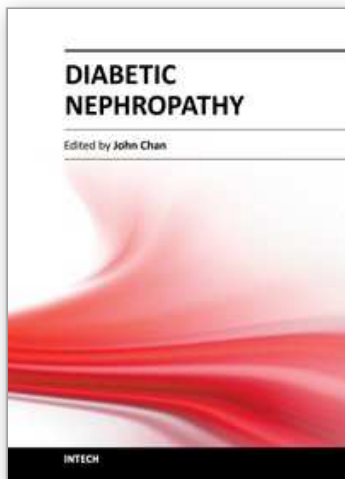
Internationally renowned experts have provided data on their own studies, and discuss the relative usefulness of their work in relation to diabetic nephropathy. The first section describes the novel role of intrarenal renin-angiotensin-aldosterone system (RAAS) and oxidative stress in the development of diabetic nephropathy and discusses the current and novel pharmacological interventions in the treatment of diabetic nephropathy. The second section discusses other important contributors outside of the RAAS in the pathogenesis of diabetic nephropathy including AGE/RAGE, epithelial-mesenchymal-transition (EMT) and immune cytokines. Features: Provides novel information on various pathophysiological determinants in the development of diabetic nephropathy Provides novel information on various pharmacological interventions of diabetic nephropathy

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