Role of inflammation in diabetic complications

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\section*{Introduction}

Diabetes mellitus, especially type 2 diabetes, is a public health problem which has reached epidemic proportions due to the rapidly increasing rates of this disease worldwide. Target organ complications, secondary to diabetes, will be one of the most important medical concerns of the coming decades. A clear example is diabetic nephropathy (DN), which has become the single most frequent cause of end-stage renal disease, in other words: ‘a medical catastrophe of worldwide dimensions’ [1].

In the last few years, our knowledge of DN and its clinical course, the factors that influence it as well as the progression of renal injury and the possibilities of effective therapeutic approaches have substantially improved. From a pathophysiological point of view, the critical importance of metabolic and haemodynamic factors for the risk of developing DN is clear. However, the intimate mechanisms leading from chronic hyperglycaemia to the development of renal injury are complex and not yet fully unravelled.

\section*{Diabetes mellitus: an evolving disease. From a metabolic disorder to an inflammatory condition}

In 1998, a hypothesis was proposed suggesting that long-term innate immune system activation, resulting in chronic inflammation, elicited disease instead of repair, leading to the development of type 2 diabetes [2]. In the last few years, numerous studies have shown that low-grade inflammation is associated with the risk of developing type 2 diabetes [3]. Furthermore, nowadays it is accepted that chronic subclinical inflammation is a part of the insulin resistance syndrome [4] and is strongly related to features of the metabolic syndrome [5].

The mechanisms by which chronic inflammation can evoke type 2 diabetes are not clear. However, it is known that adipose tissue can synthesize and
release the main pro-inflammatory cytokines, tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6), and that inflammatory markers are associated with body fat mass. Pro-inflammatory cytokines and acute phase reactants are involved in multiple metabolic pathways relevant to insulin resistance, including insulin regulation, reactive oxygen species, lipoprotein lipase action and adipocyte function [3]. Therefore, activated innate immunity and inflammation are relevant factors in the pathogenesis of diabetes, with convincing data that type 2 diabetes includes an inflammatory component [3,6].

**Inflammation and diabetic complications**

Based on this new perspective of diabetes, the next question is clear: is inflammation related to the development of diabetic complications? Recent studies have shown that inflammation, and more specifically pro-inflammatory cytokines, play a determinant role in the development of microvascular diabetic complications.

Diabetic neuropathy develops as a result of hyperglycaemia-induced local metabolic, enzymatic and microvascular changes. Pro-inflammatory cytokines are produced locally by resident and infiltrating cells. These molecules exhibit pleiotropic effects on homeostasis of glia and neurons in the central, peripheral and autonomic nervous systems. Changes induced by chronic hyperglycaemia lead to dysregulation of these cytokines. It has been demonstrated that endogenous TNF-α production is accelerated in microvascular and neural tissues, which may undergo increased microvascular permeability, hypercoagulability and nerve damage, thus initiating and promoting the development of characteristic lesions of diabetic polyneuropathy [7].

Regarding diabetic retinopathy, experimental animal investigations have shown that mRNA expression for IL-1 and TNF-α is increased in the retina early in the course of diabetes, and moreover, inhibition of TNF-α has demonstrated beneficial effects in the prevention of early diabetic retinopathy [8]. Clinical studies have shown elevated levels of pro-inflammatory cytokines in the vitreous fluid of patients with proliferative diabetic retinopathy, which are related with the activity and progression of retinal injury. These data highlighted the central and causal role of chronic low-grade subclinical inflammation in the pathogenesis of diabetic retinopathy [9].

**Diabetic nephropathy: a matter of inflammation**

A number of experimental and clinical studies have demonstrated that DN exhibits signs of inflammation. Three very recent papers illustrate the idea that inflammation plays a significant role in the pathogenesis of DN. Dalla Vestra and colleagues [10] showed that patients with type 2 diabetes and overt nephropathy exhibit the highest levels of diverse acute-phase markers of inflammation, including C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen and IL-6. Furthermore, levels of CRP, SAA and IL-6 were higher in subjects with increased glomerular basement membrane (GBM) width. More important, the authors demonstrated the association between GBM thickening, a crucial lesion of diabetic glomerulopathy, and acute phase markers (fibrinogen and IL-6). In addition, two experimental studies provide insights into the way in which renal damage in diabetes is linked to inflammation. Chow et al. [11] showed that db/db mice, a model of type 2 diabetes and DN, exhibited an increased expression of intracellular adhesion molecule-1 (ICAM-1), which promotes inflammation by increasing leukocyte infiltration and adherence, in glomeruli and tubules, along with a marked increase in macrophage infiltration. These findings strongly involve ICAM-1-induced inflammation in the development of renal injury in diabetes. In the second study, Kelly et al. [12] demonstrated in a model of diabetes and hypertension that, despite hyperglycaemia and elevated blood pressure, albuminuria was reduced and renal function was preserved in rats treated with ruboxistaurin, an inhibitor of protein-kinase C-β. Protein-kinase C has various isoforms, which are activated in diabetes and signal a number of cellular responses, including activation and expression of inflammatory mediators, such as pro-inflammatory cytokines.

The first suggestion, that pro-inflammatory cytokines could participate in the development of DN, was already made in 1991 by Hasegawa et al. [13]. These authors demonstrated that macrophages incubated with glomerular basement membranes from diabetic rats produced greater levels of IL-1 and TNF-α than macrophages incubated with membranes of normal rats.

A number of clinical studies have suggested a relationship between pro-inflammatory cytokines and DN [14,15]. Endothelial, mesangial, glomerular and tubular epithelial cells can synthesize pro-inflammatory cytokines. Importantly, these molecules have been related to significant renal effects. Interleukin-1 increases vascular endothelial permeability and has been involved in the proliferation of mesangial cells and matrix synthesis, as well as in the development of intraglomerular microcirculatory abnormalities [16,17]. Interleukin-6 affects extracellular matrix dynamics at mesangial and podocyte levels, stimulates mesangial cell proliferation, increases fibronectin expression and enhances endothelial permeability [10,18]. Finally, in recent years, most of the attention has been focused on the implications of TNF-α in the setting of DN.

**Tumor necrosis factor-α: an old actor in a new scenario**

Experimental investigations have demonstrated that mRNA expression for TNF-α is significantly increased...
in kidneys from diabetic rats compared with kidneys from normal rats [19, 20]. This cytokine is cytotoxic to glomerular, mesangial and epithelial cells, and may induce significant renal damage [21]. The direct harmful effect of TNF-α on the protein permeability barrier of the glomerulus is independent from alterations in haemodynamic factors or effects of recruited inflammatory cells [22]. From a pathogenetic perspective, it is particularly relevant that TNF-α had been suggested as a critical factor contributing to sodium retention and renal hypertrophy, important renal alterations that occur during the initial stage of DN [23]. Moreover, it has been demonstrated that increased urinary as well as renal interstitial concentrations of TNF-α precede the rise in albuminuria [24].

Clinical studies by our group have analysed the potential implications of TNF-α in DN. We have found a significant relationship between serum levels of TNF-α and urinary protein excretion in diabetic patients with normal renal function and microalbuminuria, as well as in subjects with overt nephropathy and renal failure [14,15]. On the other hand, urinary TNF-α levels were also elevated in diabetic patients with increased urinary albumin excretion, and furthermore, there was a significant rise of urinary TNF-α excretion as DN progressed. Moreover, multivariate analysis showed a significant and independent relationship between urinary TNF-α and albuminuria. Interestingly, there was no significant correlation between serum and urinary concentrations of TNF-α, suggesting that this cytokine can be produced within the kidneys [15].

Treatment of diabetic nephropathy: new perspectives, new targets

The vision of DN as an inflammatory disease triggered by altered metabolic factors opens new and important therapeutic perspectives. Diverse studies have shown that the administration of substances with anti-inflammatory properties were able to reduce the expression of mediators of renal injury and prevent the development of glomerular and tubulointerstitial damage in experimental diabetes [25–27]. More important, several of these observations, such as those related to modulation of TNF-α, have had important translational implications.

The methylxanthine derivate pentoxifylline (PTF) is a clinically available phosphodiesterase inhibitor that, in addition to its haemorheological activity, possesses significant immunoregulatory and anti-inflammatory properties. It has been demonstrated that PTF inhibits the accumulation of TNF-α mRNA and the transcription of the TNF-α gene, suppressing the production of this cytokine [28]. In vivo, PTF has shown its efficacy in different models of renal diseases, including lupus nephritis, crescentic glomerulonephritis, mesangial-proliferative glomerulonephritis and the remnant kidney model, where several inflammatory mediators, such as TNF-α or ICAM-1, were involved. Regarding DN, recent studies by DiPetrillo et al. [29] and our group (unpublished data), have found that PTF administration prevented the enhanced renal TNF-α expression, synthesis and excretion during diabetes. In addition, PTF therapy ameliorated sodium retention and renal hypertrophy, the initial pathological changes associated with DN.

Together with these experimental observations, clinical trials support the efficacy of PTF as a therapeutic agent to treat DN. Several studies have shown that PTF reduces urinary protein excretion in diabetic subjects, both with normal renal function [30,31] and renal insufficiency [14]. Interestingly, a very recent work compared the effects of PTF and captopril on proteinuria in patients with type 2 diabetes mellitus [32]. This work demonstrated that PTF (400 mg three times a day) resulted in an equivalent antiproteinuric effect to captopril (25 mg three times a day). Finally, combination of PTF with blockers of the rennin–angiotensin system, both angiotensin converting enzyme inhibitors [33] or angiotensin II receptor blockers (ARB) [34], is associated with significant reduction in urinary albumin excretion. Importantly, diabetic patients with residual albuminuria after long-term treatment with ARB may obtain a beneficial additive antiproteinuric effect after administration of PTF, which is associated with a reduction of urinary TNF-α excretion [34].

In conclusion, the pathogenetic vision of diabetes mellitus has changed in the last few years, with inflammatory pathways playing pivotal roles in the development and progression of diabetic complications. These new pathogenic factors lead to a consideration of new therapeutic approaches. Modulation of inflammatory processes in the setting of diabetes is nowadays a matter of great interest. It is possible that in the coming years the hope of new therapeutic strategies based on anti-inflammatory properties with beneficial actions on diabetic complications can be translated into real clinical treatments.

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References

24. Kalantarinia K, Awas AS, Siragy HM. Urinary and renal interstitial concentrations of TNF-α increase prior to the rise in albuminuria in diabetic rats. *Kidney Int* 2003; 64: 1208–1213