EDITORIALES

Role of TNF and IL- 1 in the development of diabetic nephropathy

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Cytokines are locally active polypeptide mediators that are secreted by not only inflammatory cells but also most cells including endothelial, epithelial and mesenchymal cells, and they play a key role in controlling growth, biosynthetic activities and functions of cells. Accumulating *in vitro* and *in vivo* evidences have implicated several cytokines as mediators of glomerular injury [1, 2]. Of all cytokines, much of the work published to date has focused on the role of interleukin-1 (IL-1) and tumor necrosis factor a (TNFα) in the pathogenesis of glomerulonephritis[3, 4].

The mechanism by which diabetic nephropathy occurs is most likely multifactorial based on the chronic metabolic disturbance. The potential participation of inflammatory cytokines on the pathogenesis of diabetic nephropathy has been hardly considered. In this paper, we describe the possibility that IL-I and TNFα could participate in the development of diabetic nephropathy.

ADVANCED GLYCATION END-PRODUCT (AGE) AND MACROPHAGE-MONOCYTE RECEPTOR SYSTEM FOR AGE

Glucose can react with protein non-enzymatically. In this reaction, glucose first form Schiff bases at a rate proportional to the glucose concentration. These Schiff bases rearrange to form the more stable Amadori-type early glycation products. Early glycation products are chemically reversible equilibrium products, and *in vivo* they increase when blood glucose levels are high and return to normal after the glucose levels are normalized [5]. This reaction is clinically applied to evaluate the condition of glycemic control in diabetic patients by measuring glycated hemoglobin.

Some of the early glycation products on long lived structural proteins such as collagen undergo a slow, complex series of chemical rearrangements to form irreversible, fluorogenic cross-linkages; fluorogenic parts of such cross-linked proteins are named advanced glycation end-product (AGE) [5-7]. AGE is a complex product with the various intermediates, and its structure has not been completely elucidated. Recently, some AGE products have been structurally characterized, and they include carboxymethyllysine, pentosidine, and crosslines [8-10]. It has been recognized that AGE could alter the structural properties of tissue proteins and reduce their susceptibility to catabolism. These alterations can contribute to the aging of tissue, and to diabetes related functional and structural changes in tissues involved in micro and macrovascular complications [7, 11]. Recently, several clinical studies have reported a link between AGE accumulation and the complications of diabetes [12-15].

Vlassara et al. identified the macrophage-monocyte receptors which specifically bind AGE-modified proteins [16, 17]. It has been suggested that these receptors uptake and degrade AGE and function as a scavenger receptors [18]. Furthermore, they demonstrated that binding of AGE-modified proteins to this receptors stimulates synthesis and release of TNF and IL- 1 from macrophages [19]. These cytokines are considered to account for the normal tissue remodeling with the removal and replacement of senescent extracellular matrix components.

TNF AND IL-1 RELEASE FROM MACROPHAGES IN RESPONSE TO DIABETIC GLOMERULAR BASEMENT MEMBRANE

Glomerular extracellular matrix consisting of collagen IV, laminin, fibronectin, and other glycoproteins

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could become a target for AGE modifications. AGE accumulation in glomerular basement membrane of experimental diabetic animals has been demonstrated [20, 21]. Furthermore, AGE were detected in sclerosed glomeruli from patients of diabetic nephropathy by immunohistochemical procedures [22]. Thus, we presumed that AGE-modified glomerular basement membrane could stimulate macrophages to release TNF and IL-1 through the binding of AGE and its receptor, and this event would result in the disturbance of homeostasis in glomerular microenviroment.

To evaluate this possibility, we carried out the experiments using streptozocin-treated diabetic rats [23]. Diabetic and normal Lewis rats were divided into two groups each; one that received daily intraperitoneal injection of sterile saline alone (DM group and C group) and one that received sterile saline containing 25 mg/kg body weight of aminoguanidine (DM-AG group and C-AG group). Aminoguanidine is a nucleophilic hydrazine compound which inhibits the progression of early glycation product to AGE formation [24]. After 12 weeks of the treatment, glomerular basement membrane (GBM) were isolated from rats of each experimental groups. Then, thioglycollate-elicited peritoneal macrophages (1×10^6) from normal rats (aged 7 to 8 weeks) were incubated with several volume of these GBM materials. After incubation for 24 hours, the supernatants were harvested and TNF and IL-I were assayed by bioasay.

As shown in figure 1, GBM from DM rats induced significantly greater levels of TNF and IL-1 production from macrophages than did GBM from other three groups at doses of 2,5 to 10 mg (wet weight). On the other hand, TNF and IL- 1 productions from macrophages induced by GBM from DM-AG rats were similar to those induced by GBM from C and C-AG rats. Early glycation products of proteins developed in GBM from diabetic rats. Treatment with aminoguanidine did not modify the level of early glycation products. Furthermore, the accumulation of fluorescent AGE in GBM from DM rats was significantly greater than those in GBM from other three groups. In aminoguanidine-treated diabetic animals exposed to identical levels of hyperglycemia, however, the level was nearly normal (table I).

These data demonstrated GBM from diabetic rats stimulated macrophages to produce greater levels of TNF and IL-1 than did normal GBM, and suggested that AGE-proteins, which were accumulated on collagen or other structural proteins of diabetic GBM, could be involved in the production of these cytokines from macrophages.

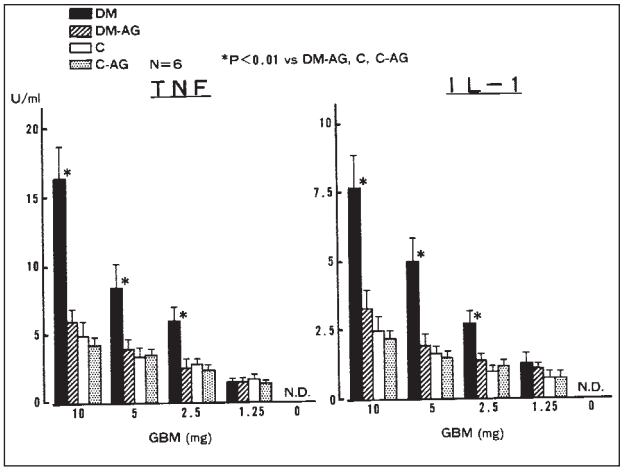

*Fig. 1.–TNF and IL-1 production by thioglycollate-elicited peritoneal macrophages in response to glomerular basement membrane (GBM) from DM (solid bar), DM-AG (hatched bar), C (open bar), and C-AG (stippled bar) group. 1×10^6 cells were incubated in the medium containing streptomycin, penicillin, and 5 % FCS in the presence of various amount of GBM from each experimental group for 24 hours at 37 °C. GBM from DM group induced significantly greater levels of TNF and IL-1 production than did GBM from other three groups with at doses of 2.5 to 10 mg. Data are shown as means ± SEM of six experiments, with a triplicate determination. *P < 0,01 vs. GBM from the other three groups. ND: not detected. From Hasegawa et al. [23].*

POSSIBLE ROLE OF TNF AND IL-1 IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY

Recently, Nakamura et al. [25] demonstrated the increased mRNA expression of some growth factors, including TNFα, in glomeruli of streptozocin-induced diabetic rats. This finding also suggests the possibility of participation of TNF in the development of diabe-

Table I. Contents of AGE and non-enzymatic glycated proteins in GBM of the four experimental groups

Groups	N	AGE content[a]	Non-enzymatic glycation[b]
DM	5	3.43 ± 0.36[c]	19.2 ± 1.9[d]
DM-AG	5	1.92 ± 0.17	18.4 ± 20[d]
C	5	1.68 ± 0.16	9.2 ± 0.5
C-AG	5	1.70 ± 0.22	8.9 ± 0.9

[a] Specific fluorescence per µmol hydroxyproline.
[b] nmoles 5-hydroxymethylfurfuralaldehyde per mg protein.
[c] p < 0.01 vs. DM-AG, C, and C-AG groups.
[d] P < 0.01 vs. C and C-AG group.

GBM samples were obtained from each group of rats after 12 weeks of the treatment as described in the text. GBM sarnples were solubilized by incubation for 72 hours at 37 °C with highly purified collagenase. The solubilized GBM fraction was analyzed by measuring the fluorescence at 440 nm upon excitation at 370 nm, and then hydrolyzed for determination of hydroxyproline levels. Portions of the GBM samples were solubilized with 1N NaOH and early glycation product was estimated by a thiobarbituric color assay. From Hasegawa et al.[23].

tic nephropathy, although they did not refer to the effector cells and mechanism for that phenomenon. Our data described above are based on the theory of macrophage-monocyte receptor system for AGE which has been suggested by Vlassara et al. [19]. However, other than AGE, immune complex or unknown factors which are deposited or trapped in GBM from plasma should be taken in consideration as one of stimulatory factors for macrophages [26-27].

TNF and IL-1, produced by macrophages in response to AGL-proteins, are considered to account for the normal tissue remodeling [19]. However, in diabetic state, it has been proposed that AGE-protein cross linkings reduce the susceptibility to digestion by nonspecific proteases and lead the synthetic and proliferative response to be enhanced with locally oversecreted cytokines [11]. It is well known that TNF and IL-1 have broad biological activities on various cells. Among them, their effects on glomerular mesangial cells to stimulate collagen synthesis [28] and prostaglandin production [29], or on endothelial cells to induce procoagulant activity and to increase permeability [30, 31], are consistent with pathophysiology of diabetic nephropathy.

Monocytes recruited from peripheral blood could become an effector cell, however, they are apparently not the only source of cytokines within glomeruli. Numerous cell culture studies have shown that glomerular mesangial cells produce TNF and IL-1 [3, 4]. Furthermore, it has been reported that these cells have a receptor for AGE, and this receptor system has a role for matrix protein regulation in the kidney [32, 33]. Although secretion of cytokines in response to AGE from glomerular mesangial cells has not been demonstrated, these cells could be potent candidates for effector cells.

We should note that TNF and IL-1 activities induced by diabetic GBM were only two-to threefold increased in comparison with the activities induced by normal GBM. This means AGE is weak stimulus for macrophages to produce these cytokines. Taken together with the difference of pathological features between diabetic nephropathy and immune-mediated glomerulonephritis, we would say IL-1 and TNF is not a main factor for the pathogenesis of diabetic nephropathy. However, it is possible that local disturbance of homeostasis induced by excessive release of IL-1 and TNF may accelerate the progression of nephropathy together with the other metabolic and hemodynamic factors.

AGE accumulates in tissue in time- and ambient blood glucose concentration-dependent manner [6, 7]. Therefore, it is considered that a larger amount of AGE accumulates in kidney of overt diabetic nephropathy on which any attempts to improve glycemic control have no beneficial effect. In this stage of diabetic nephropathy, it may be reasonable to assume that IL-1 and TNF would have pathological significance and accelerate the development of end-stage glomerulosclerosis.

CONCLUSION

In this paper, we described the possibility that IL-1 and TNF may participate in the pathogenesis of diabetic nephropathy. However, up to now, no studies have provided the direct evidence for this.

The molecular approach for diabetic nephropathy has just begun recently. Future studies should elucidate the pathological network of growth factors which are involved in the progression of diabetic nephropathy.

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