Role of Different Forms of Basic Fibroblast Growth Factor and Transforming Growth Factor in the Development of Nephrotoxic Nephritis in Rats

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The modeling of rat nephrotoxic nephritis showed that the development of matrix-associated forms of basic fibroblast growth factor and transforming growth factor-β1 can be one of the mechanisms regulating their activity. Disturbances in the development of these associated complexes may result in glomerulosclerosis.

Key Words: *basic fibroblast growth factor; transforming growth factor-β1; extracellular matrix; nephrotoxic nephritis*

Study of glomerulopathy revealed an important role of various cytokines and growth factors in the processes of damage and reparation of glomeruli, proliferation of mesangial cells, and accumulation of extracellular matrix (ECM) [8]. Experimental models demonstrating various glomerular damage mechanisms are widely used to study the role of cytokines and growth factors in the genesis of glomerulopathies. Nephrotoxic nephritis is a useful model to investigate the interactions of blood cells with glomerular resident cells, cell-matrix relationships, regulation of cell proliferation, and formation of ECM. This type of nephritis is characterized by damage to the endothelium of glomerular capillaries and glomerular basal membrane, by infiltration of glomeruli with mononuclear leukocytes, proliferation of mesangial cells, and hypertrophy of ECM. In addition to proinflammatory cytokines (interleukin-1 and tumor necrosis factor-α), cell proliferation and ECM production are regulated by basic fibroblast growth factor (bFGF) and the transforming growth factor-β1 (TGF-β1) [8]. Factor bFGF stimulates the cells of mesenchymatous and ectodermic origin; it is the major stimulator of angiogenesis [2]. Factor TGF-b1 is assumed to be a major inhibitor of proliferation of mesangial cells [6] and a stimulator of the synthesis of ECM components. It stimulates production of laminin, fibronectin, collagen IV, and heparan sulfate proteoglycan inhibits expression of proteases, and stimulates expression of protease inhibitors [1].

An important feature of bFGF and TGF-β1 is the ability to associate with various components of ECM: bFGF binds to heparan sulfate proteoglycans of ECM and basal membranes [3], while TGF-β1 binds to fibronectin [7], collagen IV [9], and to the proteoglycans decorin and betaglycan [12]. The growth factors deposited in ECM are not destroyed by ECM enzymes [5]. The deposited factors are activated by plasmin [11]. Thus, association of bFGF and TGF-β1 with ECM components opens the possibilities of local and delayed effect of these growth factors [5,10].

Our aim was to characterize soluble, membrane- and matrix-associated forms of bFGF and TGF-β1 in the model of nephrotoxic nephritis.

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MATERIALS AND METHODS

Nephrotoxic nephritis was modeled in male Wistar rats weighing 180-200 g by preimmunization with standard rabbit serum with Freund's adjuvant followed 5 days after by injection of nephrotoxic serum (110 mg/kg). The rats were divided into three groups 5 animals each: control group (physiological saline), acute nephritis (day 14 after serum injection), and chronic nephritis (day 40 after serum injection). Control rats were injected with an equivalent volume of saline. The glomeruli were isolated by the standard method. They were cultured in RPMI-1640 (Sigma) [4]. The contents of soluble, cell- and matrix-associated forms of bFGF and TGF-β1 were determined by enzyme R&D immunoassay. In addition, renal fragments were extracted for immunohistochemical examination by immunoperoxidase streptavidin-biotin method. The following substances were used: polyclonal antibodies to collagens I, III, IV, and V (Cardiology Research Center) bFGF (Sigma), monoclonal antibodies to laminin, tissue and plasma fibronectin (Sigma), biotin-conjugated secondary antibodies, and peroxidase-conjugated streptavidin (DAKO, Copenhagen, Denmark).

RESULTS

The acute stage of nephrotoxic nephritis was characterized by enhanced levels of cell- and matrix-associated bFGF (Fig. 1). At this stage soluble bFGF was absent. By contrast to bFGF, three forms of TGF-β1 were detected at this stage: soluble, cell-, and matrix-associated (Fig. 2). The content of all these forms was enhanced during the acute stage of nephrotoxic nephritis.

During the chronic stage of nephritis, the content of matrix-associated form of bFGF was drastically decreased (Fig. 1). The level of cell-associated bFGF decreased insignificantly. As in the acute stage, there was no soluble bFGF at the chronic stage. At this stage, the level of cell-associated TGF-β1 decreased insignificantly (Fig. 2), the level of matrix-associated TGF-β1 almost decreased to the control level, and the content of soluble form of TGF-β1 decreased insignificantly in comparison with the acute stage of nephrotoxic nephritis.

Thus, both acute and chronic phases of nephritis were characterized by different ratios of soluble, cell- and matrix-associated forms of bFGF and TGF-β1, mostly due to change in the level of the matrix-associated forms. Dramatic changer of the matrix-associated forms of bFGF and TGF-β1 are probably related to changes in ECM structure at the acute and chronic stages of nephritis. The acute phase is characterized by damage and detachment of endothelium from glomerular basal membrane and by entry of negatively charged plasma proteins into membrane and mesangial matrix. ECM synthesis is intensified at this stage predominantly due to heparan and dermatan sulfate proteoglycans, that bind bFGF and TGF-β1. At the chronic stage of nephritis, the synthesis of ECM is more pronounced than at the acute stage. At the same time, structural composition of ECM changes: the interstitial collagens I and III appear, while the content of proteoglycans reduces. The latter is probably related to a drastic decrease in matrix-associated bFGF and TGF-β1 at the chronic phase of nephritis.

The development of matrix-associated forms of bFGF and TGF-β1 is probably one of the mechanisms of regulation of their activity. Deposition of bFGF and TGF-β1 in ECM at the acute stage of nephrotoxic nephritis decreases activity of these growth factors, although it makes possible their local and delayed action. Disturbances in matrix-associated bFGF and TGF-β1 occurring at the chronic phase of nephritis may increase their ac-

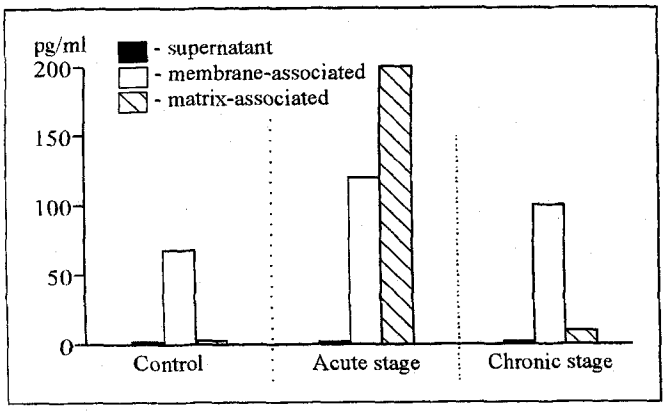

Fig. 1. Distribution of cell- and matrix-associated forms of basic fibroblast growth factor during nephrotoxic nephritis.

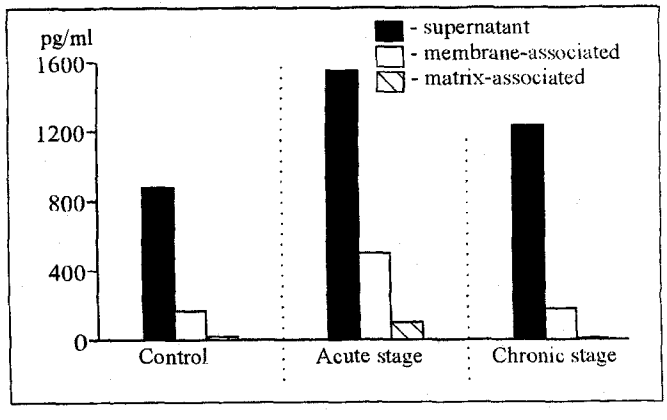

Fig. 2. Distribution of soluble, cell-, and matrix-associated forms of transforming growth factor-β1 during nephrotoxic nephritis.

tivity, which results in enhancement of mesangial cells prolifera-tion, ECM hypertrophy, and glomerulosclerosis.

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