MORPHOLOGY AND PATHOMORPHOLOGY

Effects of Immunosuppressors and Cytostatics on the Extracellular Matrix Production in Experimental Nephropathy

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Methylprednisolone increased the content of laminine, cyclosporin increased the content of fibronectin, and cyclophosphamide suppressed the extracellular matrix production in experimental nephrotoxic nephritis and puromycin aminonucleoside nephrosis. Therapy should be chosen with due consideration of the extracellular matrix structure in different morphological variants of chronic glomerulonephritis.

Key Words: *extracellular matrix; prednisolone; cyclophosphamide; cyclosporin A*

Accumulation of the extracellular matrix, a morphological indicator of the majority of renal glomerular diseases leading to glomerulosclerosis, is the key sign of progressing glomerular injury [1]. Glomerulosclerosis is the cause of stable, often irreversible dysfunctions of the kidneys, eventuating in chronic renal insufficiency.

The results of symptomatic and pathogenetic therapy with steroid hormones and cytostatics are contradictory. There are no strict indications for the choice of this or that class of drugs. The effectiveness of therapies is probably associated with pathogenetic mechanisms of injuries to the renal glomeruli, both immune and nonimmune [2,5].

We compared the ratio of the extracellular matrix components during therapy of experimental chronic glomerulonephritis by methylprednisolone (MP), cyclophosphamide, and cyclosporin A.

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MATERIALS AND METHODS

Nephrotoxic nephritis (NTN) and puromycin aminonucleoside nephrosis (PAN) were induced in Wistar rats weighing 80-100 g with antibodies to glomerular basal membrane and puromycin aminonucleoside (Sigma), respectively. Normal saline was injected to controls. On day 40 of NTN development and on day 70 of PAN, group 1 animals were exposed to three pulses of MP (70 mg/kg) for 3 days, group 2 rats received two oral doses of cyclophosphamide (15 mg/kg) once a week, and group 3 was injected intraperitoneally with cyclosporin A (Sandimun, Sandos) in a dose of 15 mg/kg for 9 days. The drug doses corresponded to those used in chronic glomerulonephritis therapy. Immunohistochemical study with antibodies to types I, III, and IV collagen, laminine, plasma and cell-associated fibronectin (Sigma) was carried out on 4-5-μ cryostat sections and in a culture of renal glomerular mesangial cells by the streptavidin peroxidase method (LSAB, DAKO). The intensity of the immunoperoxidase reaction was evaluated by the semiquantitative method. Experi-

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mental models and mesangial cell culture were prepared at the Department of Cellular and Molecular Pathology, Research Center of I. M. Setchenov Moscow Medical Academy.

RESULTS

The chronic stage of NTN and PAN was characterized by accumulation of type IV collagen; the deposition of laminine (Fig. 1, *a*) and of two forms of fibronectin was more pronounced in NTN (Table 1). Interstitial collagen (types I and III) was accumulated in the renal glomeruli of rats with NTN.

MP increased the accumulation of laminine (Fig. 1, b) and plasma fibronectin *in vivo* and *in vitro* in both types of glomerulonephritis and decreased the content of type IV collagen *in vitro*. In addition, MP suppressed the production of types I

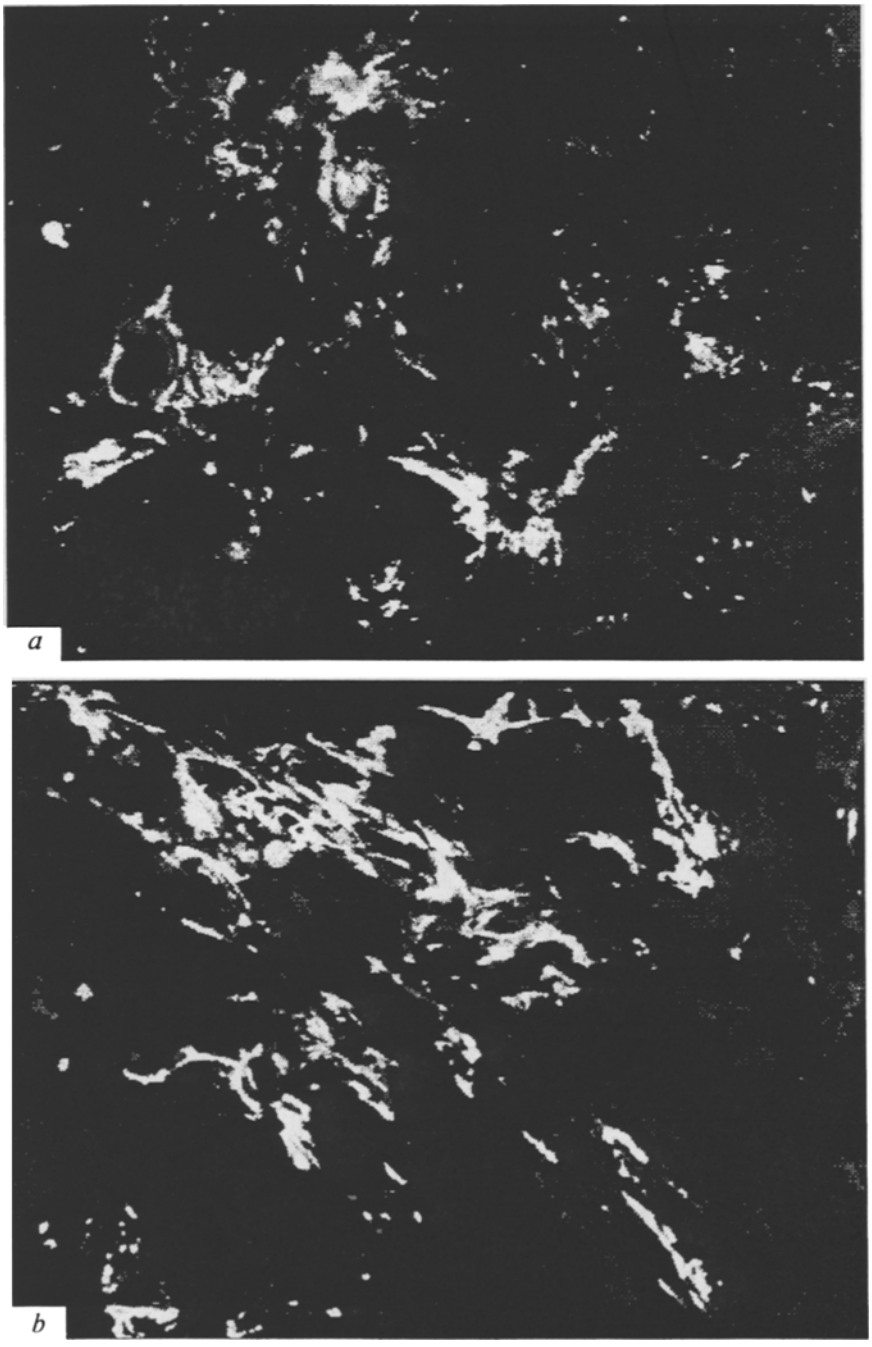

Fig. 1. Chronic stage of nephrotoxic nephritis. *a*) laminine distribution in rat mesangial cell culture; *b*) increased laminine deposition in rat mesangial cell culture under the effect of methylprednisolone. Immunofluorescent method, ×400.

TABLE 1. Distribution of the Extracellular Matrix Components during Treatment of Chronic NTN and PAN with Various Immunomodulators

Components	No treatment		Control		MP		Cyclophosphamide		Cyclosporin	
	in vivo	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*
NTN										
Collagen										
I	+-	+	—	+-	—	+-	—	+-	—	+-
III	+	+	—	+	—	+	—	+-	—	+-
IV	++	+++	+	+	++	++	++	++	++	++
Laminine	+	+	+	+	++	+++	+	+	+	+-
Fibronectin										
tissue	+	++	+-	+	++	++	+	+	++	++
plasma	+	+-	+-	—	+	+	+	+-	+	+++
PAN										
Collagen										
I	—	+-	—	+-	—	+-	—	+-	—	+-
III	—	+-	—	+	—	+-	—	+-	—	+-
IV	++	++	+	+	++	+	++	+	++	+
Laminine	+	+	+	+	++	++	+	+	+	+-
Fibronectin										
tissue	+-	+	+-	+	+	+	+-	+	+	++
plasma	+-	—	+-	—	++	+	+-	+-	+	+++

Note. "-" no immunoperoxidase reaction; "+-" slightly positive reaction in 20% examined glomeruli or visual fields in mesangial cell culture; "+" slightly positive reaction in all examined glomeruli or in the whole culture well; "++" positive staining; "+++" intensive reaction.

and III collagen in renal glomeruli in NTN and stimulated the accumulation of cell fibronectin in the glomeruli in PAN. In both models, cyclophosphamide suppressed the accumulation of type IV collagen and cellular fibronectin *in vitro* and slightly increased the deposition of plasma fibronectin in PAN *in vitro*. Cyclophosphamide suppressed the accumulation of atypical interstitial collagens in renal glomeruli in NTN.

Cyclosporin A had no cytotoxic effect on the renal glomerular and canalicular epithelial cells. It suppressed the production of types I and III collagen in the glomeruli in NTN, decreased the deposition of type IV collagen and laminine in both models *in vitro*, promoted an increase in the content of plasma fibronectin *in vitro* and of cellular fibronectin *in vivo* in NTN. In PAN, the content of cellular fibronectin and plasma fibronectin increased both *in vivo* and *in vitro*.

A comparison of the drug effects on the structure of extracellular matrix revealed the following regularities. Despite the specific features in the structure of extracellular matrix and differences in the mechanisms of injury in NTN and PAN, each drug produced identical effects in both models. Methylprednisolone increased the laminine content *in vivo* and *in vitro* and decreased the accumulation of type IV collagen *in vitro* without changing the accumulation of type IV collagen in the glomeruli.

Cyclophosphamide and cyclosporin A suppressed the appearance of interstitial collagens in renal glomeruli and decreased the deposition of type IV collagen in mesangial cell culture. Cyclosporin A suppressed the production of laminine and increased the accumulation of cell-associated and plasma fibronectin in the extracellular matrix, which was particularly pronounced *in vitro*.

Unlike other agents, cyclophosphamide had no appreciable effect on the production of laminine and decreased fibronectin deposits in the renal glomeruli and in cell culture produce and slightly increased the content of plasma fibronectin *in vitro* in PAN.

The immunomodulators used in antinephritic therapy exert different effects on the extracellular matrix production and induce qualitative and quantitative changes in its structure [6]. All extracellular matrix components support the cell-matrix interactions in the renal glomerulus through integrin receptors, thus providing cell adhesion and acting as chemoattractants [3]. Therefore, structural changes in the extracellular matrix can induce the mesangial zone infiltration with mononuclear leukocytes and stimulate mesangial cells, the main producers of the

extracellular matrix components and proteases. The imbalance between production and utilization of the extracellular matrix components leads to the development of sclerosis or recovery of normal structure of the extracellular matrix [4]. Therefore, the structure of the extracellular matrix in various morphological variants of chronic glomerulonephritis should be taken into consideration during the treatment of patients by MP, cyclophosphamide, and cyclosporin A.

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