MORPHOLOGY AND PATHOMORPHOLOGY

Ultrastructural and Biosynthetic Reactions in Cell Populations of Renal Glomeruli of the Kidney under Conditions of Glomerular Pathology

L. M. Nepomnyashchikh, G. I. Nepomnyashchikh, S. V. Aidagulova, T. A. Telegina, and N. O. Podol'skaya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 11, pp. 591-596, November, 1999
Original article submitted November 1, 1999

Proliferative and degenerative variants of structural and functional changes in the cell populations of renal glomeruli are revealed. These variants differ in the intensity of biosynthetic reactions in endotheliocytes, mesangiocytes, and parietal epitheliocytes. The degenerative variant is characterized by inhibition of RNA and DNA synthesis and ultrastructural alterations in endotheliocytes, which attests to the leading role of damage to glomerular endothelial associations in destruction of renal glomeruli.

Key Words: *glomerular pathology; nephrobiopsy; endothelium; radioautography; electron microscopy*

The leading immunological pathogenetic concept of primary glomerulopathy is based on the immunocomplex and autoimmune damage [3,11]. The damaging effect of mononuclear leukocytes plays a role in the progress of glomerulonephritis [13]. In addition, the pronounced changes in the glomerular cells are described [9] indicating that the destructive and sclerotic processes in glomeruli are related not only to the immune reactions, but also to the state of cell populations and extracellular matrix in glomeruli [5,6,8,12,15].

Because of some anatomical and physiological peculiarities (intense blood supply, complex metabolic processes, intense tubular transport, and excretion of various metabolites and toxins), the kidneys are very sensitive to damaging effect of various environmental factors [7]. The population character of renal damage is related to drug aggression, degrading quality of drinking water, and other unfavorable anthropogenic environmental factors [2], therefore the search for markers of environment-related nephropathy and development of new approach to pathogenesis of glomerular diseases are of particular importance [14].

We carried out a complex pathomorphological study of glomerular cell populations during glomerular diseases documented by a complex of clinical morphological markers.

MATERIALS AND METHODS

We examined renal bioptates from 82 patients (49 men and 33 women at the age of 14-71 years). The specimens were studied by light and electron microscopy, as well as by radioautography. For light microscopy, the major part of renal bioptate was fixed in 10% neutral formalin and processed routinely. The paraffin sections were stained with hematoxylin and eosin in combination with Perls' reaction. Elastic fibers were stained with Weigert resorcine-fuchsin according to

Laboratory of General Pathological Anatomy, Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

0007-4888/99/0011-1174$22.00

Van Gieson method and Schiff reaction with iodine acid was performed. Amyloid deposits were revealed by Congo red and gentian violet staining.

For electron microscopy, the smaller portion of specimens (<1 mm^3) was fixed in 4% paraformaldehyde and postfixed in 1% OsO_4. After routine treatment, the specimens were embedded in Epon-Araldite. Semithin sections were cut on a Tesla ultratome and stained with 1% azure II. Ultrathin sections were prepared on a LKB III ultratome, contrasted with uranyl acetate and lead citrate, and examined under a JEM-1010 electron microscope operated at 80 kV accelerating voltage.

The intensity of biosynthetic reactions in cell populations in glomeruli was assayed by *in vitro* radioautography of renal bioptates after incubation with radioactive RNA and DNA precursors [10]. The fragments of renal bioptates (<1 mm^3) were placed into the cultural flasks with media 199 containing labeled DNA and RNA precursor and incubated for 1.5 h at 37°C. The intensity of RNA synthesis was measured with ^{3}H-uridine (200 μCi/ml, specific radioactivity 26.6 Ci/mM). DNA synthesis was assessed by incorporation of ^{3}H-thymidine (100 μCi/ml, specific radioactivity 48 Ci/mM). After incubation the specimens were washed in Millonig phosphate buffer (pH 7.2-7.4), fixed in 4% paraformaldehyde, and routinely processed for electron microscopic examination. Semithin sections were covered with M-type photoemulsion and exposed for 7 and 13 days at 4°C, then photographic processing and azure II staining of the sections were performed. The label density and labeling index for the glomerular cell elements and tubules were evaluated under a light microscope at ×600.

RESULTS

Two basic variants of diffuse damage to the renal glomerular compartment were revealed: proliferative and destructive damage.

The first variant is characterized by changes in all glomerular cell populations. Glomerular endotheliocytes had signs of functional activity: large nuclei with focal marginal chromatin condensation and nucleolus, free ribosomes and polysomes in the perinuclear space and in thickened peripheral cytoplasmic processes, multiple profiles of the granular cytoplasmic reticulum, small mitochondria, and varying number of pynocytotic vesicles. On the luminal surface, long polymorphous pseudovilli (Fig. 1, *a*) formed arcades and plexuses (Fig. 1, *b*). The volume of cytoplasm increased, while the number of fenestrae decreased. In some endotheliocytes, focal destruction of membrane organelles with the formation of polymorph residual bodies was observed.

In the lumen of glomerular capillaries, which were often markedly narrowed due to hypertrophy of the peripheral processes of endotheliocytes, we observed erythrocytes, platelets, polymorphonuclear leukocytes, and monocytes that formed contacts with apical surface of endothelial associate through their cytoplasmic processes.

The basal glomerular membrane appeared intact but widened in the paramesangial regions (Fig. 1, *c*). Thickening of the basal membrane fragments, interposition of mesangial matrix with mesangial cells, and solitary deposits were seen.

Proliferative variants of glomerular damage was characterized by pronounced proliferation of mesangial cells accompanied by accumulation of mesangial matrix, widening of the mesangial zone, accumulation of membrane-like substance in the mesangium, and the appearance of electron dense agglomerates in the paramesangial region. Most mesangiocytes had signs of active function: increased number of cytoplasmic processes, numerous profiles of the granular cytoplasmic reticulum, ribosomes, well-structured small mitochondria. Occasionally we observed vacuole-like structures, phagolysosomas, and fibrillar structures. Somewhere active proliferation of mesangiocytes was accompanied by focal proliferation of endotheliocytes.

The podocytes also significantly changed. In some regions "fused" cytopodia formed continuos lining of the urinary space. Hyperplasia of fibrillar structures and sometimes villiform transformation of podocytes were observed.

The destructive variant of glomerular diffuse damage was characterized by polymorphous ultrastructural alterations in glomeruli. In most cases endotheliocytes had drastically thinned and fragmentary peripheral processes, in which fenestra were replaced by pores (Fig. 2, *a*). The cytoplasm was characterized by high electron density, the presence of single blurred organelles in the perikaryon and low pinocytotic activity. Alterations in the mesangial compartment were minor (Fig. 2, *b*), sometimes insignificantly widened mesangial regions without proliferation of mesangiocytes were seen. Degenerative changes in podocytes were common feature (Fig. 2, *c*): decrease in the volume and depletion of the cytoplasm, disorganization of the trabecular structure, reduction of the protein-synthesizing compartment, increased number of fibrillar structures, vacuolization of the cytoplasm, and frequent villiform transformation. Fusion of cytopodia and focal desquamation of podocytes from the glomerular basal membrane were most important.

Radiography analysis revealed significant difference in proliferative and metabolic activity of glomerular cells in the examined variants of glomerular alterations. Proliferative variant was characterized by

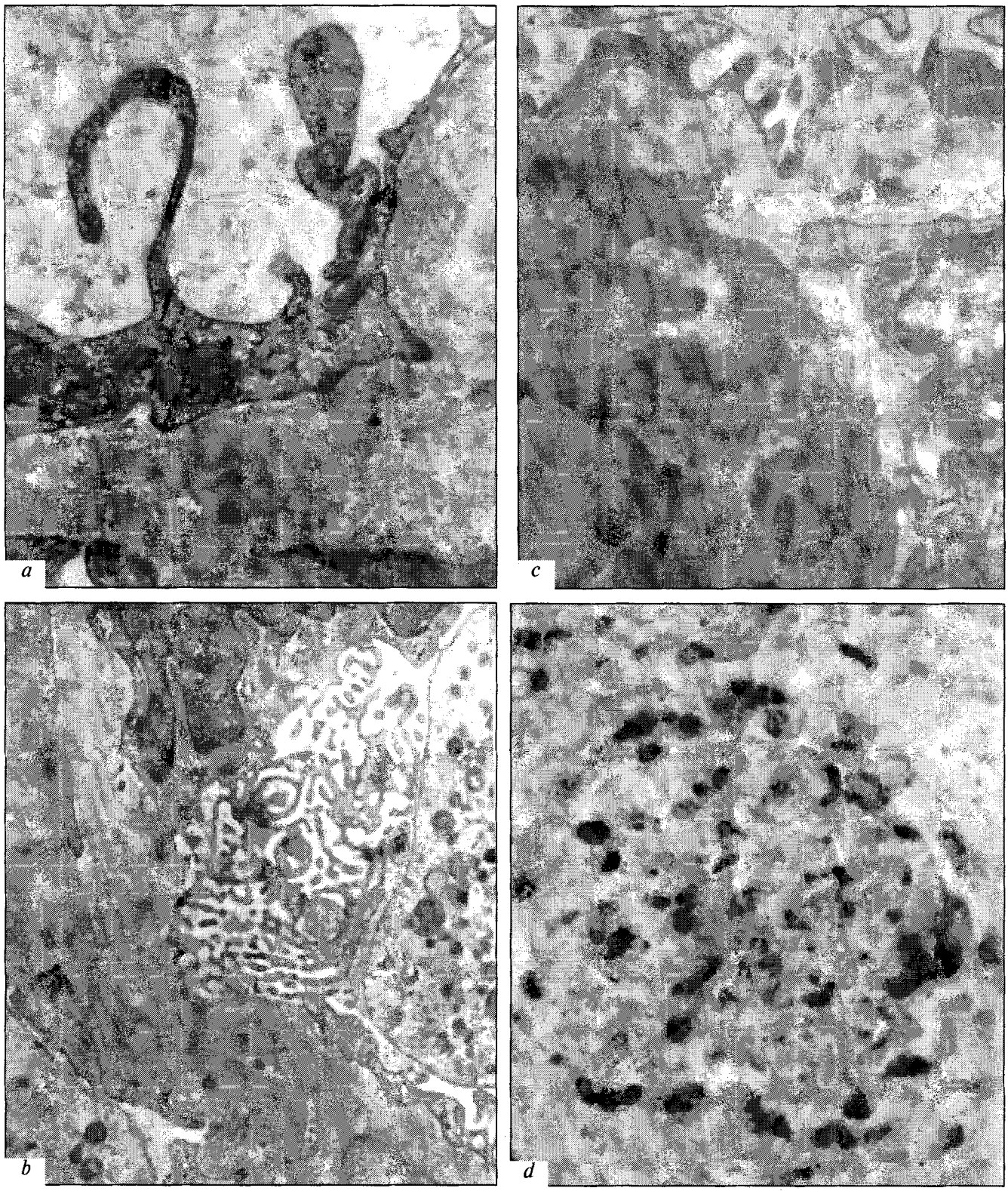

Fig. 1. Proliferative variant of alteration of glomerular cell population. *a*) thickening of cytoplasmic peripheral processes and formation of pseudovilli on the luminal endotheliocyte surface, ×10,000. *b*) formation of arcades and plexuses by endotheliocyte processes and mesangial interposition, ×6000. *c*) accumulation of mesangial matrix, ×5000. *d*) high level of RNA synthesis. Incubation with ^{3}H-uridine. Semithin section stained with azure II, ×350.

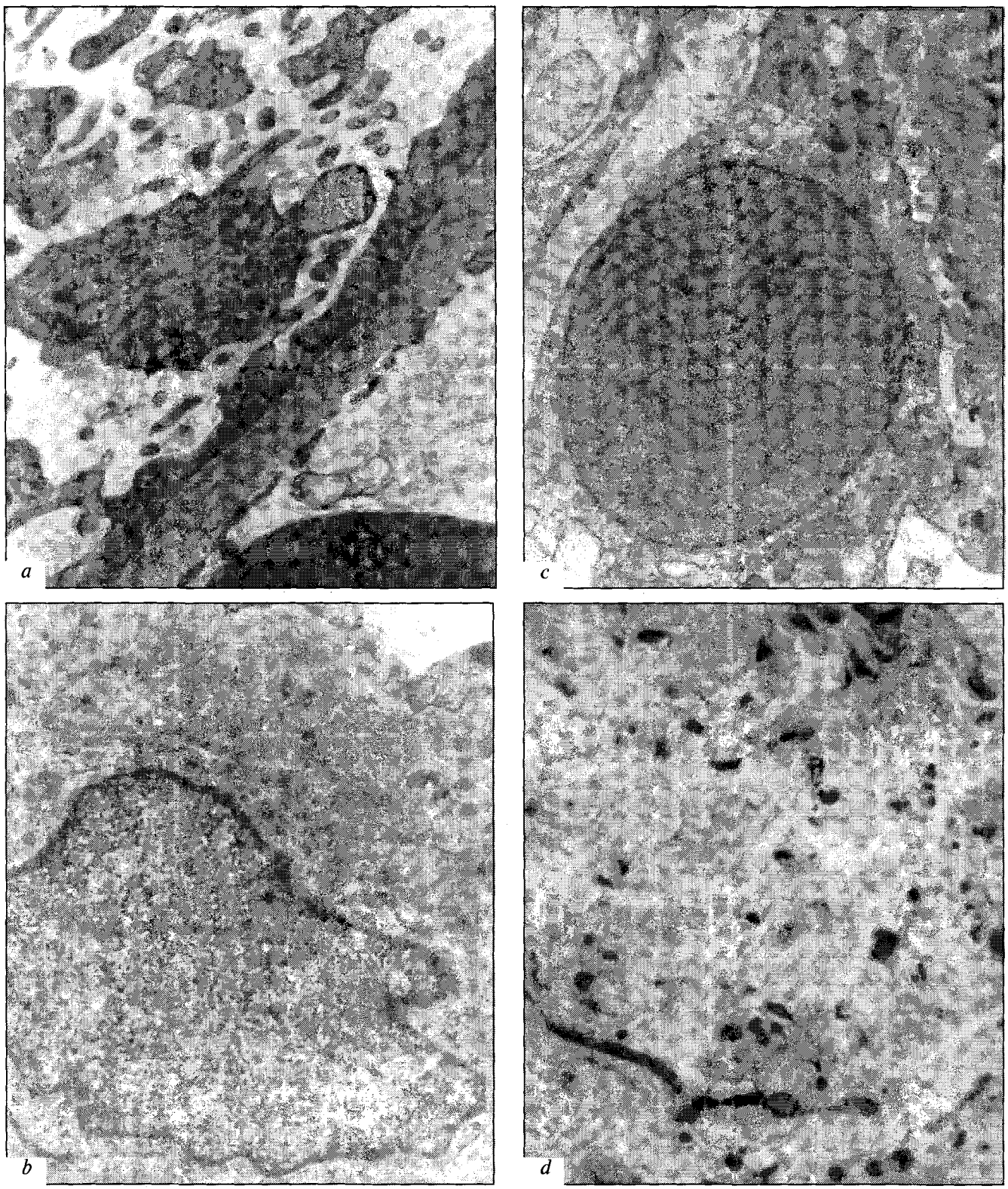

Fig. 2. Degenerative variant of alteration of glomerular cell population. *a*) drastic thinning and fragmentation of endotheliocyte cytoplasmic peripheral processes and villiform podocyte transformation, ×8000. *b*) mesangiocyte with large nucleus and low number of ribosomes, ×10,000. *c*) podocyte with reduced protein-synthesizing compartment and fused cytopodia, ×6000. *d*) low level of RNA synthesis. Incubation with ^{3}H-uridine. Semithin section stained with azure II, ×450.

intense biosynthetic processes (Fig. 1, *d*): ^{3}H-uridine labeling index was 73-92% in cells of the vascular bundle and 94-100% in parietal epitheliocytes, label density was very high. The index of labeling in epitheliocytes of proximal and distal tubules approximated 100%.

Radiography with DNA precursors revealed proliferative potency of mesangiocytes and endotheliocytes: in the glomeruli with pronounced hypercellularity (up to 180 cells per semithin section of single glomerulus) silver grains were observed in 1-3 cells. The raidoautographs with ^{3}H-thymidine were found also in parietal glomerular epitheliocytes at the stage, in which semiluna were not formed.

In degenerative variant of glomerular damage, the total ^{3}H-uridine labeling index in glomerular cells was markedly decreased (Fig. 2, *d*) and varied from 6 to 62%, while in parietal epitheliocytes it was 57-71% with low density of silver grains. In tubular epitheliocytes, the ^{3}H-uridine labeling index reached 100%, which is probably related to functional stress of the tubules that enhances reabsorption capacity to compensate proteinuria. No ^{3}H-thymidine label in cells of the glomerular and tubular compartments in renal bioptates were found.

In glomerular capillary endotheliocytes, the labeling index with RNA precursor was extremely low (by 34% in comparison with the first variant), which together with their ultrastructural alterations attested to pronounced (probably, primal) damage to the glomerular endothelial capillaries. It may be considered as a kind of endotheliopathy underlain by inhibition of synthetic function of endotheliocytes.

There are two strategies of compensation of disturbed function of the endothelial associate due to dystrophic changes of endotheliocytes in glomerular pathology. One of them is hypertrophy and/or proliferation of endotheliocytes and mesangiocytes with enhanced production of the extracellular matrix directed to membrane reparation [1], which corresponds to the proliferative variant of glomerular damage. The degenerative variant (the second strategy) is related to pronounced endothelial atrophy and podocyte metaplasia, in particular the formation of continuos cytoplasmic barrier with switching to pinocytotic filtration pathway, or villiform transformation, which reflects "regression of specialization". Evolutionary ancient functional activity of epitheliocytes was reported in some other organs (stomach mucosa, bronchi, *etc.*) [4]. All these processes are directed at maintenance of glomerular filter architectonics.

Irrespective to differences in the mechanisms of damage and compensation of cell populations in the glomerular compartment, they finally develop similar sclerotic alterations, which suggest that most nosologic forms of glomerular diseases are characterized by a common pathway in structural rearrangement.

REFERENCES

1. S. Yu. Bogomazova, O. P. Gladskikh, A. A. Ivanov, *et al.*, *Arkh. Patol.*, No. 6, 45-50 (1997).
2. M. S. Ignatova, E. A. Kharina, V. A. Spitsyn, *et al.*, *Ter. Arkh.*, **69**, No. 6, 44-49 (1997).
3. A. Coen and S. Nast, *Nefrologiya*, **2**, No. 3, 117-139 (1998).
4. G. I. Nepomnyashchikh, *Frontier Tissues (Mucosa and Skin) in Morphogenesis of General Pathology* [in Russian], Novosibirsk (1996).
5. L. M. Nepomnyashchikh, G. I. Nepomnyashchikh, S. V. Aidagulova, *et al.*, *Pathomorphology of Glomerular and Tubulointerstitial Nephrites and Nephropathies* [in Russian], Novosibirsk (1997).
6. L. M. Nepomnyashchikh, L. D. Sidorova, G. I. Nepomnyashchikh, *et al.*, *Byull. Sib. Otdeleniya Ross. Akad. Med. Nauk*, No. 1, 123-129 (1996).
7. N. A. Mukhin, I. M. Balkarov, A. N. Britov, *et al.*, *Ter. Arkh.*, **69**, No. 6, 5-10 (1997).
8. M. A. Pal'tsev and A. A. Ivanov, *Arkh. Patol.*, No. 6, 13-16 (1994).
9. Yu. L. Perov and V. I. Fedorov, *Ibid.*, No. 3, 3-10 (1980).
10. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, *Electron Microscopic Radioautography of the Cell* [in Russian], Moscow (1980).
11. V. V. Serov, in: *Inflammation* [in Russian], Eds. V. V. Serov and V. S. Paukov, Moscow (1995), pp. 518-540.
12. A. V. Cybulsky, D. J. Stewart, and M. I. Cybulsky, J. Am. Soc. Nephrol., **3**, No. 7, 1398-1404 (1993).
13. M. Nagata, Y. Akioka, Y. Tsunoda, *et al.*, *Kidney Int.*, **48**, No. 2, 527-535 (1995).
14. M. Rachman and M. C. Smith, *JAMA Rossiya*, **2**, No. 4, 46-57 (1999).
15. J. M. Rebibou, C. J. He, F. Delarue, *et al.*, *Nephrol. Dial. Transplant.*, **7**, No. 4, 288-292 (1992).