Ultrastructure of Kidney Cell Population in Patients with Markers of HCV- and HBV-Infections (Analysis of Biopsy Specimens)

N. L. Tov*, L. M. Nepomnyashchikh, S. V. Aidagulova, and A. A. Onishchenko*

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Changes in the glomerular, tubular, and interstitial compartments were revealed in kidney biopsy specimens from patients with serological markers of HCV and HBV infections. The dominant change was destruction of the tubular apparatus and atrophy of the tubular epithelium associated with progressive interstitial fibrosis. Our results indicate that kidney disorders constitute patho- and morphogenesis of systemic infection in HCV- and HBV-infected patients.

Key Words: *chronic HCV- and HBV-infections; nephrobiopsy; pathomorphology; immunohistochemistry*

One of the most important properties of hepatitis C and B viruses is replication in various organs [8,9]. Replication of these viruses occurs in hepatocytes, mononuclear cells of the blood and bone marrow, and cells of the kidneys, heart, pancreas, and other organs [7,12]. This characteristic underlies systemic damage and associated clinical manifestations of HCV- and HBV infections [1,6,10,11,13].

Here we studied ultrastructural changes in cells of renal glomeruli and tubules in patients infected with hepatitis C and B viruses.

MATERIALS AND METHODS

We examined biopsy specimens from the kidneys of 20 patients (14 men and 6 women, 16-52 years) carrying markers of HCV-infection (12 patients), HBV infection (7 patients), and mixed infection (HCV+ HBV, 1 patients). The complex study included biochemical test for aminotransferase activity, blood enzyme immunoassay for markers of hepatitis B (HBsAg, HBeAg, HBc-IgM, HBc-IgG, HBsAb, and HBeAb) and C viruses (total HCVAb and antibodies against Core- and NS-antigens), and polymerase chain reaction for HCV RNA and HBV DNA.

For light microscopy paraffin sections of kidney biopsy specimens were stained with hematoxylin and eosin in combination with Perls reaction, by the method of van Gieson with post-staining with Weigert resorcin fuchsin for elastic fibers, with Schiff reagent, and with Congo red (amyloid deposits). The samples for electron microscopy and immunohistochemical analysis were fixed with 4% paraformaldehyde. Semithin sections were stained with Schiff reagent and azure II. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope.

Immune detection of the NS3-antigen in hepatitis C virus was performed on paraffin sections by two-step indirect immunoperoxidase assay followed by visualization with streptavidin-biotin and diaminobenzidine. Mouse monoclonal IgG2b antibodies against re-

Department of General Pathology and Pathophysiology, Institute of Regional Pathology and Pathophysiology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; *Department of Internal Diseases, Novosibirsk State Medical Academy, Russian Ministry of Health. ***Address for correspondence:*** pathol@soramn.ru. L. M. Nepomnyashchikh

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combinant protein NS3 (NS3-HCV, NovoCastra Lab. Ltd.) served as primary antibodies.

Hepatitis C virus RNA and hepatitis B virus DNA were detected in nonfixed kidney biopsy specimens using QIAamp Viral RNA Kit (Cat 29504, QIAGEN), Sibenzim kit, QIAEX II Gel Extraction Kit (Cat 20021, QIAGEN), and commercial test systems of the Laboratory for Study and Synthesis of Preparations for Diagnostics of Human and Animal Diseases (Central Research Institute of Epidemiology, Russian Ministry of Health).

RESULTS

Clinical signs of chronic glomerulonephritis were found in 17 patients and included nephrotic and hypertonic syndromes (edema, proteinuria, leukocyturia, hematuria, hypoproteinemia, and hypercreatinemia). Three patients had toxicoallergic (2 patients) and drug-induced (1 patient) tubulointerstitial nephritis. The presence of serological markers of chronic HCV and HBV infections in some patients was associated with clinical manifestation of chronic hepatitis (hepatomegaly, pain, and cytolytic syndrome). Activity of aminotransferase increased in 8 patients. Blood aminotransferase activity in 1 patient exceeded the normal by 10 times.

Structural changes in kidney biopsy specimens concerned glomerular, tubular, and interstitial compartments of the kidneys. Progressive changes in the glomeruli are considered as the initial proliferative reaction of glomerular cells, increased fibroplastic process, atrophy, and total glomerular fibrosis [5]. Polymorphous structural reorganization of the glomeruli cannot be attributed to the certain morphological variant of glomerulonephritis [4]. More likely these changes reflect progression of the disease (Fig. 1, *a*, *b*).

Changes in proximal tubules were the characteristic morphological sign of bioptates from HBV-infected patients. Dystrophy of tubular epitheliocytes was revealed in 3 of 20 bioptates. In other kidney biopsy specimens prevailing features were focal or diffuse tubular necrobiosis and necrosis, desquamation of tubular cells, and formation of granular cylinders obturating the tubules (Fig. 1, *c*). Atrophy of the epithelium in proximal tubules was often seen. Most pronounced alterative changes were accompanied by edema, hyperemia, and cellular infiltration of perifocal tissue, which developed against the background of peritubular fibrosis and thickening of the basal membranes. Damage to the tubular epithelium was associated with thickening of the basal membrane and accompanied by dysfunction of reabsorption, secretion, and concentration in the tubular compartment of the nephron.

Diffuse sclerosis of the stroma of varying degree was most pronounced in the peritubular and periglomerular zone. Severe peritubular fibrosis dominated in bioptates from patients with serological markers of HBV (50%) and HCV infections (27%). Interstitial fibrosis was revealed in all bioptates and, probably, contributed to clinical manifestations of kidney disorder. Infiltration with cells (primarily lymphocytes) was least pronounced. Specific reconstruction of renal vessels was revealed in patients with arterial hypertension and resulted in arterial myoelastofibrosis and perivascular sclerosis.

Lymphoid aggregates and follicles were found in 55% bioptates from patients with serological markers of HCV infection (Fig. 1, *d*). It serves as an indirect morphological criterion of chronic HCV infection. Tubular epitheliocytes in these patients exhibited a positive immunohistochemical reaction with monoclonal antibodies against the NS3-antigen of hepatitis C virus (Fig. 1, *e*). Blood tests for HBsAg revealed annular transformation of the podocyte nucleus in 1 bioptate (structural marker of HBcAg, Fig. 1, *f*).

Electron microscopy of optically unchanged or minimally altered glomeruli in kidney biopsy specimens from patients with serological and morphological markers of chronic hepatitis C revealed ultrastructural changes in the glomerular filter. They included pronounced modification of endotheliocytes in capillary loops, presence of uneven fenestrae, sharp increase in electron density of the cytoplasmic matrix and karyoplasm, alteration of membrane organelles, and formation of high retinal arcades in the luminal plasmalemma. These changes were directed toward compensatory increase in the filtration area (Fig. 2, *a*). Several endotheliocytes had ultrastructural signs of apoptosis. The glomerular basal membrane at considerable length had normal thickness and irregular electron density. These parameters increased in the paramesangial space.

Alteration of epitheliocytes in the visceral layer of the Bowman's capsule (podocytes) was manifested in diffuse fusion of cytopodia, presence of numerous heterogeneous protein- and lipid-containing vacuoles in the podocyte cytoplasm (Fig. 2, *b*), and hyperplasia of the Golgi apparatus and fibrillar structures. Our findings illustrate changes in the cytoarchitectonics and involvement of podocyte bodies (not cytopodia) in the formation of primary urine. Signs of decompensation and degeneration (colliquation necrosis) were characteristic of several epitheliocytes.

Electron microscopy revealed loss of dimorphism in the ascending and descending tubules. Pronounced dystrophy and atrophy of tubular epitheliocytes determined dysfunction of reabsorption and secretion, which resulted in the appearance of monomorphic

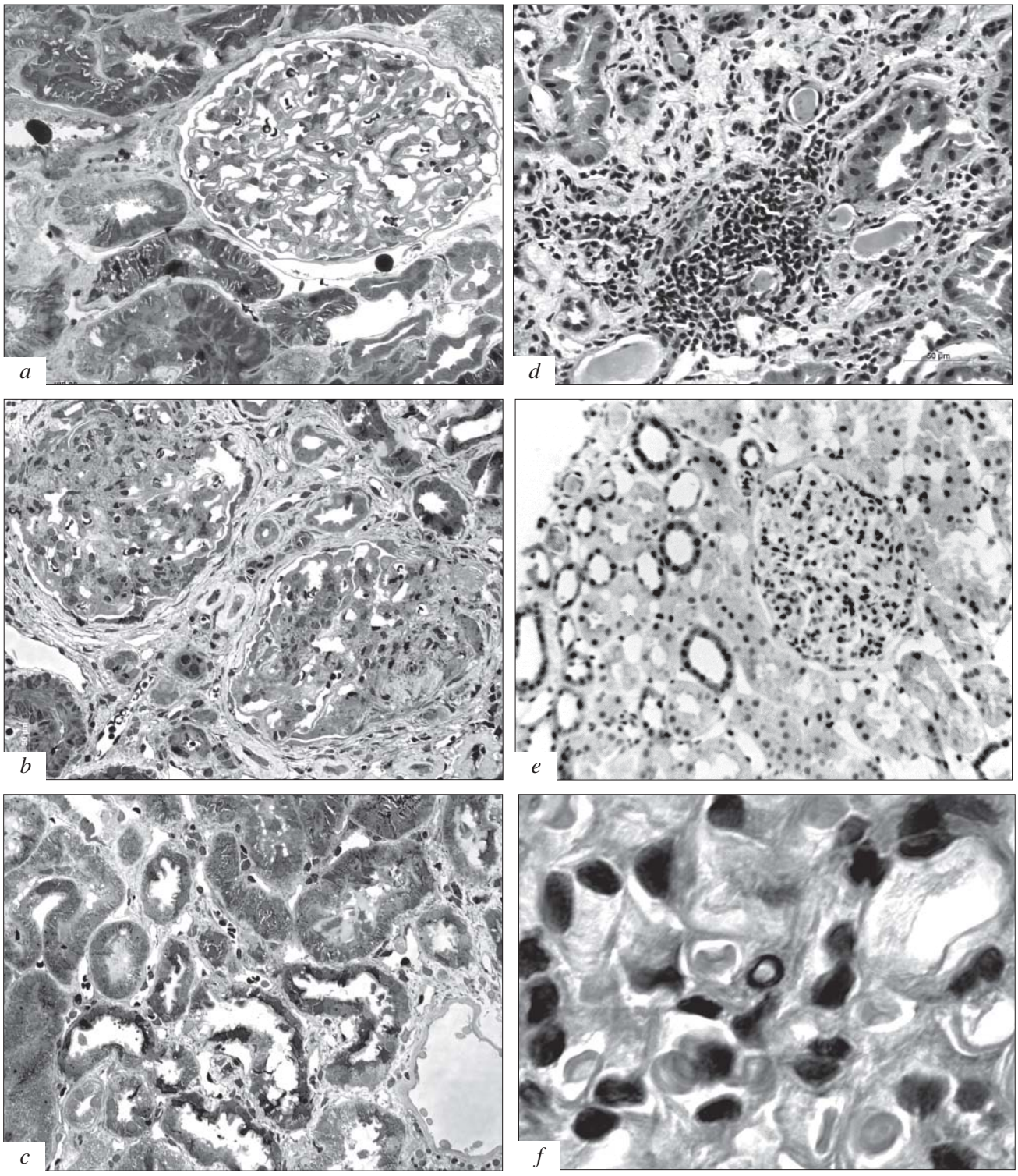

Fig. 1. Light microscopy of kidney biopsy specimens from patients with serological markers of HCV and HBV infection. Polymorphism of glomerular basal membranes and hypoplasia of glomerular cells (*a*); segmentary fibroblastic changes in glomeruli (*b*); dystrophy and focal necrosis of the tubular epithelium (*c*); lymphoid aggregate in the interstitium and numerous protein cylinders in tubules (van Gieson staining, ×600, *d*); HCV NS3Ag in the tubular epithelium (immunohistochemical detection, ×400, *e*); and annular nucleus in the glomerular podocyte (hematoxylin-eosin staining, ×1000, *f*). Semithin sections, azure II staining, ×650 (*a-c*).

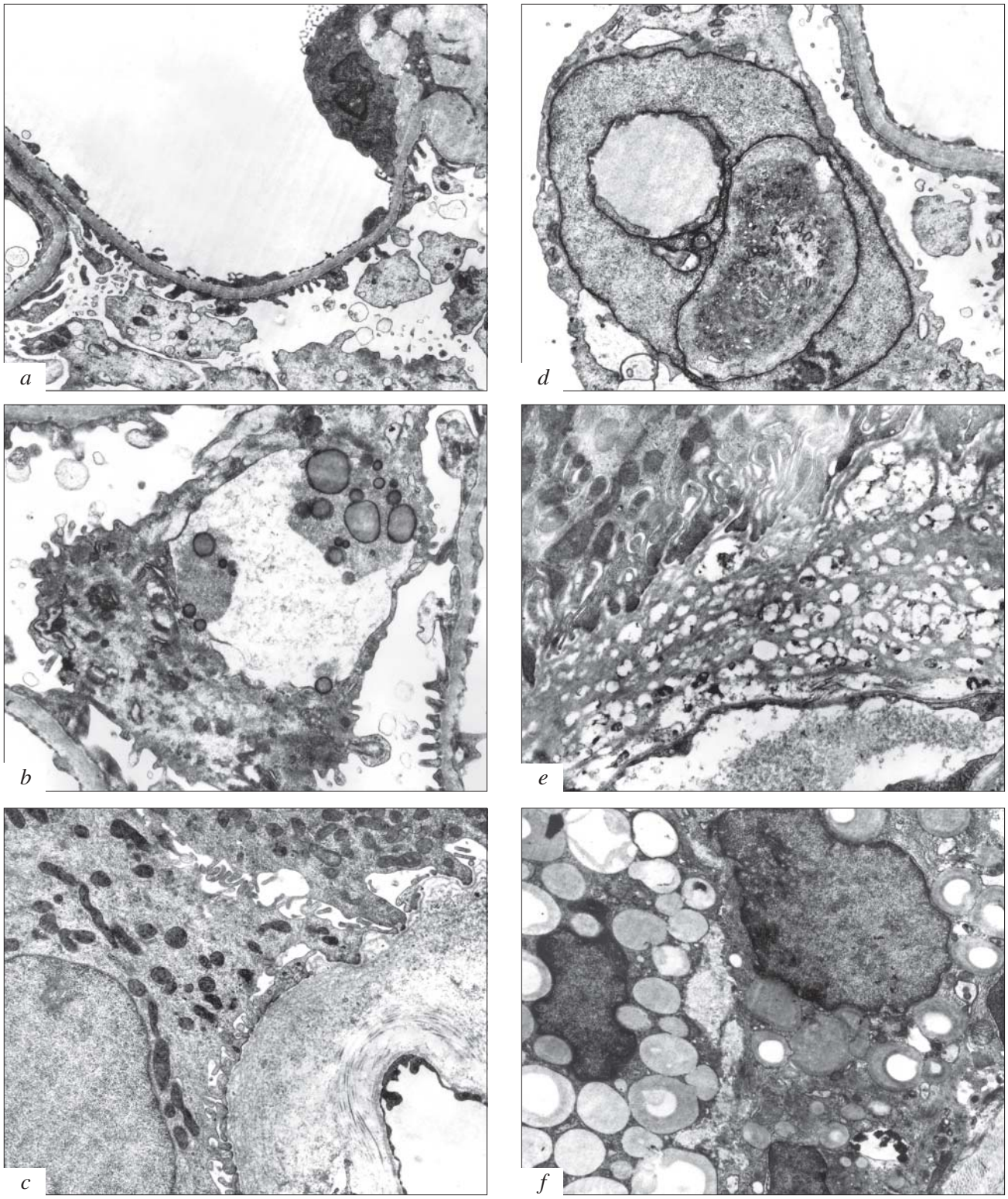

Fig. 2. Ultrastructure of kidney cells in patients with serological markers of HCV and HBV infections. Endotheliocyte with signs of degeneration (×400, *a*); focal fusion of cytopodia and destruction of the podocyte (×6000, *b*); thickening of the tubular basal membrane and pericapillary collagen fibrils (×5000, *c*); total fusion of cytopodia and destruction and sequestration of the podocyte nucleus (×5000, *d*); thickening and vacuolization of the tubular basal membrane (×6000, *e*); and interstitial cells with large heterogeneous granules (×4000, *f*).

cells in proximal and distal tubules. Most pronounced ultrastructural changes in the tubular epithelium were associated with the loss of plication in the basal cytolemma, decrease in the size and number of mitochondria, and destruction of the brush border in proximal tubular epitheliocytes. Functional disorders produced interstitial edema, which was particularly pronounced in the peritubular and pericapillary zone. Numerous collagen fibrils were formed between the epithelial basal membrane and peritubular capillaries (Fig. 2, *c*). Microcirculatory disturbances were associated with atrophy of endotheliocytes in peritubular capillaries and tubular epitheliocytes. These changes form a morphological basis of nephrotic syndrome.

Electron microscopy of kidney biopsy specimens from patients with markers of HBV infection revealed ultrastructural disorganization of the glomerular filter. The changes in endotheliocytes were less pronounced compared to those in HCV infection. These cells exhibited the increased functional activity and mild signs of degeneration. Changes in epitheliocytes of the glomerular filter (podocytes) were more pronounced (Fig. 2, *d*) and included total fusion of cytopodia, villous transformation and hypertrophy of the nuclear compartment, and presence of a considerable number of cellular vacuoles and short fibrils. The glomerular basal membrane was heterogeneous, irregularly thickened, and contained no electron dense deposits.

Tubular epitheliocytes underwent most pronounced destruction in HBV-infected patients. Considerable polymorphism of the ultrastructural organization was related to differences in the degree of tubular necrosis and intracellular regeneration providing functional activity. The brush border in proximal tubular cells was disorganized. A considerable number of small and large heterogeneous pinocytotic vesicles with the protein substrate were present in the supranuclear region. Several epitheliocytes contained large granules with high content of lipids. The epithelial basal membrane was clearly defined and thickened and included numerous vacuoles with electron dense center (Fig. 2, *e*).

Mononuclear cells with electron dense center were found in the interstitium. Their cytoplasm was filled with round granules of moderate electron density (Fig. 2, *f*). These cells are phenotypically and functionally similar to Ito liver cells and serve as lipid depots. Under the influence of fibrogenic stimuli they are transformed into fibroblast-like cells producing collagen fibrils. This process plays a role in the progression of renal failure associated with HBV infection.

Destruction of the tubular compartment and atrophy of the tubular epithelium were associated with progressive fibrosis of the interstitium and were revealed in kidney biopsy specimens from patients with HCV and HBV infection.

Kidney function correlates with the degree of tubular atrophy and severity of interstitial changes, but not with glomerular damage. A direct correlation was found between the severity of interstitial fibrosis and degree of kidney dysfunction [14]. It is important to evaluate the cellular and molecular mechanisms of accumulation of the intercellular matrix in damaged kidneys. Resident interstitial macrophages, pericytes, trans-differentiating epitheliocytes [15], and Ito-like cells are probably involved in matrix production. It remains unclear whether the control over activity of any signal molecule can prevent the progression of fibrosis.

Simultaneous analysis of bioptates from the liver and kidneys confirmed that renal disorders constitute patho- and morphogenesis of systemic infection in HCV- and HBV-infected patients [2,3]. The morphogenetic characteristic of HBV infection is the development of severe destructive changes.

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