Structural and Functional Features of Lipid-Containing Hepatocytes in Hepatitis C

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Pathomorphological studies of lipid-containing hepatocytes included in the system of major markers of hepatitis C showed that the phase of hepatitis C virus replication (PCR data) corresponds to subplasmalemmal microvesicular steatosis of hepatocytes.

Key Words: *hepatitis C; steatosis of hepatocytes; pathomorphology*

Extraordinary capacity of the virus to prolonged persistence in the host is the most important feature of HCV-infection. Despite the presence of virus-specific immune response, it does not lead to elimination of the virus in most infected individuals and does not protect against reinfection. The properties of hepatitis C virus and reactions of infected organism responsible for immune incompetence response and contributing to viral persistence and chronic course of the infectious process are now intensively studied [4].

The intensity of virus replication is extremely high. According to published data, it exceeds 10^{12} virus particles produced daily even in the chronic phase of the infection [12]. Virus replication is performed by RNA-dependent RNA polymerase lacking proofreading function, which leads to rapid evolution of different related quasispecies in the infected organism and this is the major problem for immune control of HCV [2,5,8].

Hepatitis C virus can replicate in many organs. The possibility of viral replication in many organs such as liver, bone marrow, kidney, pancreas, spleen, adrenal glands, lymph nodes, and thyroid gland was shown using highly specific modifications of PCR and *in situ* hybridization [1,6,10].

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Chronic hepatitis C is featured by hepatocyte steatosis (fatty degeneration) often filling the cytoplasm and involving the population of parenchymal liver cells.

Here we studied the structure and functions of lipid-containing hepatocytes of various types in hepatitis C (HCV-infection).

MATERIALS AND METHODS

We carried out clinical and pathomorphological examination of 199 patients with chronic HCV infection. In addition to detailed history analysis, we measured biochemical parameters of the blood and serological markers of viral hepatitides A, B, C, and D. HCV RNA in the serum and blood mononuclear cells were detected in native liver tissue by PCR. Genotyping of HCV was performed and the degree of viremia was evaluated. The study of 310 needle biopsies of the liver included immunohistochemical detection of HCV NS3 antigen in paraffin sections in addition to light microscopy of paraffin and semithin sections and electron microscopy.

Pathological changes in liver biopsy specimens were analyzed using Los Angeles classification of chronic hepatitis [7] considering etiological factor as the leading characteristic. Infection activity and the stage of fibrosis were also determined. The overall activity of the infectious process was assessed by three components: clinical picture, biochemical tests refer-

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ring to the leading role of the cytolytic syndrome, and structural changes in the liver. Light microscopic study was conducted using Leica DM 4000B with Leica DFC320 digital camera, ultrastructural study, using Jeol JEM-1010 electron microscope at an accelerating voltage of 80 kV.

RESULTS

Complex clinical and morphological study showed that in patients with HCV infection markers, chronic hepatitis C was mostly light oligosymptomatic or hidden. It was characterized by similar clinical manifestations, minimal or mild severity in the disease and, in most cases, mild fibrosis.

In patients with HCV monoinfection, minimal (33%) or mild (36%) severity of the disease was diagnosed in most cases; in 27% cases, moderately severe disease was diagnosed; only in 4% cases we observed high severity of chronic hepatitis C.

Chronic hepatitis C staging showed that in the majority of cases (85%), fibrosis was minimal or mild (activity grade A1) and was localized in the portal stroma. In 10% cases, single porto-portal septa (activity grade A2) were revealed in liver biopsies, and only in 5% cases, severe fibrosis with numerous septa (activity grade A3) and progression to liver cirrhosis was found.

Comparisons between the time of infection with hepatitis C and fibrosis staging were of great importance. We revealed that the stage of fibrosis does not depend on the probable duration of HCV infection. Mild liver fibrosis was observed in most of the observations, even in 10-20-year history of infection.

Frequent association of minimal and mild severity of chronic hepatitis C with the phase of viral replication and high viremia was a paradoxical findings. Comparisons of markers of viral replication, according to PCR, and severity of the viral infectious process showed that minimal (14.3%) and mild (55.8%) severity of the process was detected in 70% cases when HCV RNA was present in serum and/or blood mononuclear cells. Minimal pathological changes in the liver tissue also dominated in the patients with negative reaction to HCV RNA, and quantitative indicators of the activity grade were almost identical.

Special survey of 112 patients with chronic HCV monoinfection indicated that activity grade of HCV-infection is independent of viral replication and the level of viremia. Thus, the presence of HCV RNA in blood and/or liver samples (according to PCR) and the number of infected hepatocytes (defined by expression of NS3Ag HCV) have no significant association with the grade of disease activity (defined by structural changes in the liver and clinical and biochemical tests).

Low ALT activity in chronic hepatitis C can be explained by intracellular localization of the enzyme. Increased ALT indicates hepatocyte cytolysis, *i.e.* cell death by colliquation necrosis, while chronic hepatitis C extremely rarely develops this type of necrosis.

We have identified the main morphological markers of chronic HCV infection including microvesicular subplasmalemmal steatosis of hepatocytes, involutive degeneration of hepatocytes, and forming of lymphoid aggregates and follicles in the portal tracts (Fig. 1, *a*, *b*).

During pathological analysis of liver biopsies, we paid special attention to hepatocyte steatosis: one of the major markers of hepatitis C [11]. It was detected in 87% of biopsy specimens and usually had diffuse and polymorphous pattern. We have identified two main types of steatosis as microvesicular subplasmalemmal (Fig. 1, *c*-*e*) and macrovesicular steatosis (Fig. 1, *f*).

Macrovesicular steatosis of hepatocytes often occurs in liver biopsies in chronic viral hepatitis, including hepatitis C. This is a universal phenomenon arising from various toxic effects (ethanol, drugs). It has probably a compensatory character maintaining the size of cell population and organ geometry with the possible subsequent functional recovery. Polymorphous steatosis was noted in the majority of liver biopsies; it varied from minimal, which could be only identified in semithin sections, to giant drops that filled the entire cytoplasm.

Microvesicular subplasmalemmal steatosis in hepatitis C is a more specific phenomenon. We conclude (by comparing with PCR data), what it corresponds to the phase of viral replication [3] and probably reflects emergency reparative cell response to direct cytopathic effect of virus penetrating into cell by endocytosis. However, recent data indicate a closer relationship between steatosis and the life cycle of the hepatitis C virus.

The key role of lipid droplets in the production of virus particles in infected hepatocytes was determined [9,14,15] as follows: non-structural proteins and replication complexes associated with ER membranes surrounding the lipid droplet form a kind of "factory" necessary for the reproduction of active infectious particles. Core protein (nucleocapsid protein C) is essential to this process, because it interacts with the lipid droplets and recruits other viral proteins to them. In this complex, lipid droplet may provide a factor required for virus infectivity (probably, via its association with VLDL) and promoting incorporation of virus particle into the vesicular transport system of lipids and lipid-related materials to export infectious virus outside the cells [14].

In this respect, direct HCV involvement in lipid metabolism could be of great physiological significance. Direct role of this antigen in the development

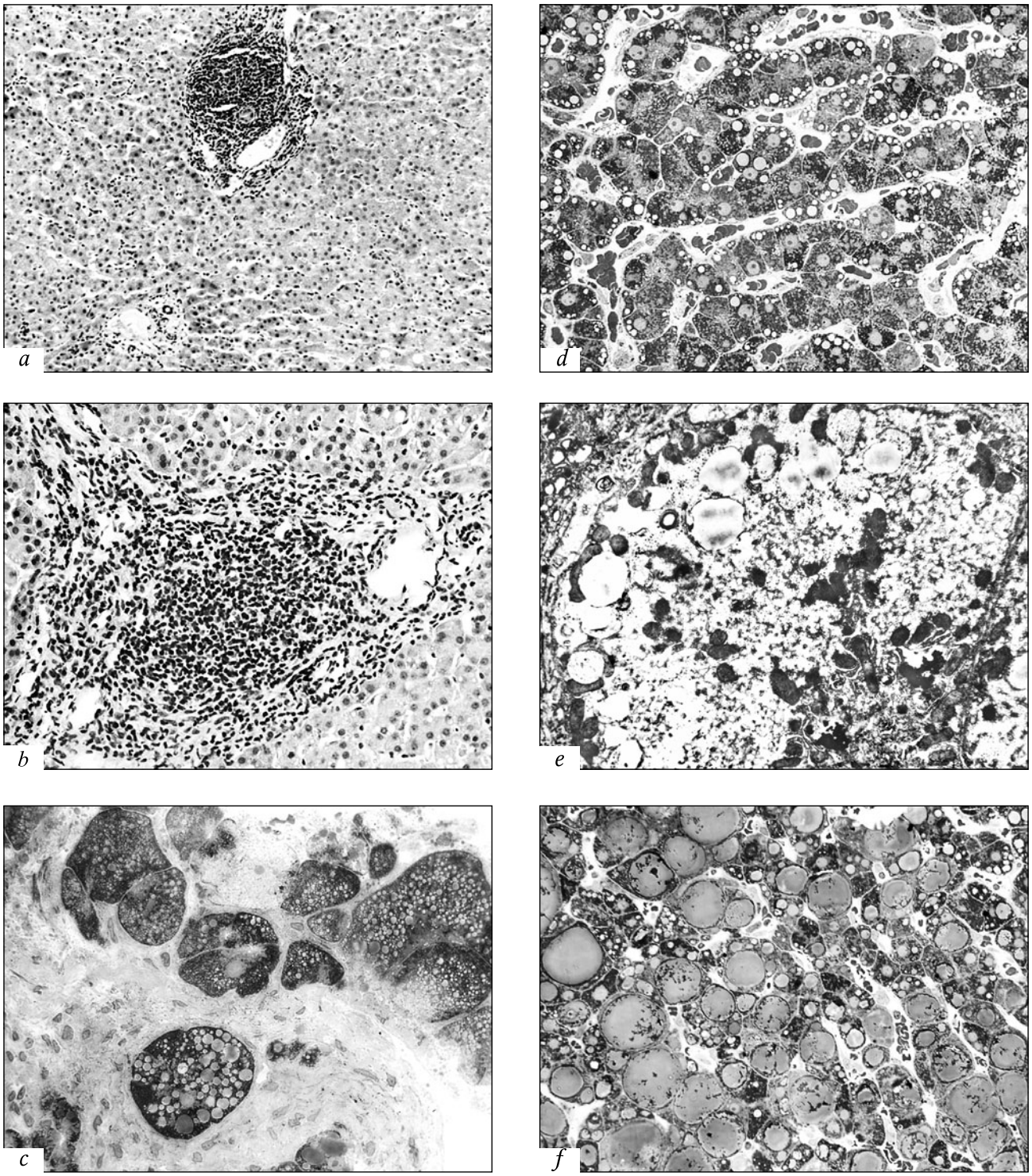

Fig. 1. Pathological characterization of liver biopsies at chronic hepatitis C (HCV-infection). *a*) compact lymphoid follicle in the portal tracts, ×160; *b*) large lymphoid aggregate in the portal tracts. Periportal distribution of cell infiltration, ×250; *c*) diffuse microvesicular steatosis of hepatocytes in the area of periportal fibrosis, ×850; *d*) microvesicular subplasmalemmal steatosis of hepatocytes, ×400; *e*) small subplasmalemmal lipid inclusions in the cytoplasm of the hepatocyte, ×8000; *f*) microvesicular steatosis of hepatocytes with fatty cysts formation, ×250. *a*, *b*: staining with hematoxylin and eosin; *c*, *d*, *f*: semithin section, staining with Schiff's reagent and azure II; *e*: electron micrograph.

of hepatic steatosis was demonstrated in transgenic mice carrying HCV core antigen [13]. Increased number of lipid droplets in the cell (small lipid vesicles) may be associated with activation of the transcription factor SREBP by core protein as well as with inhibited activity of microsomal triglyceride carrier [9]. In

this way, hepatitis C virus induces activation of lipid metabolism in the host cell for efficient production of active infectious viral particles.

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