REVIEWS

Chronic Hepatitis C: Modern Notions of Pathogenesis and Morphogenesis. Concept of Antiviral Protection in Hepatocytes

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Here we review modern notions about biological properties of hepatitis C virus, geographic distribution of its genotypes, peculiarities of immune reactions during chronic HCV infection, and role of viral replication in the infectious process. The concept of antiviral protection in liver parenchymal cells suggests suppression of biosynthetic reactions in hepatocytes resulting in inhibition of viral replication and focal degradation of the cytoplasm in infected cells followed by exocytosis and elimination of viral particles (cytosanation without cytodestruction). The recovery of structural characteristics in hepatocytes is associated with intracellular regeneration. Special accent is placed on reversibility of liver fibrosis in chronic HCV infection due to resorption of collagen fibers by hepatocytes. These data indicate that the therapy of liver fibrosis holds much promise.

Key Words: *hepatitis C; polymerase chain reaction; liver biopsy; pathomorphology*

Infections produced by hepatitis C virus (HCV) are the major cause of mortality and morbidity in various countries [28,29]. They cause a wide spectrum of liver damages from asymptomatic carriership to terminal liver diseases [3,4,9,24,36]. HCV is characterized by intensive replication, considerable genetic variance, and variability helping the virus to escape from the immune control and determining high incidence of chronization and persistence.

The incidence of immune markers for HCV infection in various groups of people varies from 1 to 10% [11]. Anti-HCV antibodies were detected in 1.8% Americans; 75% of them have viremia. Signs of active HIV infection are observed in 2.7 million Americans [74]. In Russia antibodies to HCV antigens were revealed in the serum from 2-4% donors [12]. Much attention is given to the properties of HCV and reactions of the infected organism that contribute to insufficiency of the immune response and determine viral persistence and chronic course of infection [55].

Here we review the results of clinical and morphological examination of 187 patients admitted to the Novosibirsk Municipal Infectious Clinical Hospital No. 1 and State Novosibirsk District Clinical Hospital. Observations were performed on 148 men and 39 women (16-69 years). Most patients were at a young age (30.66±1.16 years). The diagnosis of HCV monoinfection and HCV+HBV mixt infection was documented in 145 and 42 patients, respectively. More than 50% patients were intravenous drug abusers. Medicinal parenteral interventions (surgeries, blood transfusions, and stomatological and diagnostic procedures) were made in one-thirds of these patients.

Activities of alanine and aspartate transaminases (ALT and AST, respectively), alkaline phosphatase,

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γ-glutamyltransferase, and bilirubin and its fractions were measured. Serum markers of hepatitis A (HAV IgM and HAV IgG), B (HBsAg, HBeAg, HBc-IgM, HBc-IgG, HBsAb, and HBeAb), C (total anti-HCV and antibodies to Core and NS antigens), and D (anti-HDV) were detected by enzyme immunoassay. HCV RNA in the serum, blood mononuclear cells, native liver tissue, and samples of the urine and saliva was detected by polymerase chain reaction (PCR).

Genotyping was performed by comparing 5'UTR sequences and fragment of the NS5B gene in isolates of HCV with Genbank databank. These experiments were performed at the Laboratory of Molecular Biology of RNA Viruses (State Research Center for Virology and Biotechnology Vektor).

Peripheral blood immunocompetent cells were studied by flow cytometry. The total number of T lymphocytes ($CD3^+$), T helper ($CD4^+$) and T suppressor cells ($CD8^+$), and natural killer cells ($CD16^+$) was estimated. We determined the absolute and relative number of B cells carrying CD72 and CD19 markers and the ratio of $CD5^+$ cells in the population of $CD19^+$ cells. Phagocytic activity of granulocytes and monocytes was evaluated. The concentrations of various cytokines in the serum, including interleukin-2 (IL-2), IL-4, IL-10, and interferon-γ (IFN-γ), was measured by electrochemiluminescence. Immunological assays were performed in collaboration with the Institute of Clinical Immunology (Siberian Division of the Russian Academy of Medical Sciences).

Transcutaneous needle biopsy of the liver was performed under local anesthesia using Braun disposable needles. The length and diameter of tissue columns were 1.0-3.5 cm and 2-3 mm, respectively. Samples for light microscopy were fixed in 10% neutral formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin in combination with Perls reaction, by the method of van Gieson, elastic fibers were stained with Weigert's resorcin fuchsin. Periodic acid-Schiff (PAS) reaction was performed. The severity of hepatitis and liver fibrosis was determined [25,46,63]

Samples for electron microscopy were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, and embedded in epon-araldite mixture. 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.

Immunohistochemical assay of paraffin sections for HCV NS3 antigens was performed by an indirect 3-stage immunoperoxidase technique with mouse monoclonal antibodies to recombinant NS3 protein (Novocastra Lab), streptavidin-biotin visualization, and semiquantitative study of NS3 antigen expression.

Genetic Heterogeneity of HCV

HCV is a RNA flavivirus [86]. HCV encodes a single polyprotein consisting of 3011 amino acids, which is transformed into 10 mature structural and regulatory proteins. Structural components include core protein and 2 envelope proteins E1 and E2. Two regions of envelope E2 protein constituting hypervariable regions 1 and 2 are characterized by high incidence of mutations, which is probably a result of selective action of virus-specific antibodies. HCV also encodes virus-specific helicase, protease, and polymerase [61,74].

Viral replication is a very intensive process. Previous studies showed that 10^{12} viral particles appear daily even in the chronic phase of infection [78]. Viral replication involves RNA-dependent RNA polymerase lacking the function of "correction", which determines rapid evolution of various but related quasispecies in the infected organism and complicates immunological control over HCV.

Various genotypes and subtypes of HCV have different geographic distribution. Six related genotypes and numerous subtypes of HCV were identified. In Western Europe and USA genotypes 1a and 1b are most typical, while the incidence of genotypes 2 and 3 is low. Other genotypes of HCV are absent in these countries, but are revealed in other regions. Genotypes 4, 5, and 6 are widely distributed in Egypt, Southern Africa, and Southeastern Asia, respectively [74].

Molecular and genetic studies of HCV isolates in Russia started in recent years [12]. In the Siberian region HCV isolates were genotyped for the fist time from patients hospitalized at the Novosibirsk Municipal Infectious Clinical Hospital No. 1. It was of the first studies performed in Russia. These experiments were conducted at the State Research Center for Virology and Biotechnology Vektor, Center for Control and Prevention of Diseases (USA), and Novosibirsk State Medical Academy [45]. Genotyping was performed by comparing 5'UTR sequences and fragment of the NS5B gene in HCV isolates with Genbank databank. After this assay 63.4, 7.5, and 18.5% genotyped HCV samples were prescribed to subtype 1b, genotype 2 (subtypes 2a or 2c), and genotype 3 (subtype 3a), respectively. Previously unknown nucleotide exchanges, insertions, and deletions were revealed in 5'UTR sequences of 10.8% HCV isolates.

Genetic heterogeneity of HCV determines differences in the course and outcome of the infection and efficiency of treatment. In HCV-infected patients a more favorable response is associated with genotypes 2 and 3 (as differentiated from genotype 1) [74]. The development of chronic HCV infection after acute disease is observed in 92% patients with genotype 1b and 33-50% patients with other genotypes. Infection

with genotype 1b is associated with more severe damage to the liver and more aggressive course of the disease compared to other genotypes [90]. Genotype 1b often occurs in patients with liver cirrhosis.

The clinical value of genotyping is not fully understood. It is associated with various methods and approaches to studying the influence of each genotype on progression of liver disease or efficiency of interferon therapy. Progression of liver disease depends not only on the genotype of HCV, but also on viremia, alcohol consumption, and duration of infection. Although some authors noted the important role of HCV genotypes and clinical significance of genotyping, this problem requires further investigations.

Role of HCV Replication in Infectious Process

Replication in various organs is an important characteristic of HCV [53,59]. Highly specific modifications of PCR and *in situ* hybridization revealed HCV replication in the liver, blood mononuclear cells (monocytes, macrophages, and B lymphocytes), bone marrow cells, oral mucosa, kidneys, heart, pancreas, intestine, lymph nodes, adrenal glands, thyroid gland, and spleen [49,73,83].

In HCV monoinfection we found viral replication in the serum (77%), blood mononuclear cells (73%), or serum and mononuclear cells (55%). In 18% patients viral replication was observed in blood cells, but not in the serum. HCV RNA was detected in the saliva, urine, and both substrates in 7, 5, and 3 of 34 patients, respectively.

PCR for HCV RNA demonstrated viral replication in 26 samples of native liver tissue (79%). Complex analysis revealed replication of HCV in the serum, blood mononuclear cells, and liver tissue from 52% patients. In 15% patients HCV replicated in 4 substrates (taking into account samples of the saliva and urine). HCV replication in 5 substrates was observed in only 1 patient.

Study of liver biopsy specimens for HCV replication markers and severity of chronic HCV infection revealed no correlation between the presence of HCV RNA in the blood and type of structural changes in the liver. Moreover, no correlation was found between the presence of HCV RNA in the blood and liver tissue. The degree of viremia did not correlate with the severity of infection. Moreover, the severity of liver injury did not depend on the number of infected hepatocytes (immune detection of HCV NS3Ag) [14,17,18].

In general, liver injury during chronic HCV infection was not related to the number of infected hepatocytes and HCV RNA content in the blood and liver tissue. This can be explained by morphogenetic peculiarities of chronic hepatitis C. This phenomenon is related to the antiviral reaction of parenchymal cells during HCV infection, which protects hepatocytes, suppresses viral replication, and reduces its heterogeneity and variability.

Immunological Peculiarities of Chronic HCV Infection

The immune response to HCV infection consists in nonspecific (interferons, other cytokines, and natural killer cells) and subsequent specific phases (humoral and cellular factors). The main role of humoral immune factors during HCV infection is elimination of free viral particles and formation of antibodies. Heterogeneity and variability of HCV antigens and temporal blockade of the neutralizing effect of isolate-specific antibodies determine the absence of specific antibody-mediated protective reactions [5,6,57,72].

Present studies of cellular immune factors are focused on T helper cells ($CD4^+$), T killer/suppressor cells ($CD8^+$), and their cytokines (IFN-γ, TNF-α, TNF-β, IL-2, IL-4, and IL-10) [55,58]. Recent experiments were performed on transgenic mice, Daudi cells, and HCV-infected chimpanzees [55,77,80,85]. Virus-specific cytotoxic T lymphocytes play a major role in the control over HCV. These cells can produce protective or adverse effects, which depends on the microenvironment and functional activity. HCV differentially modulates the influence of cytokines via production of inhibitory factors and persists even under conditions of active $CD8^+$ cytotoxic response, which underlies the development of liver injury.

Flow cytometry revealed changes in the subpopulation of immunocompetent cells (*i.e.*, increase in the number of T lymphocytes, T suppressor cells, and B lymphocytes carrying the CD19 marker) and high activity of T helper type 2 cells in patients with viral hepatitis C. In patients with HCV+HBV mixt infection the count of T lymphocytes increases, while the relative number of T suppressor cells decreases. The similarity of immune reactions during chronic hepatitides C and C+B is manifested in a considerable decrease in the number of natural killer cells and phagocytic activity of granulocytes and macrophages.

Electrochemiluminescence study showed that changes in serum cytokines during chronic hepatitis C are limited by overproduction of IL-4. Acute hepatitis C is accompanied by an increase in the content of IFN-γ, IL-2, and IL-4 [37]. It should be emphasized that cytokines play a role in the mechanisms of viral clearance. Previous experiment showed that IFN-γ and TNF-α selectively destruct the replicating genome of HCV, but not infected hepatocytes [68].

Immunogenetic study of Europeans with hepatitis C in Western Siberia revealed high incidence of HLA-A10 (RR=2.56) and HLA-DR5 antigens (RR=1.98)

and combinations DR2-DR5, DR5-DR7, and DR1-B27 and the absence of HLA-DR4 (RR=-39.82) [36]. These data indicate that the resistance and predisposition to the development of this disease is associated with major histocompatibility complex genes. Patients with chronic HCV infection are characterized by significant changes in 8 parameters: low incidence of HLA-DR4 antigen and high incidence of HLA-A10 (RR=2.68), HLA-DR1 (RR=2.6), A9-A10, DR2-DR5, A9-DR5, and B27-DR1. Studying of the role of immunogenetic markers in the predisposition of patients with viral hepatitis to drug abuse revealed a decrease in the incidence of HLA-A11 (RR=-2.12) and increase in the incidence of HLA-B7 (RR=2.87), HLA-B40 (RR= 11.26), and their combinations [8].

Pathomorphology of the Liver and Morphogenesis of Chronic Hepatitis C

Biopsy of the liver is one of the major diagnostic procedures for HCV infection. Studies of this disease are performed by various methods, including high-resolution electron microscopy, immunohistochemical assay of the structural and nonstructural viral proteins, and detection of HCV RNA in fixed and native samples by means of PCR [79,84,88].

Morphogenesis of chronic viral hepatitides has 3 constituents: hepatocyte injury, reaction of nonparenchymal cells in the liver, and remodeling of the connective tissue (fibrogenesis, Fig. 1). The major and general reaction of parenchymal cells to damage is de-

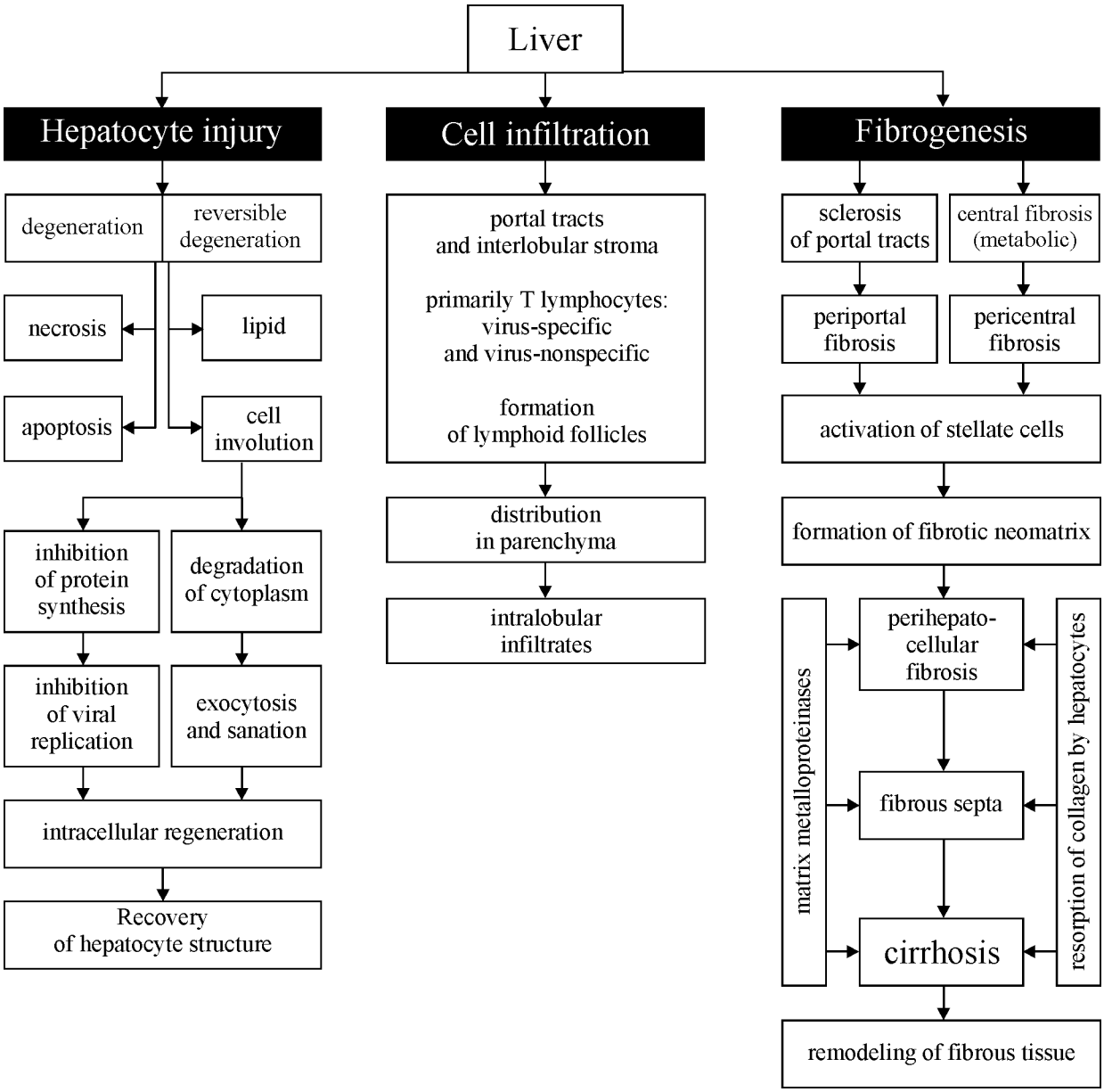

Fig. 1. Morphogenesis of chronic hepatitis C (HCV infection).

generation, which sometimes produces necrobiosis and necrosis of hepatocytes. Apoptosis in cells (*e.g.*, Councilman's bodies) was described in recent years [2,67, 81]. Infiltration with cells, particularly with virus-nonspecific T lymphocytes, is initially observed in portal tracts and then extends to hepatic lobules.

Evolution of fibrosis in viral hepatitides appears as primary fibrosis of portal tracts, distribution towards the central vein and adjacent portal tracts, and formation of porto-portal and porto-central crypts. Metabolic fibrosis is observed during alcohol addiction and long-term drug treatment. These changes develop in the region of a pronounced metabolic load (central hepatic vein) and then involve portal tracts, which results in the formation of porto-central crypts.

Our studies of the morphogenesis of chronic hepatitis C revealed not only previously described structural changes in the liver [10,33,34,43,44], but also a new form of hepatocyte degeneration [23]. Involutory degeneration is the general morphological phenomenon characterized by exhaustion of the cytoplasm, considerable decrease in glycogen content, and hypochromicity of the nuclei (Fig. 2, *a*).

Electron microscopy showed that involutory degeneration is manifested in focal or total exhaustion of the cytoplasmic matrix (Fig. 2, *b*). Clusters of osmiophilic membrane structures sequestering cytoplasmic regions played a role in the genesis of exhaustion (Fig. 2, *c*). Tubular and reticulotubular structures (clusters of small vesicles with electron dense content) that serve as a pathognomonic ultrastructural criterion for HCV infection were revealed in individual hepatocytes [85]. Degeneration of membrane cytoplasmic organelles was accompanied by the formation of heterogeneous residual bodies (Fig. 2, *d*). They migrated to the vascular pole of hepatocytes and were revealed in the Disse's space. The nuclear compartment (intact nucleus and perinuclear complexes of intracellular regeneration) remained unchanged in hepatocytes with loosened cytoplasm. Therefore, these changes were reversible [21].

Exhaustion of the cytoplasm and preservation of the nucleus and perinuclear regenerative compartment can be considered as a preventive cellular inhibition playing a general protective role. Experiments showed that this process protects cells from pathogenic factors, including viruses and toxins. Exhaustion can serve as a stage of hepatocyte sanation and removal of destructed organelles, which is followed by the recovery of ultrastructural characteristics [19]. Sanation of hepatocytes does not require massive lysis of infected cells (rare necroses in biopsy specimens). As differentiated from immune-mediated cytodestruction, sanation has no effect on the functional integrity of the organ (Fig. 1).

It should be emphasized that the immune system is characterized by considerable strain. It is manifested in the unique morphological phenomenon, hyperplasia of the lymphoid tissue. Most liver biopsy specimens (1:1000 w/w) include lymphoid follicles and aggregates [19] with signs of periportal lymphodiapedesis. Electron microscopy sometimes revealed signs of peri- and emperipolesis, which reflects the interaction of cytotoxic T lymphocytes with the target cell, development of immune-mediated injury to hepatocytes (killer effect), and their apoptotic death (Fig. 2, *e*).

Insufficiency of immune reactions to hepatitis C virus characterized by high genetic variability probably promotes expression of a protective response in liver parenchymal cells, *i.e.* their evolutionary developed mechanisms of antiviral protection. Infected hepatocytes play an important role in the antiviral response, react to cytokine signals, and activate specific intracellular antiviral reactions breaking virus life cycle [60]. These data seems contrary to the modern notions that infected hepatocytes serve only as a target for infection [55].

Pathomorphological assay of liver biopsy specimens in HCV infection gave special attention to lipid infiltration of hepatocytes (major criterion for hepatitis C) [71]. Diffuse and polymorphic changes were found in 87% biopsy specimens. PCR showed that replication of HCV is associated with small-vesicular subplasmalemmal lipid infiltration (Fig. 2, *f*) resembling a pearl collar [14]. Lipid infiltration of hepatocytes probably reflects an alarm reparative reaction of cells to the direct cytopathic effect of the virus [52] entering the cells by endocytosis [47]. This reaction maintains the population of parenchymal cells and architecture of the organ. The relationship between viral proteins and expression of phenotypic changes was studied on transgenic mice carrying HCV core antigen [77]. It was shown that this antigen plays a role in the development of liver steatosis.

Morphogenesis of liver injury during chronic hepatitis C+B was primarily associated with acidophilic degeneration of hepatocytes and formation of individual apoptotic bodies (aggregation of membrane organelles and small-vesicular modification of the cytoplasmic reticulum). In chronic hepatitis C+B the number of lipid-containing hepatocytes was much lower than that observed during HCV monoinfection. Cell-involutory degeneration was characterized by a lower volume of involved hepatocellular compartment. In biopsy specimens of the liver obtained during mixt infection dystrophic changes were more severe and extensive, hepatocyte injury was more pronounced (monocellular and large focuses of necrobiosis), and formation of lymphoid follicles in the stroma was less common (27%) compared to HCV monoinfection.

Examination of most liver biopsy specimens (64%) in mixt infection revealed typical changes in periportal hepatocyte nuclei, which included central lightening of the nucleoplasm (signet ring-like nuclei). This sign serves as a morphological criterion for HBcAg. Light microscopy showed that the formation of signet ring-

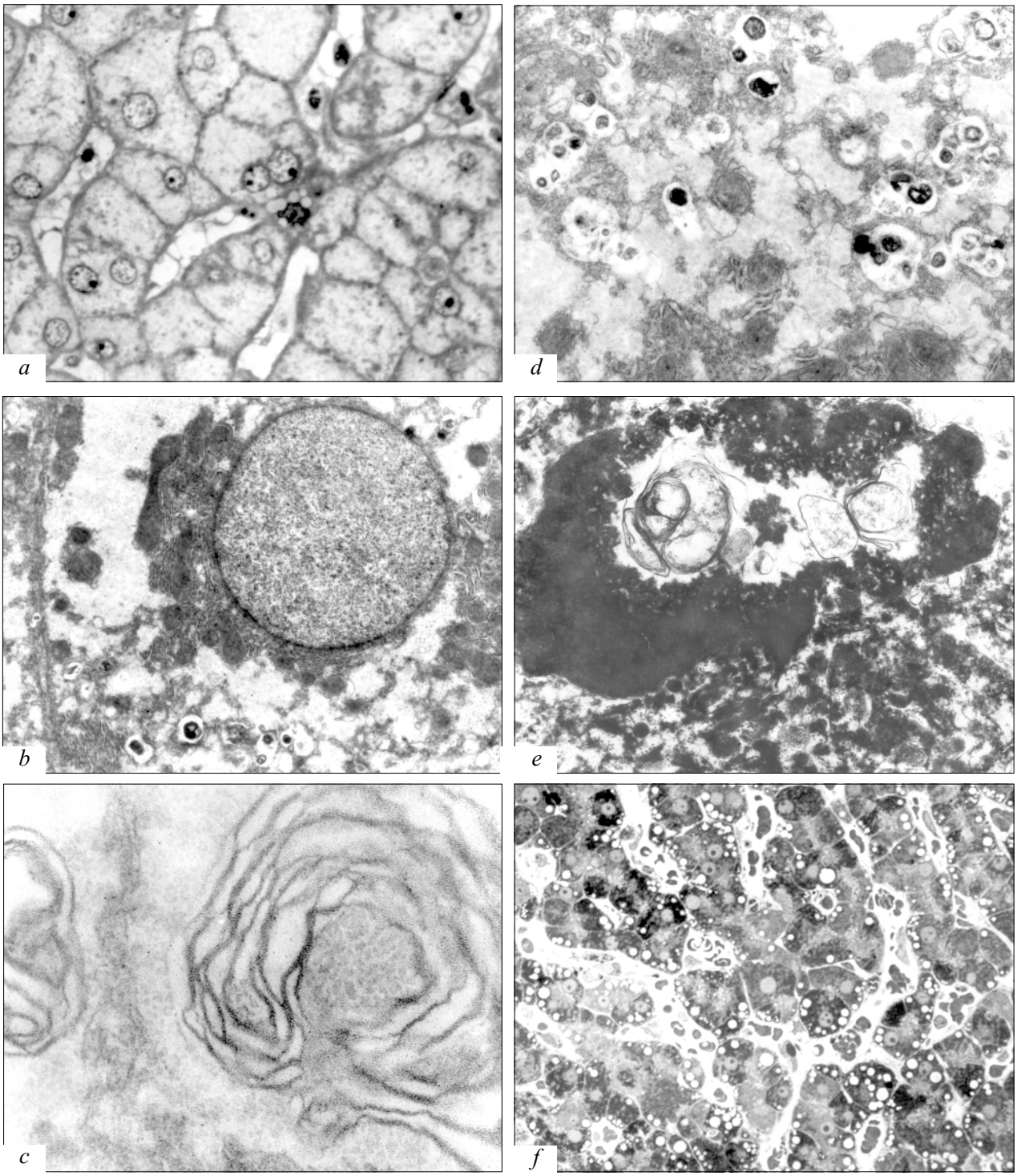

Fig. 2. Chronic hepatitis C. Liver biopsy specimens. Dystrophic changes in hepatocytes. Exhaustion of the cytoplasm in hepatocytes, semithin sections, staining with Schiff reagent and azure II (×600, *a*); exhaustion of the cytoplasm in hepatocytes, perinuclear conglomeration of mitochondria and granular cytoplasmic reticulum (×8000, *b*); sequestration of cytoplasmic regions in hepatocytes with complexes of osmiophilic structures (×20,000, *c*); focal degradation of the cytoplasm with the formation of numerous residual bodies (×12,000, *d*); apoptotic hepatocyte, degradation of the nucleus, conglomeration of cytoplasmic organelles (×8000, *e*); small-vesicular lipid subplasmalemmal infiltration of hepatocytes, semithin sections, staining with Schiff reagent and azure II (×250, *f*). Electronograms (*b*-*e*).

like structures proceeded in several stages: marginal conglomeration of heterochromatin lumps and widening of nuclear pores, progressive lysis of the nucleolemma with condensation and dislocation of the chromatin, and formation of condensed chromatin. It should be emphasized that transformation of nuclei was not necessarily associated with changes in the cytoplasm [16]. DNA viruses of animals rarely cause cell lysis: viral DNA is incorporated into the host chromosome and replicates with cell DNA over numerous cycles of cell division [31]. Cytolysis results only from chromosomal damage or cytotoxic reaction of T lymphocytes.

These data show that HCV+HBV mixt infection is characterized by phenotypic heterogeneity of hepatocytes associated with a variety of cytopathic effects produced by complex virus exposure. RNA virus of hepatitis C violates cytoplasmic organelles, but not nuclei. DNA virus of hepatitis D primarily produces degradation of the nuclear compartment and causes the appearance of signet ring-like hepatocyte nuclei (light microscopy data) [20].

Of particular interest is the combined effect of opiates and viruses of hepatitides C and B. Light microscopy showed that under these conditions structural changes in the liver did not differ from those observed during chronic hepatitides C and C+B. Several tissue and ultrastructural changes were associated with metabolism of narcotic drugs [62]. Specific fibrous changes in the liver included sclerosis of central veins and centrolobular zone (metabolic fibrosis) and mild sclerosis of portal and periportal tissues. Ultrastructural reorganization of the liver was manifested in hyperplasia of the smooth cytoplasmic reticulum (strain of detoxification activity in the liver), appearance of giant mitochondria in hepatocytes, and collagenation of the Disse's space [22].

Morphogenesis of Liver Fibrosis

Liver fibrosis is a typical reaction to chronic injury produced by various factors, including alcohol, persistent viral and helminth infections, and hereditary disturbances in metabolism of metals [27,40,41,50,75, 82,89]. Studies performed over the last 15 years elucidated the cellular and molecular mechanism of liver fibrosis [13,26,66]. It was shown that accumulation of the extracellular matrix during liver fibrosis is a dynamic and regulated, but not static and/or irreversible process. The central event in fibrogenesis is activation of stellate liver cells (Ito cells). They are transformed from passive vitamin A-accumulating cells into contractile, proliferating, and fibrogenic cells [56]. Cytokines and their receptors, products of lipid peroxidation, and other paracrine and autocrine signals are the major mediators for activation of stellate cells [51,64].

For a long time liver fibrosis and cirrhosis were believed to have a fatal outcome. This concept is currently undergoing revision [54]. It was shown that liver fibrosis regresses after biliary decompression [69]. Experimental studies demonstrated that even diffuse fibrosis of the liver is reversible [70]. Much progress in the therapy of liver diseases indicates that conservative treatment can be successful even in patients with pronounced histological changes, including cirrhosis. Moreover, fibrosis and cirrhosis of the liver sometimes regress after therapy [56].

Identification of factors associated with slow progression of fibrosis or favorable results of treatment is an important clinical problem.

Examination of biopsy specimens in chronic hepatitis C revealed diffuse fibrosis in the portal stroma (Fig. 3, *a*), walls of central veins (Fig. 3, *b*), and perivenular tissue (Fig. 3, *c*) that spread perisinusoidally and was accompanied by hyperplasia of Ito cells (Fig. 3, *d*). It was important to estimate the period from the onset of HCV infection and development of fibrosis. Most biopsy specimens were characterized by mild liver fibrosis (stage F0-1) even in long duration of the infection (more than 10-20 years). Stage II fibrosis (F2) was primarily observed in long duration of infection. In rare instances stage III fibrosis (F3) was related to infection with HCV for 1-5 years. We compared the severity of the infectious process and the stage of fibrosis. It was shown that the degree of fibrosis tended to be higher in patients with active viral hepatitis.

Examination of paired biopsy specimens in the dynamics of antiviral therapy showed that fibrous changes in the liver could undergo reduction: decrease in the volume of fibrous tissue in the portal stroma, centrolobular and, particularly, perisinusoidal zones (Fig. 1). Electron microscopy sometimes revealed resorption of collagen fibers by hepatocytes [1]. It was manifested in the unique ultrastructural phenomenon, reconstruction of the sinusoidal pole in hepatocytes with the formation of numerous plasmalemmal microvilli and plicae and concentration of mitochondria (Fig. 3, *e*). Expression of macrophageal properties in hepatocytes was probably accompanied by phagocytosis of fragments of collagen fibers; they were sometimes found in hepatocytes (Fig. 3, *f*). Previous experiments revealed similar structural changes in parenchymal liver cells [7].

These clinical and experimental observations indicate that diffuse fibrosis or cirrhosis is not irreversible in patients with preserved functions of the liver.

Liver Regeneration

The concept of intracellular regeneration suggests that most hepatocytes are capable of undergoing prolifera-

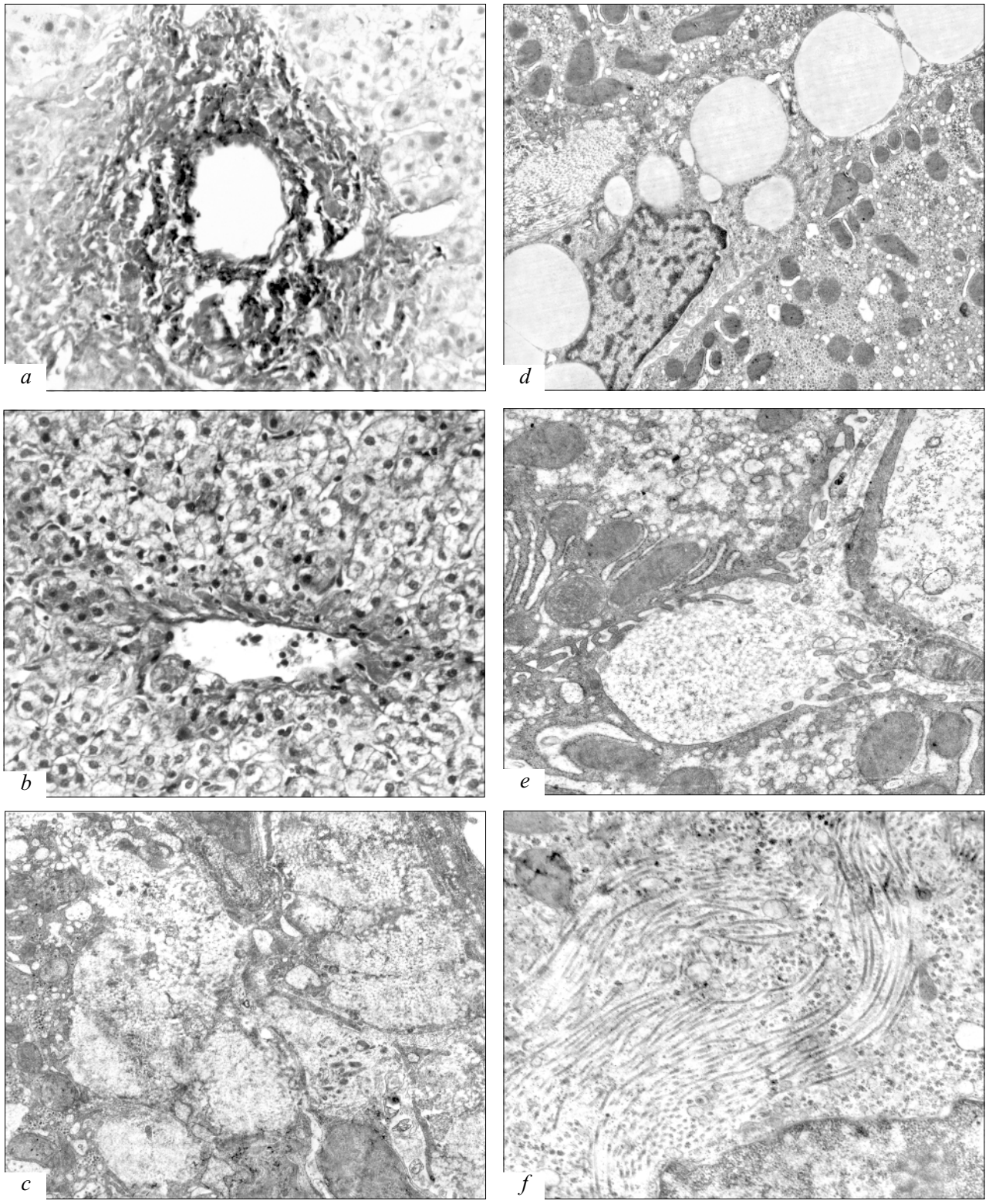

Fig. 3. Chronic hepatitis C. Liver biopsy specimens. Fibrogenesis and resorption of collagen. Portal and periportal fibrosis, van Gieson staining (×150, *a*); central and pericentral fibrosis, van Gieson staining (×150, *b*); thick bundles of collagen fibers in the wall of the central vein and perivenularly (×800, *c*); Ito cell with large lipid drops, numerous collagen fibers in the Disse's space (×2000, *d*); phagocytosis of collagen fibers, activated sinusoidal pole of the hepatocyte (×15,000, *e*); collagen fibers in the cytoplasm of hepatocytes (×10,000, *f*). Electronograms (*c*-*f*).

tion under conditions of injury [32]. Studies of cell proliferation after exposure to chemical carcinogens revealed the existence of bipotent stem cells differentiating into hepatocytes and cholangiocytes [87]. These cells are localized inside or near Hering ducts (anatomical junction of hepatocyte canaliculi and terminal branches of the biliary system). The proposed stem cells have phenotypic signs of cholangiocytes and hepatocytes.

Oval cells develop from pluripotent stem cells of the liver [48] and are often detected during chronic hepatitis C. A positive correlation was found between the severity of infection and the count of oval cells, which indirectly confirms the hypothesis that their proliferation plays a role in the development of hepatocellular carcinoma [76]. Proliferation of these cells uncompensated by adequate apoptosis (*e.g.*, at the stage of transformation into liver cirrhosis) increases the risk of tumor growth [65]. The appearance of fibrous tissue that is topographically close to these cells serves as a specific component for the induction of oval cell proliferation [30].

Our results indicate that the main signs of the regenerative processes in the liver during chronic hepatitis C are intracellular regeneration and changes in the nuclear compartment of hepatocytes. The number of binucleate and, more rarely, trinucleate cells increased in 44% samples. Polyploid nuclei and mitotic figures were rarely seen. Sometimes we revealed rosette-like structures that were predominantly localized in capillary sinusoids. In 1 patient repeated biopsy of the liver after therapy and withdrawal from ethanol intake revealed intensive proliferation of oval cells. These changes are usually observed during death of parenchymal cells in the liver [38,39]. Processes of regeneration in chronic HCV infection are associated with minimal morphogenetic destruction (as confirmed by low activity of cytolytic enzymes ALT and AST), which triggers cell proliferation involving stem cells.

A perspective approaches to the therapy of liver insufficiency is transplantation of stem cells capable of proliferating and differentiating into specialized parenchymal cells [35]. Fetal bipotent stem cells of the liver, oval cells, and mature hepatocytes proliferate under certain conditions and serve as the possible source of stem cells. It can be hypothesized that pluripotent fetal stem cells and bone marrow precursor cells perform a function of reserve stem cells.

Conclusion

Complex studies of chronic hepatitis C yielded the concept of antiviral protection in liver parenchymal cells, which suggests inhibition of biosynthetic reactions in hepatocytes, suppression of viral replication, focal degradation of the cytoplasm, exocytosis and elimination of viral particles (sanation), and structural recovery of hepatocytes due to intracellular regeneration. The distribution of these changes indicates that cell-involutory degeneration plays an important role in the morphogenesis and outcome of the disease.

Studies of fibrogenesis during HCV infection showed that fibrous changes in the liver can undergo reduction. A possible mechanisms underlying resorption of collagen fibers is expression of macrophageal properties by hepatocytes and their involvement in endocytosis of collagen.

PCR for HCV RNA and biopsy of the liver during chronic HCV infection showed that viral replication does not necessarily correlate with the severity of the infectious process. This phenomenon is related to a pronounced antiviral reaction of liver parenchymal cells. This reaction suppresses HCV replication and reduces its heterogeneity and variability, which contributes to insufficiency of the immune response.

It should be emphasized that modern notions of extremely hazardous and short-term interrelations between infectious agents and host organism contradict ecological principles and do not reflect reciprocally beneficial relationships in the evolutionary developed ecosystem "macroorganism-microorganism" [42]. The natural outcome of persistent infections is not rapid and obligatory eradication of infectious agents from the organism, but the formation of protective reactions in the macroorganism suppressing excessive division of the infectious agents and providing conditions for the most favorable type of symbiosis, commensalism.

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