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In Vivo and *in Vitro* Modelling of Hepatitis C Virus Infection

A. B. Bychenko, E. I. Samokhvalov, N. V. Markova, O. S. Rusanova, A. A. Rzhaninova, Yu. Yu. Tentsov, E. K. Artemov, and G. T. Sukhikh

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The possibility of replication of hepatitis C virus in mice and cultured mouse neurocytes was demonstrated. The elaborated models simplify investigation of this infection.

Key Words: *hepatitis C virus; virus isolation; infection models; neurotropism*

Hepatitis C virus (HCV) belongs to the flaviviruses family and is one of the most common infectious agents causing liver diseases. Clinical by liver damage varies from mild chronic hepatitis to cirrhosis and hepatocellular carcinoma [1,4,7].

In contrast to other human hepatotropic viruses, HCV causes chronic process (in 60-80% cases) associated with persistent viral replication [1,4,5,9]. The prognosis of this infection is difficult because its pathogenesis is little studied and the factors affecting viral replication are not determined. However, the possibility of *in vitro* replication of viral RNA was previously shown [1,2,6,8,15]. The authors used cells from primates, because it was believed that only humans and some apes can serve as a source of infection. Until recently the possibility of *in vivo* passages of HCV was shown only for chimpanzee [6,12,14]. In these studies viral RNA was detected in hepatocytes and peripheral blood mononuclear cells (lymphocytes and monocytes). Correspondingly, *in vitro* replication of HCV RNA was demonstrated in cultured primate hepatocytes and lymphocytes [1,2,6,8,15]. All described models have a number of disadvantages: low replication of viral RNA, long incubation period, and the absence of cytopathic effect. Thus, HCV tropism to hepatocytes and mononuclear blood cells was regarded as the key factor determining pathogenesis of the disease.

The purpose of the present study was to isolate and adapt HCV to alternative cell cultures and laboratory animals (mice).

MATERIALS AND METHODS

The following cell lines were used: PLC/PRF/5 (human hepatoma), MT-4 (human lymphocytes), and NB41A3 (mouse neurocytes). Cells were cultured in DMEM with 10% fetal calf serum (FCS). The cells were infected by incubation with 5% infectious serum (4 h, 37°C), thereafter the monolayers were washed and incubated in DMEM with 2% FCS.

In vivo experiments were performed on BALB/c mice. One-2-day-old newborn mice were injected intracerebrally with 100 μl infected serum.

The sera were obtained from 7 patients with viral hepatitis C (acute stage) and 2 pregnant HCV-infected women without clinical manifestations of hepatitis, in whom abortion was induced by social recommendations on weeks 20 and 22 of pregnancy and sera from infected fetuses were also used as infectious material.

D. I. Ivanovskii Institute of Virology; Research Center of Obstetrics, Gynecology and Perinatology, Russian Academy of Medical Sciences; Moscow

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TABLE 1. Genotyping of HCV Isolates

Serum No.	HCV genotype in serum	Isolates	HCV RNA in brain suspensions	HCV genotype in isolates
1	1b	CIM-1	+	1b
3	1b	CIM-2	+	N. d.
4	1b	CIM-3	+	N. d.
6	1b	—	—	N. d.
7	1b	CIM-4	+	1b

Note: here and in Tables 2 and 3: N. d. — not determined.

Sera were tested for the presence of all known hepatitis markers (A, B, C, D, E, and G). Viral antigens and antiviral antibodies were detected with enzyme immunoassay kits ("Litex" and "Preparat"). The presence of HCV and HGV was confirmed by PCR with reverse transcriptase (RT-PCR).

RNA was isolated from 200 μl culture medium or brain suspension by guanidine-isothiocyanate-phenol-chloroform extraction [3]. RT-PCR was performed with specific primers for 5'-nontranslated HCV region (5'-NTR) as described elsewhere [10].

HCV infectivity was expressed in LD_{50}/ml for 10% mouse brain suspension and in tissue cell infectious dose ($TCID_{50}$) per 1 ml culture medium and cell lysates.

HCV genotype in sera and cultured cells was determined by RT-PCR with specific primers to core proteins [11]. The set of primers included genotypes 1a, 1b, 2a, 2b, and 3a. Nucleotide sequence was determined by 2 chains for each PCR-product from 2 independent reactions [13].

RESULTS

HCV RNA was present in all sera used for infection, 1b genotype was revealed in 5 of them. Damage to the central nervous system (CNS) were revealed in 4 cases on days 5-10 after primary intracerebral infection by all types of sera; 20-30% animals died. Brain

TABLE 2. HCV Passage in Newborn Mice

Isolate, passage		Mean survival after infection, days	Lethality, %	Infectivity of brain suspensions, LD_{50}
CIM-1	1st	5-10	20	10^4
	5th	2-3	100	10^8
	20th	2-3	100	10^8
CIM-2	1st	10-14	20	10^4
	5th	3-4	100	10^6
	20th	2-3	100	N. d.
CIM-3	1st	7-10	25	10^4
	5th	2-3	100	10^8
	20th	2-3	100	N. d.
CIM-4	1st	5-11	30	10^5
	5th	2-3	100	10^8
	20th	2-3	100	10^8

TABLE 3. Determination of RNA and HCV Genotype in Passaged Cells

Cell line	Cytopathic effect	HCV RNA	HCV genotype
PLC/PRF/5	—	—	N. d.
MT-4	—	—	N. d.
NB41A3	+	+	1b

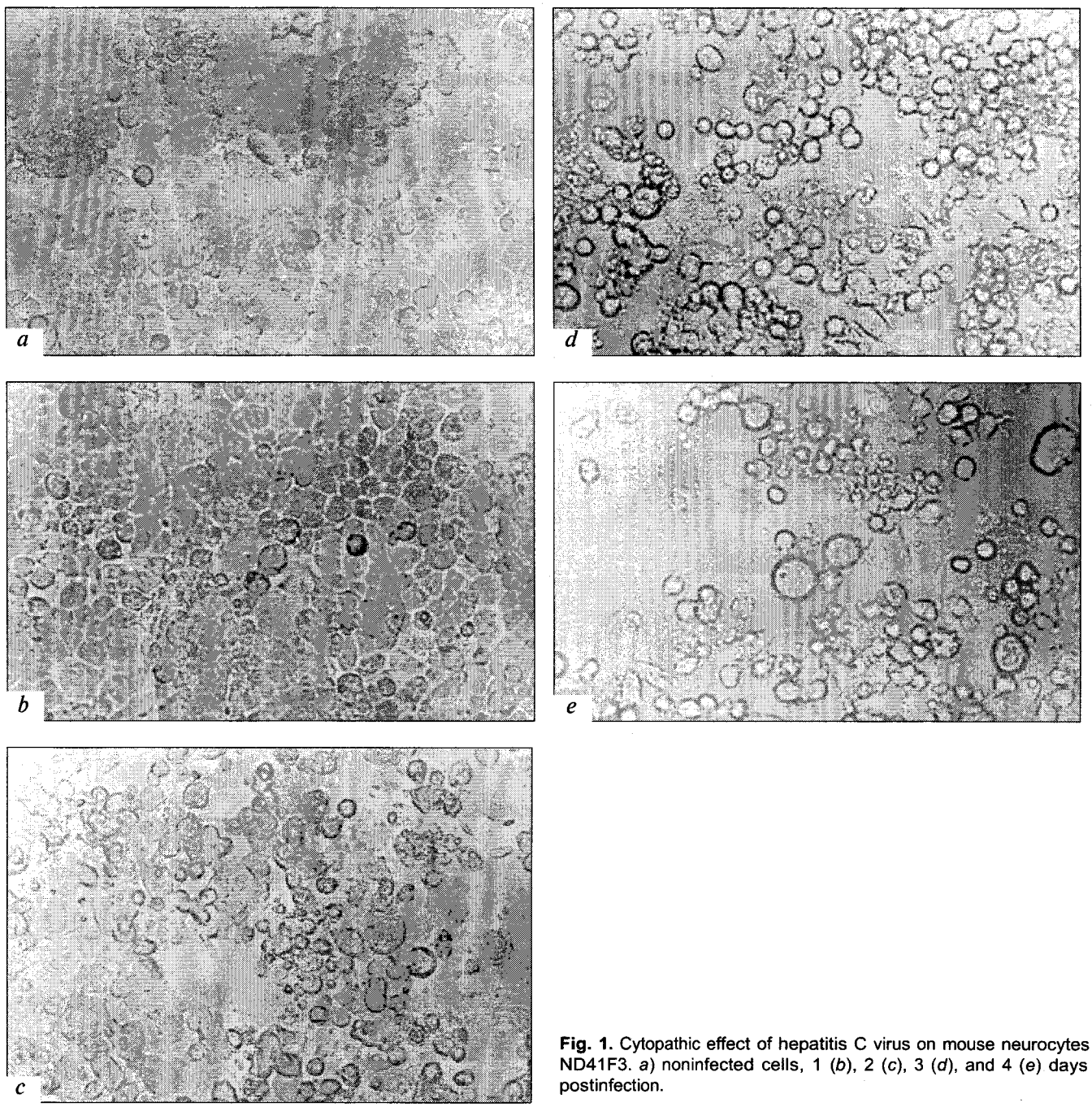

Fig. 1. Cytopathic effect of hepatitis C virus on mouse neurocytes ND41F3. *a*) noninfected cells, 1 (*b*), 2 (*c*), 3 (*d*), and 4 (*e*) days postinfection.

suspension (1:10) from each dead animal was analysed and used for subsequent infection. Similar symptoms were induced by repeatedely passaged HCV. Practically all animals died on days 2-4 postinfection. The presence of HCV RNA in brain suspensions was confirmed by RT-PCR with primers to 5'NTR-region. Viral RNA and viral genotype were examined in all passages. HCV isolates were designated as CIM-1, CIM-2, CIM-3, and CIM-4. Genotype 1b was revealed in all primary sera causing CNS damage, but only in 2 cases (CIM-1 and CIM-4) it was present in brain suspensions (Table 1). CIM-1 and CIM-4 were passaged 10 and 20 times, respectively. Infectivity of 10% brain suspensions was 10^5-10^6 LD_{50}/ml (Table 2).

Infection of cultured cells with sera from patients with hepatitis C resulted in only 1 case (serum No. 6) of acute (permissive) *in vitro* infection. NB41A3 cells were sensitive to HCV (Table 3). During passage I cytopathic effect (degeneration of 90-100% cells) was observed after 9 days. During subsequent passages cytopathic effect developed after 2-4 days (Fig. 1). HCV RNA was detected by RT-PCR in cell lysates and culture medium.

In cells infected by CIM-5, 1b genotype was detected. This isolate was passaged 30 times. Infectivity of culture medium by the end of each passage reached 10^4-10^5 $TCID_{50}$/ml.

Nucleotide sequence of PCR-products to 5'-NTR regions of CIM-4 and CIM-5 isolates confirmed the specificity of HCV infection. RNA nucleotide sequences of HCV isolates within 5'-NTR 86-230 fragment (nucleotide positions are indicated in accordance with HCV-1 prototype) corresponded to the sequences of other isolates. Homology between these regions of viral RNA in CIM-4 and CIM-5 reached 90.1%.

Thus, the possibility of *in vivo* and *in vitro* replication of HCV in mouse neuronal cells was established. Clinical manifestations of acute infection and high percent of primary isolation from mouse neurons confirmed neurotropism of this virus and high phenotypic similarity between flavi- and pestiviruses. The elaborated model of HCV culturing in mice and NB41A3 cells simplify investigation of this infection.

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