PRIMATOLOGY

Spontaneous Infection of Lower Primates with Hepatitis C Virus

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We present serological and virological evidence of spontaneous infection in Old World lower monkeys with human hepatitis C virus or, maybe, antigenically related but genetically different simian virus strain. These data is of both theoretical and practical importance and can serve as the basis for further development of experimental model of hepatitis C on lower monkeys.

Key Words: *spontaneous hepatitis C; classes G and M antibodies to HCV, HCV RNA, monkeys*

It is now accepted that chimpanzee are the only primate species sensitive to human hepatitis C virus (HCV) and other viruses causing parenteral hepatites; these monkeys are now used for modeling this infection [2,4]. However they are not always available and expensive, and therefore other, cheaper and more available monkey species are searched for this purpose. Relevant published reports are scanty. One report informs about negative results of intravenous HCV infection of some lower monkey species (*Macaca mulatta*, *M. fascicularis*, *Cercopithecus aethiops*, *M. fuscata*, and *Papio anubis*) [3]. Another report informs about positive results of experimental HCV infection of small tree monkeys (tupaias) [7]. Spontaneous HCV infection of monkeys was virtually never studied. Published data on the absence of anti-HCV antibodies in rhesus macaques and baboons were obtained on a small sample of animals (25 per species), and no reliable conclusion can be made on this point [6].

Our Institute has a unique collection of Old World monkeys, presented by different species, and therefore we could carry out a wide-scale serological screening of monkeys for anti-HCV and detect virological (HCV RNA) markers of spontaneous hepatitis C.

MATERIALS AND METHODS

The study was carried out in Adler Monkey Breeding Center in 1999-2001. Blood sera from 1398 monkeys of different species kept in different parts of the breeding center were tested: 744 *Macaca mulatta*, 338 *M. fascicularis*, 63 *M. nemestrina*, 81 *Cercopithecus aethiops*, 138 *Papio hamadryas*, and 34 *P. anubis*. For comparative study of immune response to HCV in humans, we used 26 anti-HCV-positive sera: 14 from patients with viral hepatitis C and 12 from donors tested at blood transfusion center in Sochi.

Anti-HCV antibodies were detected in human and simian sera by enzyme immunoassay with GepaSkrin (Vektor-BiAlgam) and IFA-ANTI-HCV (Diagnosticheskie sistemy) commercial kits. Positive results of screening for anti-HCV were confirmed using Anti-HCV-IFA-confirming system (core, NS3 and NS4 antigens) and IFA-ANTI-HCV-Spektr system (core, NS3, NS4 and NS5 antigens). IgM antibodies to HCV core antigen were detected mainly using IFA-ANTI-HCVc-M and in some cases with RekombiBest anti-HCV IgM (Vektor-Best). Human and simian sera were used

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in dilutions 1:2 and 1:5 according to the manufacturers' instructions. Positive sera were titered selectively. The results of enzyme immunoassay were analyzed on a Uniscan spectrophotometer (Flow) using a filter with a wavelength 492 nm.

HCV RNA in anti-HCV-positive sera from humans and monkeys was detected by the PCR using Russian commercial test systems (DNK-Tekhnologiya) and AmpliSens HCV-240/BKO-440 (Institute of Epidemiology, Ministry of Health of Russian Federation) in accordance with the manufacturers' instructions. Conservative site in 5'-nontranslated domain of viral genome was used as the amplification target.

RESULTS

Anti-HCV antibodies were detected in monkeys of virtually all tested species, their incidence varied from 2.9 to 16.0%. In *M. mulatta* and *M. fascicularis* (which were the most numerous) the percentage of positive animals was 12.1 and 8.3, respectively. Monkeys of other species were less numerous, but some of them were also anti-HCV-positive, the greatest number among *M. nemestrina* and *C. aethiops* (16%) and the least among Papios of both species (less than 6%). The general percentage of monkeys infected with HCV was 10.5, which is notably higher than in healthy population [2]. Table 1 presents the data confirmed by analysis in one or two test systems. It is noteworthy that if the results were confirmed in parallel in two test systems, they completely coincided in about 70% cases. This fact requires explanations, one of which can be the use of antigens of different protein composition in the test systems [1]. The number of false-positive results which were not confirmed in any of the test systems was 1.8% (25 of 1398).

The spectrum of anti-HCV in the monkeys varied within a wide range: from production of a complex of antibodies to the major diagnostic HCV proteins (structural core and 1-3 nonstructural NS3, NS4, and NS5), which was observed in 23.3-76.9% sera to the presence of antibodies either to the core antigen (1.1-50.0%) or to NS proteins alone (23.1-100%). The percentage of animals whose sera were reactive with regard to the core and 1-3 NS proteins was the highest among *C. aethiops*, *M. fascicularis*, and *M. nemestrina* (76.9, 46.4, and 40.0%, respectively). By contrast, the majority of *M. mulatta* and *P. hamadryas* (75.6-100%) had antibodies only to NS proteins. HCV-positive human sera contained mainly a complex of antibodies to the core and NS proteins (25 patients, 96.4%); antibodies to the core antigen were detected in only 1 patient. It is noteworthy that the titers of anti-HCV in the majority of simian sera were not high in comparison with human sera (1:5-1:40).

The percentage of seropositive monkeys depended not only on the species, but on the place of their residence as well. Anti-HCV-positive animals were detected in only 13 (50%) of 26 tested groups of *M. fascicularis*, 27 (79.4%) of 34 groups of *M. mulatta*, and 8 (72.7%) of 11 groups of *C. aethiops*. The percentage of seropositive animals in these groups varied from 5.9 to 35.5% in *M. mulatta* and *M. fascicularis* and from 23.5 to 44.5% in *C. aethiops*.

Anti-HCV-positive animals were aged 11 months to 23 years. The group of seropositive *M. fascicularis* included 36% adolescents (1-3 years), 21% young (3-5 years), and 43% adult (over 5 years) monkeys, the corresponding values for *C. aethiops* being 7, 33, and 60%.

It was important to test simian sera for anti-HCV IgM, as the presence of these antibodies is evidence of a "fresh" infection. A total of 401 monkeys were tested with this aim in view; IgM were detected in 34 (8.5%). The total number of monkeys with IgM antibodies of those IgG-positive to this virus was 17.5% (18 of 103) and 5.4% of those IgG negative (16 of 298). The incidence of IgM in the sera varied, depen-

TABLE 1. Spectrum of Anti-HCV in the Sera of Monkeys of Different Species

Monkey species	Percentage of positive*	Antibodies to HCV proteins		
		core and NS	core	NS
Macaca mulatta (*n*=744)	90 (12.1)	21 (23.3)	1 (1.1)	68 (75.6)
Macaca fascicularis (*n*=338)	28 (8.3)	13 (46.4)	5 (17.9)	10 (35.7)
Macaca nemestrina (*n*=63)	10 (15.9)	4 (40)	1 (10)	5 (50)
Cercopithecus aethiops (*n*=81)	13 (16.0)	8 (76.9)	0 (0)	3 (23.1)
Papio hamadryas (*n*=138)	4 (2.9)	0 (0)	0 (0)	4 (100)
Papio anubis (*n*=34)	2 (5.9)	0 (0)	1 (50)	1 (50)
Total (*n*=1398)	147 (10.5)	48 (32.7)	8 (5.4)	91 (61.9)

Note. *Of the total number of positive sera.

TABLE 2. Characterization of HCV RNA-Positive Sera

Monkey species	Age, years	Cage/aviary	HCV markers						
			anti-HCV	anti-core	anti-NS3	anti-NS4	anti-NS5	anti-HCV core IgM	HCV RNA
M. mulatta									
No. 26,102	9.1	Aviary 31	+++	++++	+	+++	+++	++	++++
No. 33,291	1.6	Cage 5	++++	+	++	++++	+	++	++
No. 3101	12.8	Aviary 23	++++	+	++++	+	—	+	++
No. 32,273	3.8	Aviary 23	++++	++	++	++++	+	—	++++
No. 32,740	2.4	Area No. 1	+++	+++	++++	++++	—	—	++
No. 32,967	6.0	Aviary 67	++	+	+++	+	+	—	++++
No. 33,406	1.6	Aviary 67	+++	—	++	++++	—	—	++++
No. 21,945	14.1	Aviary 25	+++	—	++++	+	+	—	++
M. fascicularis									
No. 30,598	8.5	Aviary 11	++++	++++	++	—	—	+	++
No. 31,030	8.2	Aviary 11	++	++	+	—	—	—	++
M. nemestrina									
No. 17,184	22.4	Aviary 54	++++	++++	—	—	—	—	++++

Note. Number of pluses corresponds to the intensity of reaction.

ding on the monkey species, being the highest in *M. mulatta* and *M. nemestrina* (9.6 and 7.9%, respectively). It is noteworthy that the titer of anti-HCV IgM was not high (1:5-1:20).

We tested the sera of anti-HCV-positive monkeys for HCV RNA by the PCR method. This test showed the presence of viral genetic material in the sera and its active replication in monkeys. By the present time 80 seropositive and 6 seronegative simian sera are tested. According to preliminary data confirmed in two Russian test systems, HCV RNA was detected in 11 (14.1%) of 78 anti-HCV-positive sera (from 8 *M. mulatta*, 2 *M. fascicularis*, and 1 *M. nemestrina*). The sera of RNA-positive monkeys were characterized by different spectra of anti-HCV: 8 (72.7%) of 11 had antibodies to the core and 1-3 NS antigens, 1 (9.1%) to the core antigen alone, and 2 (18.2%) to NS antigens (Table 2). It is noteworthy that IgM antibodies were detected in 4 (36.4%) of 11 HCV RNA-positive sera. The age of RNA-positive monkeys varied from 1.6 to 22.4 years.

As for the intensity of antibody response, it was lower in monkeys than in humans, in whom the intensity of reaction to all HCV markers corresponded to four pluses. We should also like to emphasize that RNA was detected in at least two monkeys in yards Nos. 23, 67, and 11, where the number of seropositive animals was 23-28%. Animals of these groups are particularly interesting from epizootological and epidemiological viewpoint and should be regularly tested.

Search for the probable source of HCV infection and evaluation of epidemiological hazards of seropositive animals for man was one more aspect of our investigation. Preliminary serological testing of 80% staff of the Breeding Center and laboratories showed that none of them had hepatitis C markers.

Hence, we obtained serological and virological evidence of spontaneous HCV infection of Old World lower monkeys for the first time. However these results require thorough analysis and further studies. First of all, we must solve the key problem, whether the immune response in monkeys is directly associated with human HCV or with antigenically related but genetically different strain of simian virus. As even human HCV strains are characterized by exclusive genome heterogeneity [2], HCV RNA specimens isolated from *M. mulatta*, *fascicularis*, and *nemestrina* require additional genotyping or sequencing.

It is also essential to analyze the causes of a great variety of antibody response to HCV in monkeys and its lower intensity in comparison with humans, and the presence of anti-HCV to only NS proteins in rather many monkeys. It is noteworthy that similar data were obtained in testing of chimpanzee 2.5-10 years after their experimental infection with HCV strain isolated from man [5]. Further monitoring is needed in order to trace the formation of humoral immunity to HCV in monkeys; this monitoring is important from epizootological and epidemiological viewpoints. One more important aspect is search for biochemical and mor-

phological signs of hepatitis C in monkeys for evaluation of its clinical forms.

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