REVIEWS

Monkey Models of Hepatitis A (Results and Prospects)

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The current status of animal models of hepatitis A using various species of monkeys is reviewed, and it is shown that these models can reveal new aspects of its pathogenesis.

Successful research into a number of human viral infections depends in large measure on the availability of appropriate animal models. Work on human infections that can be reproduced only in nonhuman primates (monkeys) incurs additional problems. One such infection is hepatitis A (HA), and the role of monkeys in its study is hard to overestimate. A great deal of time and effort has gone into the development of animal models for human HA.

Attempts to produce HA in monkeys, begun in the 1940s, were not successful until the late 60s. At first, Bearcroft succeeded in reproducing signs of liver damage typical of this disease by infecting red monkeys (*Erythrocebus patas*) [12], but these studies were unfortunately discontinued. Shortly afterward, Deinhardt successfully infected South American marmosets (*Saguinus nigricollis* and *S. fuscicollis*) with material from human patients [19]. Characteristic morphological changes in the liver and elevated levels of serum transaminases were observed in five passages. Monkeys that had sustained the disease were resistant to reinfection. It was also shown that the sera of patients cured of HA neutralized the virus isolated from infected marmosets [42]. Marmosets, however, proved to be insensitive to the infective agent under natural conditions and fell ill only when infected experimentally with it in large doses. Nonetheless, the marmoset model mentioned above made a valuable contribution to the body of knowledge on HA because this was its first animal model widely used during the period when the HA virus (HAV) had not yet been discovered and methods for a specific diagnosis of the disease were unavailable.

More recently, other marmoset species (*S. mystax*, *S. labiatus*) were found to respond to oral or parenteral administration of infective material (liver tissue from human patients) by morphological changes in the liver characteristic of acute hepatitis and by elevated activities of serum transaminases, although they did not develop clinical manifestations of hepatitis such as jaundice. It was proposed to use marmosets for detecting the virus in infective materials, i.e., as a test system.

For a number of years, until cultured HAV strains were produced in the 80s, the liver and feces of infected monkeys were the only virus-containing materials accessible for study. They were utilized both to examine morphological, physical, and chemical properties of the virus [43] and as antigen in immune reactions. Using such an antigen, serological surveys were conducted among human and monkey populations [20,24,36]. Also, marmoset livers were used to prepare the first anti-HA vaccine, whose efficacy was demonstrated by immunizing and challenging these marmosets with a virus strain pathogenic for them [40]. In addition, the marmoset model was employed to study the pathogenesis of HA infection, virus replication sites, and the mechanism at the root of the damage caused by the virus. Some researchers came to the conclusion that the liver is the only site where the virus replicates and that liver damage is the result of its direct action

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[30,35]. Other investigators obtained important information on extrahepatic localization of HAV. With immunofluorescence assays, the viral antigen was detected not only in hepatic cells but also in the mucous membrane of the upper small intestine and in membranes of renal and splenic cells [28,37]. Furthermore, the marmoset model provided convincing evidence for the involvement of an immunopathological mechanism in the pathogenesis of HA [27]. A series of studies was carried out to test the efficacy of killed cultured vaccine [6,41] and to verify the attenuation of virus strains intended for use in live vaccine [25,29,39]. The model also proved very useful for a comparative genetic analysis of the attenuated and wild-type HAV strains [17].

Another animal model became available in 1975, when HA was reproduced in chimpanzees after 30 years of unsuccessful attempts. This species of nonhuman primates proved highly susceptible to HAV, could be readily infected with it, sustained the natural infection without any apparent clinical manifestations, and acquired immunity. Previous failures to infect chimpanzees were retrospectively blamed on the use of immune animals in the experiments. With the advent of appropriate immunological tests, only seronegative animals in which HA infection could be produced were selected for experimentation. The use of chimpanzees as an experimental model of HA has been the subject of numerous publications. Early studies were aimed at characterizing the experimentally induced infection and the virus itself [14,21, 42,49]. The disease developed after oral as well as intravenous administration of fecal filtrates from human patients. After an incubation period of 2 to 4 weeks, the HAV antigen appeared in the urine of infected chimpanzees. Virus excretion also continued for 2 to 4 weeks. Sera from infected animals contained elevated aminotransferase levels and liver biopsy specimens from them showed histopathological changes typical of acute hepatitis. The disease was accompanied by the appearance of anti-HAV IgM and IgG antibodies. External manifestations of the infection were either absent or limited to nonspecific symptoms such as listlessness and anorexia; jaundice was not observed. With immunoelectron microscopy and immunofluorescence methods, the HAV antigen was detected in the cytoplasm of hepatocytes and Kupffer's cells during the 4th week of infection [38].

The chimpanzee model adequately reproduces human HA and is regarded as an indispensable tool for checking the virulence of attenuated strains selected as candidates for live vaccines [22,29]. This model is also of much value in studies on the pathogenesis of HA infection, which have yielded evidence suggesting extrahepatic replication of the virus [16]. After oral infection of chimpanzees, the HAV antigen was sequentially detected in their serum (on day 14 postinfection), tonsils (on day 16), saliva and pharyngeal washings (on day 18), and liver (in the 3rd week). Another possible site of virus replication is thought to be the oropharynx [16]. Furthermore, the model has been used to examine the contribution of immunopathological mechanisms to the development of HA. Circulating IgM immune complexes were found in 8 of 9 infected chimpanzees during the period when they exhibited a set of pathological changes characteristic of this infection [34]; the formation of such complexes is considered to reflect the viremic phase of infection, but further research is needed to elucidate the role of this phase in the pathogenesis of HA.

Our review of the literature attests that the marmoset and chimpanzee models have greatly contributed to our knowledge of HA. However, investigators are up against serious problems, especially in procuring animals. Chimpanzees are very expensive and, moreover, special permission is required to use them in experiments. Marmosets are also hard to obtain: the natural population of these South American monkeys has drastically declined, and it is extremely difficult to breed them in nurseries because of their exacting demands as regards maintenance conditions. Attempts were therefore made to reproduce HA in other monkeys, but did not meet with success for many years. In infection experiments, various species of Old and New World monkeys have been used, including macaques, talapoins, baboons, bushbabies, and woolly, squirrel, and night monkeys [18,44,45,48]. Reports of positive infection developing in some of those species began to appear in the 80s; the disease was successfully produced in American bushbabies (primates of the genus *Galago*) [23]. Somewhat later, a spontaneous outbreak of HA among night monkeys was described; using a virus strain isolated from diseased animals and from human patients, a similar disease was induced in these monkeys experimentally [32,50]. Night monkeys were exploited for testing killed vaccines and one variant of attenuated vaccine [13,33].

The view was expressed in the literature that Old World monkeys, which are more readily available and reproduce well in captivity, respond to infection only by producing antibodies without developing any signs of the disease and so are unsuitable for use as a model of human HA [44,48]. These monkeys have also been deemed unfit for this purpose in recent reviews. Yet our findings, as well as those obtained later by other workers, contradict this view. Indeed, a natural infection with signs and symptoms typical of HA was identified among brown macaques of the

Sukhumi Nursery [2], and outbreaks of spontaneous HA were recorded among rhesus and Javan macaques and green monkeys imported to the Sukhumi Nursery from their natural habitats in 1985-86 [4,8,46]. Almost at the same time, other investigators also reported signs of naturally evolving HA in rhesus macaques [31], Javan macaques [47], and green monkeys [1]. Contrary to the prevailing opinion, monkeys of these species proved to be highly susceptible to HAV. On arrival at the Sukhumi Nursery, most monkeys had neither anti-HAV antibodies nor any other signs of infection, although each batch contained a few individuals already in the acute phase of the disease. This phase proceeded without overt clinical manifestations (e.g., jaundice was never noted), but was accompanied by typical signs of HA such as HAV excretion in the feces, elevated serum alanine aminotransferase (ALT), histological changes in liver biopsy specimens, and the appearance of anti-HAV IgM and IgG antibodies. Comprehensive examinations of the monkeys revealed that the seronegative individuals were infected one after another over a period of 2 to 4 months in each batch, and that the infection was accompanied by the above-mentioned laboratory signs of HA. From the diseased rhesus macaques, green monkeys, and hamadryas baboons, virus strains designated as HAV-RM, HAV-GM, and HAV-HB, respectively, were isolated and found not to differ from human HAV in morphological, physicochemical, or antigenic properties. With these strains, an experimental HA with manifestations similar to those of spontaneous and human HAV was produced in monkeys and proposed as a model [5]. However, there remained the important question of whether or not human HAV is pathogenic for macaques and green monkeys. In addition to being of theoretical interest, this question is of great practical importance because strains of human HAV pathogenic for monkeys must be used to infect monkeys when protective properties of candidate vaccines are being tested. Models using such a strain were developed by collaborative efforts at the Institute of Experimental Pathology and Therapy (Sukhumi) and the D. I. Ivanovskii Institute of Virology of the Russian Academy of Medical Sciences (Moscow). A disease resembling human HA was first induced in Javan and rhesus macaques and green monkeys using an isolate, designated as HAV-41, from the feces of a patient, after which an HAV strain with stable properties was obtained through passages in rhesus macaques and proposed for practical use [7].

Monkeys of the above-mentioned species proved susceptible to both oral and intravenous infection, and the disease they developed was accompanied by a set of characteristic signs and symptoms [9]. The findings from these studies enabled the spontaneous and experimental HAs seen in Javan and rhesus macaques and green monkeys to be regarded as adequate models of human infection not inferior to the similar models developed earlier. We used them to clarify several issues related to the pathogenesis and epidemiology of HA infection. Comprehensive examinations of the monkeys over a two-year period enabled us to follow the time course of virological, biochemical, morphological, and serological signs of the infection, their persistence, and the correlations between them. Both the acute and chronic forms of the disease were observed in the macaques and green monkeys with spontaneous or experimentally induced HA [10]. The acute form lasted 1.5 to 2 months or, less frequently, 3 to 4 months. The earliest sign of disease was fecal virus excretion, which started on days 4-6 after the monkey was infected. Elevated activity of serum ALT and morphological changes in liver biopsy specimens were usually recorded several days later (but sometimes also on days 4-6). Virus excretion and elevated ALT activity were cyclic, correlated in time, and lasted from 3-4 days to 2-4 weeks. Morphological changes in the liver escalated over a period of 1 to 2 weeks and then gradually subsided and disappeared in 1.5-2 months (less often in 3-4 months). When the disease became chronic, two variants were observed, with or without relapses, but in both cases the first phase of the infection took on an acute form and its manifestations were similar in all monkeys and did not differ much from those seen in the acute form described above. In cases of chronic recurring HA, the acute phase was succeeded by a period lasting from 1 to 7.5 months during which none of the signs of infection were evident. This period was followed by one or, less often, more (2 or 3) recurrences of 1 to 5.5 months' duration with virological, biochemical, and morphological signs of HA. The second variant was long-lasting and comprised several (3 to 6) phases. After the acute phase, virological and biochemical signs of the disease were no longer observed, but morphological changes in the liver, while much less pronounced than before, persisted for 7 to 16 (or even 28) months and, moreover, fecal HAV excretion and elevated ALT activity were concurrently recorded from time to time. Occasionally, morphological changes in the liver were intensified during that period. Virus in the feces of monkeys with chronic HA was still detectable 7 to 15 months (20 months in some cases) after their infection [11]. The continuing pathogenicity of persisting HAV was demonstrated in an experiment where seronegative macaques were inoculated with a suspension of feces collected during a

relapse occurring about 13 months postinfection. It should be stressed that the virus was observed to persist during a time when the level of IgG antibodies was high.

The models described above demonstrated, for the first time ever, the ability of HAV to persist in primates while retaining its pathogenicity. That such persistence is a regular phenomenon was indicated by detection of the virus in both experimental and natural HAs in monkeys of three species. These findings suggested that the virus can also persist in the human body.

Recently developed models using macaques and green monkeys have thus yielded results shedding new light on what had seemed to be a well-known disease. These results are in tune with the recent dismissal of the concept that HA is an acute infection of short duration. The number of reports that the disease can run a protracted course with two or three phases and even with late recurrences is growing [15,26]. However, they have failed to confirm the etiological role of HAV and thus leave open the issue of whether or not infection with other hepatotropic viruses is added in cases of HA progression to chronicity [3]. Our findings from monkey models of HA have furnished evidence of an etiological link of HAV with the protracted forms, phases, and late recurrences of the disease, and have established a framework for conducting human studies to address this issue. Confirmation of the ability of HAV to persist in the human body will at last provide an affirmative answer to the question as to whether the virus can persist in individuals during an interepidemic period. This question is of great epidemiological importance because, in the absence of evidence that the immune organism can still harbor the virus, HA has so far been viewed as a peculiar exception in the epidemiology of anthroponoses.

The findings obtained with monkey models pose new questions for investigators, such as where in the body the virus is located during its prolonged residence there, what mechanisms promote its persistence and progression of the disease to its chronic form, and what role the factors of cell-mediated immunity play in these mechanisms. Other crucial points also remain unclear; for example, it is not known how immunopathological processes contribute to the damage suffered by liver cells, how long the immunity lasts, and how strong it is. These questions can all be addressed by making use of the models we have developed and described, given that these models (macaques and green monkeys) are just as good as their predecessors (marmosets and chimpanzees), but are more readily available and more cost effective. Their use should also provide broad opportunities for testing new vaccines and various drugs.

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