VIRUS HEPATITIS A IN LOWER OLD-WORLD MONKEYS (POSSIBLE MODEL FOR VACCINE TESTING)

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Manifest forms of spontaneous hepatitis A have been described only in chimpanzees and South American owl monkeys [10, 12]. The disease has been reproduced experimentally only in chimpanzees and marmosets [4, 5]. As regards the lower old-world monkeys, the view is held that they are subject to an asymptomatic infection, accompanied only by seroconversion [8]. Clinical and morphological manifestations of the disease have not been described in these monkeys, whether due to natural or to experimental infection [6, 11]. Meanwhile long experience of the study of spontaneous pathology of the lower old-world monkeys, kept in the Sukhumi nursery, has demonstrated that they are prone to liver lesions very similar in their morphological characteristics to human virus hepatitis. The frequency of these attacks of hepatitis have increased considerably in the last 5 years.

In this paper we give morphologic, virologic, and serologic data on spontaneous hepatitis A in macaques and green monkeys, and also the results of experimental reproduction of a disease similar to human hepatitis A in Javanese macaques.

EXPERIMENTAL METHOD

The investigation was conducted on lower old-world monkeys delivered to the Sukhumi nursery in 1985-1986 from their natural habitat: 93 rhesus monkeys (from Vietnam), 20 Javanese macaques (from Indonesia), and 200 green monkeys (from Kenya). In the experiments, virus-containing material (a mixture of suspensions of liver and feces from rhesus monkeys) was injected into three seronegative adolescent Javanese macaques, intravenously, and also was given perorally. The monkeys were examined before and after infection: liver biopsy was carried out and sera were tested for alanine aminotransferase (ALT) and the feces were studied by immunoelectron-microscopy. Organs of dying monkeys, fixed in 10% neutral formalin, were studied histologically. Paraffin sections were stained with hematoxylin and eosin. Frozen sections were stained for fat by Goldman's method. Hepatitis A virus (HVA) antigen and antibodies to it were determined by two methods: enzyme immunoassay (EIA) (jointly with A. G. Andzhaparidze and M. S. Balayan, Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR), and radioimmunoassay (RIA) (jointly with N. V. Doroshenko and V. M. Stakhanova, D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR). Materials were investigated by immunoelectron-microscopy (IEM) by the method described previously [9], in the JEM-100B electron microscope with instrumental magnification of 50,000-100,000. ALT activity was determined by a micromethod [3] and expressed in micromoles of substrate per milliliter of serum per minute.

EXPERIMENTAL RESULTS

A considerable mortality was observed in all groups of monkeys imported from their nautral habitat. In the course of 5 months 23 of 93 rhesus monkeys died. Of 200 green monkeys 112 died in the course of 4 months, and so did 9 of 20 Javanese macques in the course of 3 months. The largest number of monkeys died during the first month of their stay in the nursery.

Post mortem examination of the dead monkeys showed liver damage in 21 rhesus monkeys, 56 green monkeys, and all the Javanese macaques, in the form of acute hepatitis, similar in

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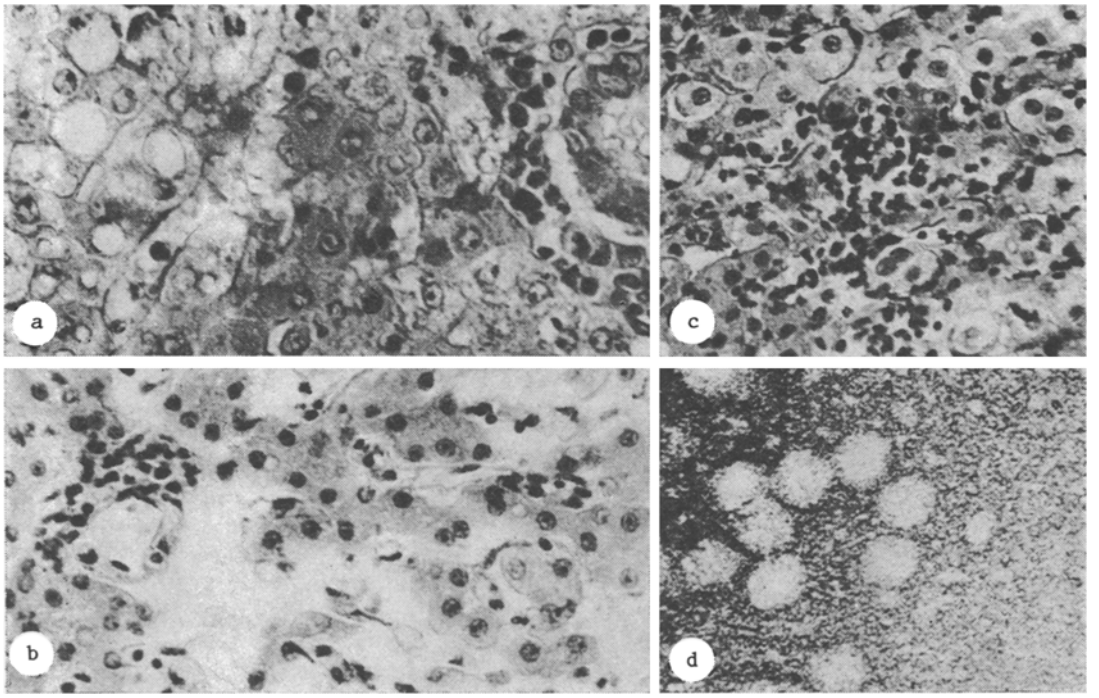

Fig. 1. Experimental hepatitis in Javanese macaques. a) Degeneration of hepatocytes, infiltration of portal tract by lymphocytes in biopsy material taken on the 27th day after infection from monkey 21030, 800×; b) foci of lysis, disintegration of hepatic laminae in monkey 21120, dying on the 27th day after infection, 600×; c) degeneration of hepatocytes, intralobular lymphohistiocytic infiltration in focus of death of hepatocytes from the same monkey, 600×; d) complete (measuring 27 nm) virus particles, covered with antibodies after interaction with antiserum in fecal sample from the same monkey taken on the 10th day after infection. a, c, d) Stained with hematoxylin with eosin.

its manifestations to human virus hepatitis A. The most typical picture was observed in the rhesus monkeys. The changes were characterized by circulatory disorders in the liver, on which were superposed cloudy-swelling and fatty degeneration of the hepatocytes, with excessive eosinophilia of the cytoplasm. In the region of the portal tracts, and also in the outer limiting lamina, concentrations of hepatocytes with typical balloon degeneration were observed. Hypertrophy and hyperplasia of the Kupffer cells, with the appearance of numerous binuclear hepatocytes and hepatocytes with large, hyperchromic nuclei, were observed. In some animals areas of necrosis were seen, with infiltration by lymphocytes, macrophages, and polymorphonuclear leukocytes in foci of necrosis. Lymphocytic infiltration was observed in the portal tracts.

The lesions in the liver of the Javanese macaques was similar to that in the rhesus monkeys, but more severe in character. No large areas of necrosis were observed, and cells with typical balloon degeneration were less frequent. Hypertrophy and hyperplasia of the Kupffer cells and lymphocytic infiltration of the portal tract were well defined.

Unlike the macaques, bone degeneration of hepatocytes was not found in the green monkeys, but disturbances of the circulation and fatty degeneration of the hepatocytes around the periphery of the lobules were more marked.

In six rhesus monkeys and five green monkeys the liver damage could be considered to be the cause of death, because no other pathological processes were found in the animals. In the remaining monkeys, however, besides hepatitis, dysentery, pneumonia, corona virus infection, and parasitic infestation also were observed, as are characteristically found in the period of acclimatization of monkeys to conditions of captivity in nurseries.

As a result of the virologic investigation, involving both EIA and RIA, the HVA antigen was found in the intestinal contents and liver of the macaques which died with signs of acute hepatitis, and also in fecal samples collected during life. Virus-specific antigen also was discovered in suspensions of the liver and feces of the green monkeys. In the course of 3

months of observation a statistically significant increase in the serum ALT level was observed in several monkeys. Serological investigation of the monkeys at intervals showed that in all groups of animals seroconversion took place, with elevation of the level of class M antibodies to HVA in the majority of them, evidence of a recent attack of hepatitis A.

In all three macaques a disturbance of the general state (loss of appetite, reduced motor activity) was observed with effect from the 2nd week after infection. In the 2nd to 3rd week all the animals showed a marked increase in serum ALT activity. One macaque died on the 27th day after inoculation, and biopsy material taken from the liver of two other macaques investigated at these times revealed lymphocytic infiltration of the portal tracts and hypertrophy and hyperplasia of the Kupffer cells. Cloudy swelling degeneration, hypereosinophilia of the cytoplasm, and balloon degeneration were observed in the hepatocytes (Fig. 1a). At autopsy on a monkey which died the liver was enlarged a little and unevenly stained, whereas the meninges and brain tissue were hyperemic. Histological examination of the brain revealed hyperemia, stasis, thrombosis, and perivascular hemorrhages, mainly in vessels of the microcirculatory bed. Areas of lysis with disturbance of the complex structure of the hepatic laminae (Fig. 1b), hypertrophy and hyperplasia of the Kupffer cells, and various changes in the hepatocytes, including balloon degeneration, were observed in the liver. Lymphohistiocytic infiltration was observed in the foci of necrobiosis (Fig. 1c). The portal tracts were infiltrated by lymphocytes.

IEM-revealed aggregates of empty and full virus particles (measuring 27 nm), covered with antibodies, were found in samples of feces on the 9th, 10th, and 11th days after infection (Fig. 1d).

The present investigation showed that during acclimatization of monkeys (with corresponding depression of immunity) outbreaks of hepatitis A arise, with a similar course to that of human hepatitis A, and in some cases they end in death. In the three species of monkeys studied, the disease most closely resembled in its course human hepatitis A in rhesus monkeys. The virus found in lower old-world monkeys has antigenic and morphological similarity with human HVA. On infection of Javanese macaques with this virus, a disease with the clinical and morphological manifestations typical of hepatitis A was produced. Further investigations will show whether or not this agent is identical with human HVA, or whether it is a virus peculiar to monkeys. Irrespective of the origin of the virus, experimental hepatitis A in macaques may be used as a model with which to test vaccines against human hepatitis A.

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