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RHESUS MONKEYS INFECTED WITH HEPATITIS E VIRUS (HEV) FROM THE FORMER USSR ARE IMMUNE TO SUBSEQUENT CHALLENGE WITH AN INDIAN STRAIN OF HEV

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Summary. - Two rhesus monkeys (*M. mullata)* of approximately two years of age were inoculated intravenously with a 10 % suspension of hepatitis E virus (HEV) positive stool from Kirghistan as evidenced by immuno-electron microscopy. Evidence of HEV infection was demonstrated by rise in serum alanine transaminase (ALT) levels and seroconversion of these monkeys to anti-HEV after 1-1/2 months post-inoculation as evidenced by immunoblot. One year after the primary inoculation, these monkeys were challenged with an Indian strain of HEV. No rise in serum ALT levels was noted during an observation period of 6 months. The same inoculum produced HE in two rhesus monkeys. The results showed that strains from India and Kirghistan were antigenically closely related and rhesus monkeys infected with one strain of virus were immune to another strain.

Key words: rhesus monkeys; hepatitis virus strains; challenge

Introduction

Large scale epidemics of enterically transmitted non-A, non-B hepatitis (ET-NANBH) are reported every year from different parts of India (Ramalingaswami and Purcell, 1988; Arankalle *et al.*, 1988; Chadha *et al.*, 1988; Chadha *et al.*, 1991; Risbud *et al.*, 1992). The Asian states of former USSR also experience epidemics of this disease (Favorov *et al.*, 1986; Favorov *et al.*, 1992). The agent responsible for ET-NANBH has been recently cloned and referred to as HEV (Tam *et al.*, 1991). For the development of vaccines or suitable diagnostic tests, it is important to know the variations of the strains isolated from different countries. With this in view, we have infected rhesus monkeys with one HEV strain

(Kir-88) from the Kirghistan Republic (former USSR). These monkeys were subsequently challenged with an Indian strain of HEV (PW-90). Our findings are reported here.

Materials and Methods

Two rhesus monkeys (M. mullata) of approximately 2 years of age were inoculated intravenously with 2 ml of Millipore filtered (0.45 μm) of 10 % suspension of a virus-positive stool (as evidenced by immune electron microscopy, IEM) obtained from a patient suffering from the disease during an epidemic of ET-NANBH in Kirghistan during 1988. Serum ALT levels were monitored twice a week for two months prior to and six months after inoculation. One year after inoculation with the Kir-88 strain, these monkeys were challenged with 2 ml of 10 % suspension of a stool sample collected from a patient suffering from ET-NANBH during an epidemic of the disease at Akluj, Maharashtra in 1990 PW-90. This stool sample was positive for HEV as evidenced by IEM. Simultaneously, two more rhesus monkeys were inoculated intravenously with 2 ml of the 10 % stool suspension (PW-90). Serum ALT levels were monitored for all these monkeys, 2 months prior to and 6 months post-inoculation.

Serum samples collected prior to and 1-1/2 months after virus inoculation were checked by immunoblot analysis for anti-HEV antibodies. The procedure used was similar to Favorov *et al.* (1992) with slight modification, i. e. washings of the nitrocellulose strips was carried out at 37 °C.

Results and Discussion

Since 1989, we have been trying to infect rhesus monkeys with different strains of HEV. From our experience we feel that the rhesus monkey is a fairly good model to study HEV infection. Two monkeys (M6 and M7) were infected with the Kir-88 strain obtained from Kirghistan. Fig. 1 shows the serum ALT profile of the infected monkeys. A biphasic curve was noted for M6, the first peak being on the 21st post-inoculation day (134 IU/l) and the second on the 36th day (122 IU/l). A distinct biphasic curve was not observed for M7. Peak serum ALT level was noted on the 24th day. After normalization of serum ALT levels (40 IU/l), no rise was noted for both the monkeys for a period of six months post-inoculation. Pre-inoculation serum samples from both the monkeys were negative for anti-HEV antibodies as evidenced by immunoblot analysis. However, blood samples collected 1-1/2 months post-inoculation were strongly reactive for the presence of anti-HEV antibodies. Thus, both the monkeys were infected with HEV and developed anti-HEV antibodies. Since a limited number of immunoblot strips was available, blood samples collected at different time points could not be tested.

These monkeys (M6 and M7) were challenged with an Indian strain (PW-90) after 1 year of infection with the Kir-88 strain. It should be noted that both the inocula contained a large number of HEV particles as evidenced by IEM. No rise in serum ALT levels was observed in either of the monkeys during an observation period of 6 months. When inoculated into two other rhesus monkeys the challenge inoculum (PW-90) produced typical serum ALT rise.

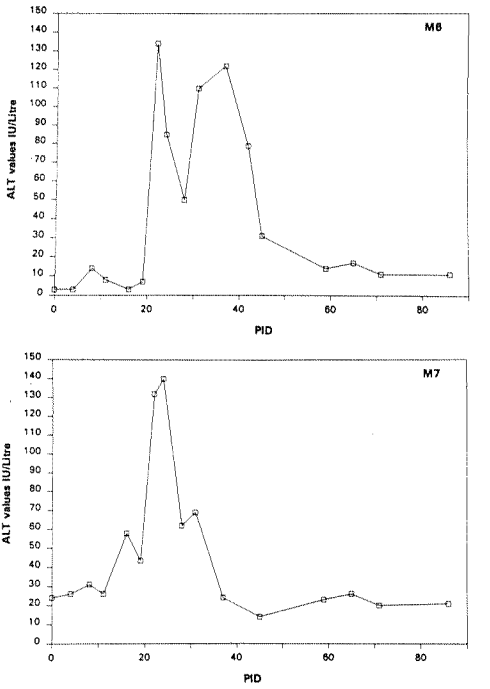

Fig. 1
Serum ALT profile of rhesus monkeys (M6 and M7) infected with Kir-88 strain of HEV

Peak serum ALT levels were observed on the 35th (76 IU/l) and the 37th day (54 IU/l) post-inoculation. Biphasic curve was not seen in either of the monkeys. These monkeys showed seroconversion to HEV as evidenced by immunoblot analysis. These results clearly indicate that rhesus monkeys infected with a strain of HEV from USSR were immune to subsequent challenge with an Indian strain one year after primary inoculation. It seems therefore that the strains of HEV circulating in 1988 in Kirghistan and in 1990 in India were antigenically closely related. Cross-challenge studies employing additional strains are needed before we can conclude that different strains of HEV share common antigens which are protective in nature. We would like to add here that by employing IEM, we had shown immunoaggregation of virus particles of an Indian strain (Ahm-84) with immune sera from different geographical areas including in USSR. Employing similar techniques, Tashkent-1435 (another strain from USSR) was shown to react with sera collected from ET-NANBH epidemic at Kolhapur, India in 1981 (Arankalle and Banerjee, 1988). In conclusion, in this communication we provide a direct evidence of resistance of monkeys previously infected with the Kirghistan strain of HEV to a challenge with the strain from India.

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