Effect of Exogenous Mouse Interferon on Murine Fulminant Hepatitis Induced by Mouse Hepatitis Virus Type 2

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We investigated the effect of exogenous mouse α- + β-interferon produced by mouse L cells on the growth of mouse hepatitis virus type 2 (MHV-2) in the liver, the development of liver cell necrosis, and survival in murine fulminant hepatitis induced by MHV-2. Murine fulminant hepatitis was induced in 4-week-old male ICR mice by intraperitoneal inoculation of MHV-2. Mouse interferon (10^3 IU/mouse/day) was intraperitoneally injected every day. Exogenous mouse interferon suppressed both the growth of MHV-2 in the liver tissue and development of liver cell necrosis, and prolonged the survival. It was also found that the earlier mouse interferon was administered, the greater was the prolongation of survival.

Activation of the interferon system is a defense mechanism against viral infections (1). Recently, interferon has been used in the treatment of various viral diseases. Levin and Hahn indicated that, in patients with fulminant hepatitis, the antiviral interferon system was grossly defective (2). Five patients with fulminant hepatitis in grade III or IV coma were given 3 million units of human α-interferon intramuscularly daily in that study; Three of these 5 patients survived. So far, however, the efficacy of interferon therapy on fulminant hepatitis has been in dispute.

Mouse hepatitis viruses are pathogenic RNA viruses belonging to the coronavirus group and about a dozen viral strains have been identified (3). Mouse hepatitis virus type 2 (MHV-2) is highly virulent for inbred ICR mouse strain, producing fulminant hepatic necrosis (4, 5). In the present study, we investigated the effect of exogenous mouse interferon in this fulminant hepatitis mouse model.

MATERIALS AND METHODS

Growth of MHV-2. For quantitative determination of the growth of MHV-2, delayed brain tumor (DBT) cells derived from a mouse brain tumor were used (6). For the culture medium, minimum essential medium (MEM) fluid was supplemented with 10% each of calf serum and tryptose broth. The culture medium was discarded when DBT cells became a monolayer on a 25-cm² culture dish. The cells were then cultured with 0.2 ml of a crude suspension of MHV-2 at 37°C for 4 hr. Thereafter, the DBT cells infected with MHV-2 were further cultured in 5 ml of the culture medium at 37°C for 15 hr. Five milliliters of the culture broth thus obtained was frozen and stored at −80°C until used for the experiment.

MHV-2 was quantitated according to the method of Hirano et al (6). The DBT cells monolayered in a petri dish (35 mm in diameter) were infected with 0.2 ml of the stored culture broth or liver homogenate at 37°C for 40 min. The DBT cells were then washed with fresh culture broth and further cultured in a medium containing 50% nobel agar for 2 days. After this culture, the number of plaques formed was counted.
Murine Fulminant Hepatitis Model. The fulminant hepatitis model was prepared by a single intraperitoneal inoculation of 0.2 ml of a dilute MHV-2 fluid (prepared to contain $10^3$ plaque-forming units (PFU)/10 ml MHV-2) to 4-week-old male ICR mice, a strain susceptible to MHV-2.

Administration of Mouse Interferon. The highly purified mouse interferon (containing both α- and β-interferon) used in this experiment was provided by Toray Basic Research Laboratory (Kamakura, Japan) (7). It was prepared from mouse L-cells and had a specific activity of $3 \times 10^4$ IU/mg protein. It was dissolved in physiological saline and was adjusted to have a concentration of $10^3$ IU/0.2 ml. The mice given interferon were divided into four groups based on the time of inoculation: group IF1, one day before MHV-2 inoculation; group IF2, at the same time as inoculation; group IF3, one day after inoculation; and group IF4, two days after inoculation. To each group, $10^3$ IU/mouse/day of the exogenous mouse interferon was administered intraperitoneally until the 4th day after MHV-2 inoculation. No treatment was given to the control (group C) after inoculation. The dosage of interferon ($10^3$ IU/mouse/day) used in this experiment was that used by Levin and Hahn (2), calculated on a weight-conversion basis to correspond to several million units/day in man.

Liver Histology and Quantitation of MHV-2 in Hepatic Tissue. Three animals each of group C (control) and IF2 were sacrificed daily until the third day after MHV-2 inoculation. Liver sections stained with hematoxylin and eosin were studied by light microscopy. When mice died during the study, liver histology was examined immediately. In addition, the amount of MHV-2 in the hepatic tissue of one animal in each group was determined and correlated with the light microscopic findings. The MHV-2 count was determined by the plaque-assay method as described after homogenization of 0.2 g of wet hepatic tissue with 0.8 ml of culture medium.

Survival Time. The survival of mice in group C ($N = 20$), IF1 ($N = 20$), IF2 ($N = 20$), IF3 ($N = 20$) and IF4 ($N = 25$) was assessed to the seventh day postinoculation. The cumulative survival curves in each group were determined by the method of Kaplan-Meier and statistical analysis was made in accordance with the generalized Wilcoxon test (8).

RESULTS

Microscopic Histology of the Liver. In group C (control), spotty necrosis was observed in the liver in one of three animals one day after inoculation; submassive hepatic necrosis was seen in the three animals on the second day; and massive necrosis in the three animals on the third day. In group IF2, there was no necrosis of hepatic cells one day after inoculation; spotty necrosis was found in one of three animals on the second day; and on the third day focal necrosis was seen in two of three animals and submassive necrosis in one. In group IF2, the degree of necrosis of the hepatic cells at each examination time was milder compared with group C.

MHV-2 Count in Hepatic Tissue and Liver Histology. In group C, the MHV-2 count in the hepatic tissue one day after MHV-2 inoculation was $2.0 \times 10^4$ PFU/g wet tissue, and $1.5 \times 10^7$ PFU/g wet tissue on the second day (Figure 1). Necrosis of the hepatic cells was progressive such as from spotty necrosis on day 1 to submassive necrosis on day 2, as MHV-2 grew in the liver. In group IF2, the MHV-2 count in the hepatic tissue one day after MHV-2 inoculation was $5.0 \times 10^4$ PFU/g wet tissue, and $7.5 \times 10^4$ PFU/g wet tissue on the second day. Necrosis of the hepatic cells was suppressed, such
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Fig 2. Cumulative survival rates of groups C (N = 20), IF1 (N = 20), IF2 (N = 20) IF3 (N = 20) and IF4 (N = 25) on the basis of the life-table method.

that there was no necrosis on day 1 to focal necrosis on day 2, as MHV-2 did not grow in the liver.

Survival Time. Death occurred in group C (N = 20) beginning the second day after inoculation. All animals had died by the third day. In contrast, in group IF1 (N = 20), 17 died between four and six days after inoculation, and three were still alive after seven days; in group IF2 (N = 20) one lived more than seven days and 19 died between days 4 and 6; in group IF3 (N = 20) none lived more than seven days and 10 died between days 4 and day 5; and in group IF4 (N = 25) only six lived for four days. When analyzed by cumulative survival rates, the survival for the IF1-IF3 groups was found to be significantly prolonged compared with the control ($P < 0.01$, Figure 2).

DISCUSSION

In 1978, Virelizier and Gresser inoculated C3H/He mice with MHV-3 and produced chronic hepatitis (semiresistant) in these animals (9). When a mouse anti-interferon antibody was administered to these mice starting from shortly after MHV-3 inoculation, they developed fulminant hepatitis and died. These observations suggested that the interferon system has an important role as an in vivo defense mechanism against MHV-hepatitis (9). On the other hand, according to the report of Schindler et al (10) in 1982, serum interferon levels increased significantly after inoculation with MHV-3 in C57BL/6 mice (susceptible) which died of fulminant hepatitis after infection with MHV-3, whereas A/J mice (resistant) did not develop fulminant hepatitis, and the increase in serum interferon was insignificant. They concluded that the interferon system did not seem to be important as defense mechanism in vivo (10). Thus, there is no established opinion as to how the interferon system is involved as MHV hepatitis progresses, nor have there been any reports assessing the effect of exogenous mouse interferon on MHV hepatitis.

In the present study, we investigated the effect of exogenous mouse $\alpha$- + $\beta$-interferon produced by mouse L cells on a murine fulminant hepatitis model. The effect was assessed from the histological changes of the liver, the MHV-2 content in hepatic tissue, and the survival time. The degree of hepatic cell necrosis, in comparison with the control group, was much milder histologically in group IF2, in which mouse interferon was administered at the time of MHV-2 inoculation. It indicates that the administration of mouse interferon, in doses similar to those used in man without side effects (2), suppresses the progress of hepatic cell necrosis in this murine model.

The mechanism of hepatic cell necrosis by MHV has been recognized to involve both host-response mechanisms and direct viral cytopathology (3, 11). Since the MHV-2 count in the hepatic tissue was remarkably lower in group IF2 than in the control group at each time it was measured, exogeneous mouse $\alpha$- + $\beta$-interferon may suppress hepatic cell necrosis in this group by its direct antiviral action and/or interferon-dependent immune responses (natural killer cell, cytotoxic T cell, macrophage, etc).

The activity of macrophage, which is genetically restricted in mice, is widely accepted to be the important defense mechanism against MHV-2 infection (12, 13). Bang et al (12) reported, in 1960, that MHV-2 grew well in a culture of macrophages taken from the liver and peritoneum of MHV-2-susceptible ICR mice, whereas no growth of MHV-2 was observed in a similar system using macrophages from resistant C3H. In this regard, it is speculated that exogenous mouse $\alpha$- + $\beta$-interferon might enhance the antiviral activity of macrophages for MHV-2 in susceptible ICR mice.

The survival time was significantly prolonged in groups IF1-IF3, in which mouse interferon was started before the mice developed fulminant hepatitis. Even in group IF4, in which the administration...
of interferon was started when some of the mice had already died, the survival time was somewhat prolonged compared with the control. These observations clearly demonstrate the value of early treatment with mouse α- + β-interferon produced by mouse L cells in a murine model of fulminant hepatitis, although the relevance of these findings to human fulminant hepatitis, which is caused by different viruses, is unclear.

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