Protective Effect of Recombinant Interferon (IFN)- α/β on MHV-2cc-induced Chronic Hepatitis in Athymic Nude Mice

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Abstract: The protective effects of recombinant IFN- α/β on MHV-2cc-induced chronic and persistent hepatitis in athymic nude mice were examined. The mice intraperitoneally (ip) inoculated with MHV-2cc at day 0 of experiment were divided into 4 groups. Three of them were administered ip with recombinant IFN- α/β at a daily dose of 1×10^3 IU from -1 (-1D-group), 0 (0D-group), and +1 day of experiment (+1D-group), respectively, for 3 consecutive weeks. The remaining one (control group) was given 0.1 ml/mouse of PBS from +1 day of the experiment in the same way. Three mice in each group were killed at 1, 2 and 3 weeks post inoculation (WPI) with MHV, respectively. The liver virus titer in the control group increased gradually and maintained high levels throughout the experimental period. In the IFN-groups, particularly in the -1D- and 0D-groups, the virus titers were significantly lower than that in control group. Histopathologically, focal hepatic lesions were observed at 1WPI and large irregular inflammatory lesions developed at 3WPI in the control group. Similar but somewhat less severe lesions were observed in the +1D-group. In the -1D- and 0D-groups, lesions were not observed at 1WPI and only small organized lesions with mononuclear cell infiltration were seen at 3WPI. In conclusion, it was clarified in the present study that the progression of MHV-2cc-induced chronic hepatitis in athymic nude mice was effectively prevented by extrinsic IFN- α/β when administered from -1 day and 0 day of the virus infection.

Key words: athymic nude mice, chronic viral hepatitis, interferon- α/β , MHV-2cc

Interferon (IFN) is intrinsically induced during the virus infection, especially in its early phase, and plays an important role in the host-defense mechanism [1]. Today, recombinant IFNs are produced on a large scale and applied to the therapy of various viral diseases such as human type B or type C hepatitis [2–4, 9, 10, 17,

19], but extrinsic IFN-administration is not always effective and the exact protective mechanisms are not fully clarified. In the field of experimental medicine, the effects of extrinsic IFN on various viral infections have been studied in mice [12, 18]. Especially in influenza A/HK virus-infection, the *in vivo* effects of

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exogenously administered interferons have been widely studied. Although the administration of hybrid recombinant human alpha interferon A/D (rIFN A/D) had only a limited degree of effectiveness on the mortality in mice infected with influenza virus in the *in vivo* test, it was effective in inhibiting the replication of the virus *in vitro* in primary mouse embryo cells [20].

It is reported that extrinsic IFN- α/β -administration had a significant protective effect on MHV-2-induced acute and fulminant hepatitis [11]. MHV-2 is a hepatotropic and high virulent strain of mouse hepatitis virus (MHV), and MHV-2cc which was isolated from SR-CDF1-DBT (DBT) cell cultures persistently infected with MHV-2 is a low virulence variant of MHV-2 [7]. MHV-2 induces acute hepatitis in the infected mice and killed mice in a few days. On the other hand, MHV-2cc induces only a little change in euthymic mice, but chronic persistent hepatitis in athymic nude mice. In this study, we studied the protective effects of recombinant IFN- α/β on MHV-2cc-induced chronic and persistent hepatitis in athymic nude mice, which is expected to be a useful model for human chronic viral hepatitis such as type C hepatitis [5, 6].

Thirty-six 6-week-old female athymic BALB/c-*nu/nu* mice (Japan Clea Co. Ltd., Tokyo) were used. The mice were serologically confirmed to be free from MHV-infection before being used. They were kept under controlled conditions (temperature, $23 \pm 2^\circ\text{C}$; relative humidity, $55 \pm 5\%$) in an isolator casing system (Niki Shoji Co., Tokyo) and fed autoclaved pellets (MF, Oriental Yeast Co., Tokyo) and water *ad libitum*. The mice were equally divided into 4 groups and intraperitoneally inoculated with 10^5 plaque-forming units (PFU)/mouse of MHV-2cc on day 0 of the experiment. Three of 4 groups were intraperitoneally administered with recombinant human IFN- α/β (Japan Chemical Research Co. Ltd., Kobe) at a daily dose of 1×10^3 IU/mouse from days -1 (-1D-group), 0 (0D-group), and +1 of the experiment (+1D-group), respectively, for 3 consecutive weeks. The remaining group was given 0.1 ml/mouse of phosphate buffered saline from day +1 of the experiment in the same way and served as controls. Three mice from each group were killed by exanguination under ether anesthesia at 1, 2 and 3 weeks post inoculation (WPI) of MHV.

At necropsy, the activities of L-aspartate aminotransferase (AST) and L-alanine aminotransferase (ALT),

which are main parameters of hepatocyte damage, were colorimetrically measured on serum samples obtained from each animal by using test reagents (Wako Pure Chemical Industries Ltd., Osaka) and an autoanalyser (Olympus Co. Ltd., Tokyo). At the same time, virus titration by plaque assay on DBT cell cultures was done on the liver tissues obtained from each mouse according to the method of Hirano *et al.* [8]. All data were expressed as the mean $\pm$ standard error, and statistical analysis was done by Student's *t*-test.

In addition, the liver tissues obtained from each mouse were fixed in 10% neutral buffered formalin, and paraffin sections ($4 \mu\text{m}$) were stained with hematoxylin and eosin (HE) for histopathological examinations.

During the experimental period, no animals died in any group. As shown in Fig. 1, the liver virus titer in the control group increased gradually in the course of time. On the other hand, those in the IFN-groups were significantly lower than that in the control one, and the suppressive effect on the liver virus titer was somewhat weaker in the +1D-group than in the other 2 groups. In addition, in the -1D-group, no significant viral proliferation occurred until 1WPI.

As shown in Figs. 2 and 3, the serum AST and ALT activities in the control group were maintained at a very high level, but those in the -1D-group were at apparently lower level throughout the experimental period. Such serum enzyme activities in the 0D- and +1D-groups were at different levels in the control and -1D-groups.

In the control group, numerous large foci of collapsed hepatocytes were observed at 1WPI (Fig. 4), and were connected with each other and formed large irregular inflammatory lesions with regenerative hepatocyte masses at 3WPI (Fig. 5). Among IFN-groups, similar but somewhat less severe lesions were observed in the +1D-group at 1 (Fig. 6) and 3WPI (Fig. 7) as compared with those in the control group. In -1D- and 0D-groups, lesions were not observed at 1WPI, and those at 2WPI were apparently smaller than those in the control and +1D-groups (Fig. 8), and small organized lesions with mononuclear cell infiltration were left at 3WPI (Fig. 9). These histopathological findings corresponded closely with the liver virus titers and the ALT activities. Though the AST activities of 0D- and +1D-groups at 1WPI did not reflect the pathological

Fig. 1. Changes in virus titers in the liver. $\circ$; IFN day -1; $\bullet$; IFN day 0; $\square$; IFN day+1; $\blacksquare$; control group. Each point represents the mean value and the vertical bar represents the standard errors.

Fig. 2. Changes in serum AST activities. $\circ$; IFN day -1; $\bullet$; IFN day 0; $\square$; IFN day+1; $\blacksquare$; control group. See the footnote to Fig. 1.

Fig. 3. Changes in serum ALT activities. $\circ$; IFN day -1; $\bullet$; IFN day 0; $\square$; IFN day+1; $\blacksquare$; control group. See the footnote to Fig. 1.

changes well, the activities at 2 and 3WPI correlated with hepatic lesions.

In this study it was clarified that the progression of MHV-2cc-induced chronic hepatitis in athymic nude

mice was effectively prevented by extrinsic IFN- α/β when administered from day -1 and day 0 of the virus infection, but IFN- α/β -treatment from +1day was not so effective. This result is the same as in a previous report stating that IFN- α/β and IFN- β had significant protective effects on MHV-2-induced murine acute and fulminant hepatitis only when IFNs-administration had been started before the viral infection [11, 15]. In this study, extrinsic IFN also exerted significant protective effects on MHV-2cc-induced chronic hepatitis only when IFN-administration had been started before the viral inoculation. In both chronic hepatitis and acute hepatitis in MHV-infected mice, *in vivo* protective effects of exogenous IFNs were exerted only when they were administered before viral infection. This may be explained by the well-known fact that IFN suppresses viral replication at a very early stage of infection both in *in vivo* [13, 14] and *in vitro* systems [21]. On the other hand, there is another report that IFN could not inhibit the proliferation of MHV-2 *in vitro* [21]. Although it is not certain that IFN can inhibit the proliferation of MHV-2cc *in vitro*, Taguchi *et al.* showed that there was a difference in the *in vitro* suppressive effects of IFN on virus replication among the strains of MHV according to the virulence [21]. They

Fig. 4. Liver of a mouse in the control group at 1 WPI. HE, $\times 100$.

Fig. 5. Liver of a mouse in the control group at 3 WPI. HE, $\times 100$.

Fig. 6. Liver of a mouse in the day+1 group at 1 WPI. HE, $\times 130$.

Fig. 7. Liver of a mouse in the day+1 group at 3 WPI. HE, $\times 100$.

Fig. 8. Liver of a mouse in the day-1 group at 2 WPI. HE, $\times 100$.

Fig. 9. Liver of a mouse in the day 0 group at 3 WPI. HE, $\times 100$.

suggested that the IFN treatment suppresses virus replication at several stages and the suppressive effects may be related to the pathogenicity of these viruses.

In conclusion, recombinant IFN- α/β exerted a protective effect on MHV-2cc-induced chronic persistent hepatitis in athymic nude mice, and this protective effect seems to be mainly due to its suppressive effect on virus replication in the liver, though some other factors, for example, activation of natural killer cells by IFN [16], may also play a role in this protection. Further investigation is now in progress to clarify details of the protective mechanism.

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