Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Virus Research, 1 (1984) 557–563
Elsevier

VRR 00146

Kupffer and endothelial liver cell damage renders A/J mice susceptible to mouse hepatitis virus type 3

Carlos A. Pereira, Anne-Marie Steffan and André Kirn

Laboratoire de Virologie, Faculté de Médecine, Université Louis Pasteur, et Unité I.N.S.E.R.M. U 74, 3 rue Koeberlé, 67000 Strasbourg, France

(Accepted 21 July 1984)

Summary

Damage to the Kupffer and endothelial cells of the liver sinusoids induced by the administration of sublethal doses of frog virus 3 (FV 3) renders A/J mice which are genetically resistant to mouse hepatitis virus type 3 (MHV 3) highly susceptible to this virus. Liver histopathology of these animals revealed typical necrotic foci containing MHV 3-specific antigens. FV 3-pretreated mice, after MHV 3 infection, showed higher levels of serum transaminase (GPT) than controls, and MHV 3 replicated more rapidly and to higher titres. Our results bear out the important role of the liver sinusoidal lining in protecting against hepatocyte infection and its direct involvement in the resistance of A/J mice to MHV 3 infection.

sinusoidal liver cell, frog virus 3, mouse hepatitis virus type 3 susceptibility

Introduction

The sinusoidal cells have been singled out as being the key liver cells involved in the resistance of mice to virus infection (Kirn et al., 1982b, 1982c, 1983; Steffan and Kirn, 1979; Taguchi et al., 1983). Although the hepatocytes constitute the main site of virus replication, the virus particles carried by the blood have to cross this sinusoidal barrier, made up of endothelial and Kupffer cells, in order to attain the liver parenchyma. Under experimental conditions, damage of these cells provoked by frog virus 3 (FV 3), an iridovirus, leads to breakages in the sinusoidal lining, allowing vaccinia virus to infect and replicate in the hepatocytes (Steffan and Kirn, 1979). We have shown previously that the partial resistance expressed by Kupffer and endothelial cells from A/J mice to in vitro replication of mouse hepatitis virus

type 3 (MHV 3), a coronavirus, may be an important factor in their resistance (Pereira et al., 1984a). The data presented here are the result of further investigations concerning the importance of the sinusoidal lining in the resistance to virus infections.

Materials and Methods

Viruses

MHV 3 and FV 3 were cultivated and titrated respectively on L 929 at 37 °C and BHK 21 cells at 26 °C as previously described (Aubertin et al., 1973; Pereira et al., 1984b). In all the experiments 10^4 PFU of MHV 3 were inoculated intraperitoneally (i.p.), and 1.5×10^7 PFU of FV 3 intravenously (i.v.).

Mice

Six- to eight-week-old mice of the inbred A/J Orl strain from the Centre d'Elevage d'Animaux de Laboratoire, CNRS, Orléans, France, were used in the experiments.

MHV 3 infection of FV 3-pretreated mice

Six hours after inoculation of a sublethal dose of FV 3, mice were infected with MHV 3. At different intervals after infection the animals were sacrificed, their livers removed, and the sera obtained immediately frozen. Livers were used for histopathological examinations and virus titration, and sera for transaminase (GPT) activity. Liver sections were prepared for light microscopy in a Tissue-Tech II cryostat and fixed with acetone. Some sections were stained with hematoxylin–eosin and others treated with anti-MHV 3 hyperimmune A/J mouse serum (1/20) for 30 min, carefully washed with saline, and treated again with a 1/100 dilution of fluorescent anti-mouse gamma-globulins (Institut Pasteur, Paris) for another 30 min. The sections were rewashed and observed in a Reichert Diavar microscope under U.V. light. Controls treated with normal mouse serum instead of mouse MHV 3 antibodies were included in each series. The livers were also observed under a transmission electron microscope (TEM). These techniques have been previously described (Kirn et al., 1982a). For virus titration, livers were ground and the titer of MHV 3 determined in each specimen by plaque assay on L 929 cells; the results are given as plaque-forming units per gram of liver (PFU/g liver). The level of transaminase (GPT) serum activity was established by microdetermination (Testomar GRT Alat, Behringwerke A.G., F.R.G.).

Sheep red blood cells (SRBC) antibodies production and in vivo immunity

Six hours after FV 3-pretreatment mice were injected i.p. with 10% of SRBC. Fifteen days later they were sacrificed and sera obtained and frozen separately. SRBC antibody titration was performed in each specimen by the hemagglutination technique. The immunity was studied by MHV 3 immunization (10^4 PFU) of mice 30 days before FV 3-pretreatment and MHV 3 infection. Fifteen mice used in this

TABLE 1

SUSCEPTIBILITY OF A/J MICE TO MHV 3 INFECTION AFTER PRETREATMENT WITH FV 3

Treatment [a]	Infection [a]	Mortality		Mean survival time
FV 3	–	0/24	0%	–
–	MHV 3	0/20	0%	–
FV 3	MHV 3	28/34	82.35%	6 to 8 days

[a] A sublethal dose of FV 3 (1.5×10^7 PFU i.v.) was administered 6 h before MHV 3 infection (10^4 PFU i.p.).

experiment were observed for 20 days and the mortality was recorded. The same number of non-immunized mice were used as controls.

Results

Effect of FV 3 pretreatment on the resistance of A/J mice infected with MHV 3

Groups of A/J mice were inoculated with a sublethal dose of FV 3 six hours prior to MHV 3 inoculation and control groups received either MHV 3 or FV 3 only. The animals were observed for 20 days and the mortality rate recorded. The results expressed in Table 1 show that A/J mice became susceptible after FV 3-pretreat-

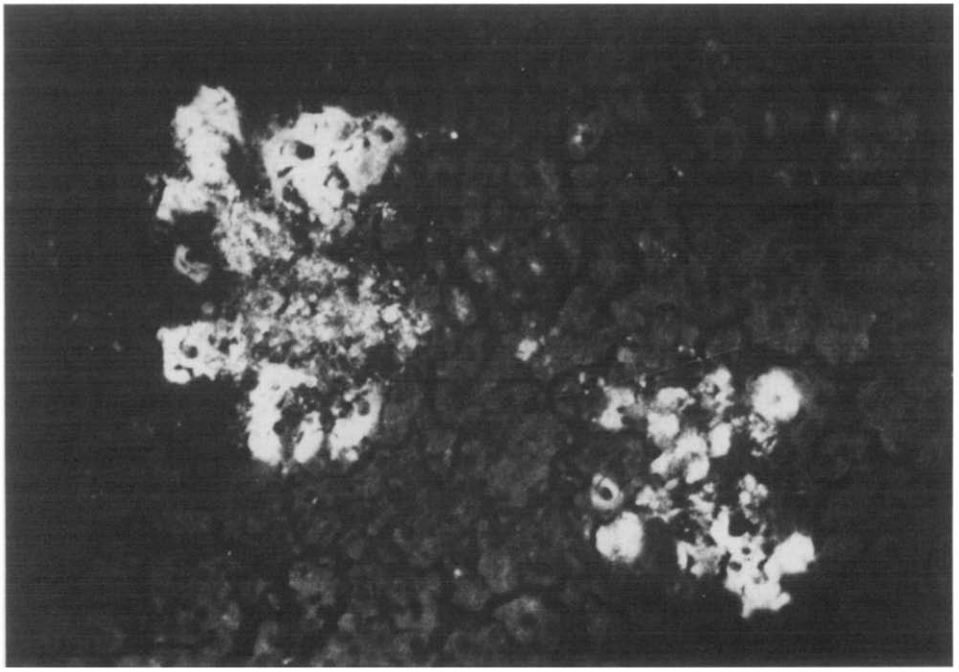


Fig. 1. Immunofluorescence in the liver of a FV 3-pretreated mouse 48 h after MHV 3 infection. Necrotic foci showing the MHV 3 specific antigen. ×220.

ment. The mean survival time was 7 days. Following MHV 3 infection, FV 3-pretreated mice showed hepatocellular damage with numerous foci of infection, in contrast to non-pretreated animals presenting only a few small foci. An immunofluorescence test revealed the presence of MHV 3 antigens (Fig. 1). TEM examinations showed that the hepatocytes of FV 3-pretreated mice displayed a large number of cytoplasmic vacuoles containing numerous virus particles (Fig. 2).

Virus replication in livers of infected mice

At different intervals after MHV 3 inoculation, FV 3-pretreated or non-pretreated mice were sacrified and their livers collected for virus titration. The time course for MHV 3 replication is represented in Fig. 3. In the livers of FV 3-pretreated mice, 40 h after infection, virus titers are about ten times higher and remain elevated until the animals die, in contrast to non-pretreated mice where the titers are lower and decrease 72 h after inoculation (results not shown). As can be seen in Fig. 4, an increase in the GPT-serum activity was observed 72 h after MHV 3 infection of FV 3-pretreated mice. The levels of GPT-serum activity in mice inoculated only with sublethal doses of FV 3 or MHV 3 remained low. These results indicate that the

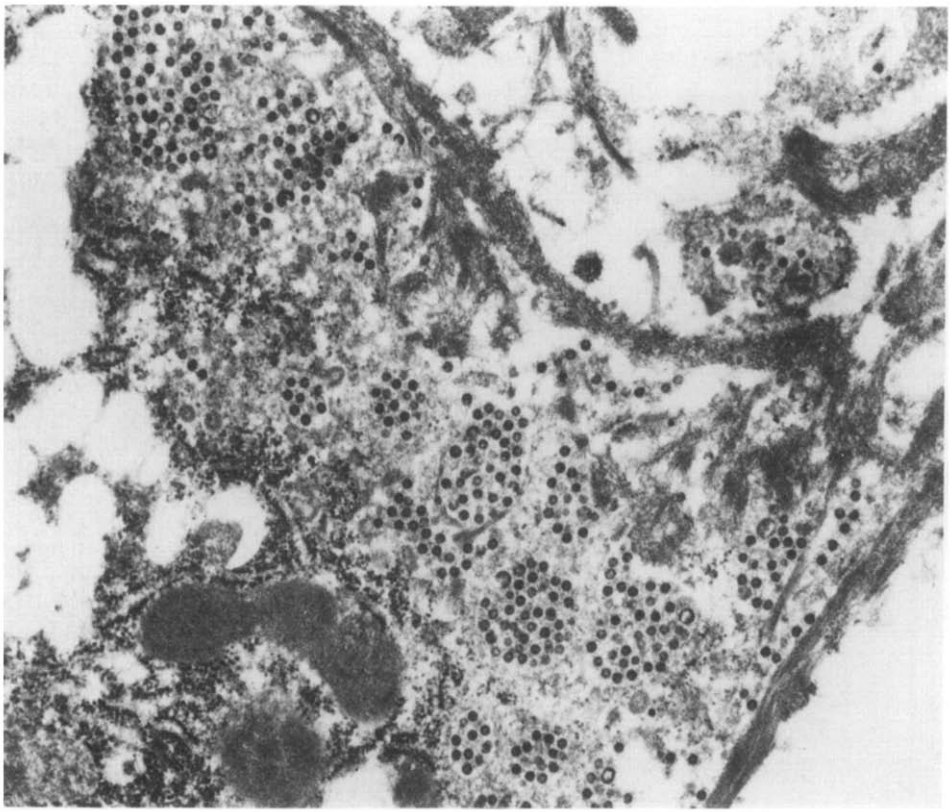
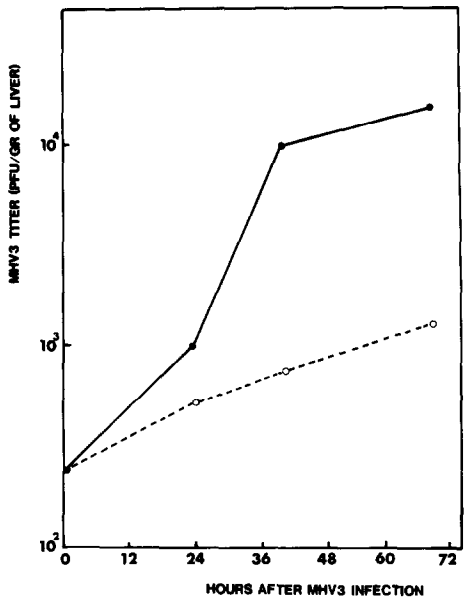


Fig. 2. Virus-containing cytoplasmic vacuoles in hepatocyte of FV 3-pretreated mouse 72 h after MHV 3 infection. ×26000.



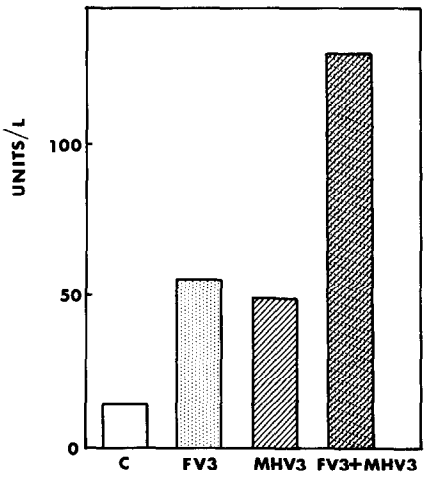


Fig. 3. Kinetics of virus multiplication in livers of control and FV 3-pretreated mice. Mice were pretreated with sublethal dose of FV 3 (1.5×10^7 PFU i.v.) 6 h before infection with MHV 3 (10^4 PFU i.p.). ———, FV 3-pretreated mice; - - - - - -, control mice.

Fig. 4. GPT-serum activity in controls and FV 3-pretreated mice after 72 h MHV 3 infection.

hepatocellular damage provoked by MHV 3 is more extensive in FV 3-pretreated mice than in normal ones.

Effect of FV 3-pretreatment on SRBC antibody production and in vivo immunity

In order to investigate the ability of FV 3-pretreated mice to respond to an antigenic stimulation, we studied the SRBC antibody production in sera of FV 3-pretreated and normal mice injected with SRBC. Both groups of mice showed the same titer of SRBC antibodies (from 64 to 128 in three different experiments). When mice were immunized against MHV 3 30 days before FV 3 pretreatment and MHV 3 infection, all the mice survived. Non-immunized mice, which were used as controls, showed 80% mortality. These results show that mice develop immunity to MHV 3 infection and can then survive the double infection, and that FV 3 pretreatment does not interfere with the particular immune response tested.

Discussion

After intravenous inoculation of FV 3 into mice, there is a hepatocellular necrosis secondary to sinusoidal cell damage (Kirn et al., 1983). FV 3 does not multiply in mice, but produces a toxic effect on liver cells (Kirn et al., 1983). When sublethal doses are inoculated there is no damage to the liver parenchyma whereas breakages

in the sinusoidal lining are evident (Gendrault et al., 1977). We have shown that the destruction of this lining has dramatic consequences for the parenchymal cells, which develop the capacity of taking up infectious particles (Steffan and Kirn, 1979) or toxic substances directly from the blood.

Hepatitis caused by MHV 1 is more severe in weaning mice previously inoculated with *E. coccoides* (Gledhill, 1956) or certain leukemia viruses (Gledhill, 1961). It has been postulated that the increase in MHV 1 pathogenicity is related to an augmentation in the susceptibility of the Kupffer cells whose phagocytic capacity, as measured by the carbon clearance test, is raised after the inoculation of these agents (Gledhill et al., 1965). This is certainly not the case in FV 3-preinfected mice where the phagocytic capacity of the liver RES is severely depressed (Kirn et al., 1978).

As a consequence of MHV 3 replication in hepatocytes, some strains of mice develop an acute hepatitis which leads to a high mortality. After MHV 3 infection, the resistant A/J mouse strain reveals only a very small number of well-defined necrotic foci in the liver, which disappear 8 to 12 days later (Virelizier, 1979). Our results in the present investigation show that A/J mice become susceptible to MHV 3 infection, with a mortality rate of 80%, when Kupffer cell damage and breakage in the sinusoidal lining is induced by sublethal doses of FV 3. That the hepatitis which develops will turn out to be fatal is indicated by the increase in the titer of the transaminases, and is further borne out by the histopathological findings, which reveal necrotic foci containing MHV 3-specific antigen. The virus-containing cytoplasmic vacuoles, seen by TEM, show that virus replication takes place in the hepatocytes.

As it has been established that the immunological response determines the outcome of MHV 3 infection (Dupuy et al., 1975; Pereira et al., 1984b; Virelizier, 1979), it was of interest to determine whether FV 3-pretreated mice were able to develop a normal systemic immune response to an antigenic stimulus. FV 3-pretreated animals, like normal mice, were able to produce SRBC antibodies, and MHV 3 immunization enabled the pretreated mice to resist MHV 3 infection. These results indicate that FV 3 treatment causes no drastic impairment of the systemic immune response.

In previous work we have shown that sinusoidal liver cells are able to produce interferon (Kirn et al., 1982c), which may diffuse into the space of Disse and induce an antiviral state in the hepatocytes. In the case of sinusoidal cell damage by FV 3-pretreatment, the lack of interferon production by these cells may render the hepatocytes more susceptible to MHV 3 infection with consequences for the resistance of the animal as a whole. Some authors have suggested that Kupffer cells play a role in the development of the immune response (Heil and Garvey, 1982; North, 1969; Richman et al., 1979; Rogoff and Lipsky, 1981). It is possible that after FV 3-pretreatment, which induces damage in numerous Kupffer cells, a decrease in local immune response in the liver takes place without affecting the systemic immune response. In order to clarify whether FV 3-pretreatment does in fact influence the immune response, further experiments need to be performed investigating the role of Kupffer cells in the local immune response, as well as several other aspects of the systemic immune response. In conclusion, the data presented in this paper demon-

strate that, once the sinusoidal barrier is damaged, MHV 3 is able to replicate in the liver parenchyma of the resistant A/J mouse strain inducing fatal hepatocyte injury. The results thus show that the intactness or otherwise of the sinusoidal lining has a direct influence on the degree of resistance of A/J mice to the MHV 3 infection.

References

Aubertin, A.M., Hirth, C., Travo, C., Nonnenmacher, H. and Kirn, A. (1973) Preparation and properties of an inhibitory extract from frog virus 3 particles. J. Virol. 11, 694–700.

Dupuy, J.M., Levy-Leblond, E. and Le Prevost, C. (1975) Immunopathology of mouse hepatitis virus type 3 infection II – Effect of immunosuppression in resistant mice. J. Immunol. 114, 226–230.

Gendrault, J.L., Steffan, A.M., Bingen, A. and Kirn, A. (1977) Interaction of frog virus 3 (FV 3) with sinusoidal cells. In: Kupffer cells and other liver sinusoidal cells. (Wisse, E. and Knook, D.L., eds.), p. 223, Elsevier/North-Holland Biomedical Press, Amsterdam.

Gledhill, A.W. (1956) Quantitative aspects of the enhancing action of Eperythrozoa on the pathogenicity of mouse hepatitis virus. J. Gen. Microbiol. 15, 292–304.

Gledhill, A.W. (1961) Enhancement of the pathogenicity of mouse hepatitis virus (MHV-1) by prior infection of mice with certain leukemia agents. Brit. Cancer 15, 531–538.

Gledhill, A.W., Bilbey, D.L. and Niven, J.S.F. (1965) Effect of certain murine pathogens on phagocytic activity. Br. J. Exp. Pathol. 46, 433–442.

Heil, M.F. and Garvey, J.S. (1982) Accessory function of aging Kupffer cells with different lymphocyte populations. J. Reticuloend. Soc. 32, 287–296.

Kirn, A., Steffan, A.M., Anton, M., Gendrault, J.L. and Bingen, A. (1978) Phagocytic properties displayed by mouse hepatocytes after virus induced damage of the sinusoidal lining. Biomedicine 29, 25–28.

Kirn, A., Bingen, A., Steffan, A.M., Wild, M.T., Keller, F. and Cinqualbre, J. (1982a) Endocytic capacities of Kupffer cells isolated from the human adult liver. Hepatology 2, 216–222.

Kirn, A., Gut, J.P. and Gendrault, J.L. (1982b) Interaction of viruses with sinusoidal cells. In: Progress in Liver Diseases VII (Popper, H. and Schaffner, F., ed.), p. 373, Grune and Shelton, New York.

Kirn, A., Koehren, F. and Steffan, A.M. (1982c) Interferon synthesis in primary culture of Kupffer and endothelial cells from the rat liver. Hepatology 5, 676.

Kirn, A., Gut, J.P., Bingen, A. and Steffan, A.M. (1983) Murine hepatitis induced by frog virus 3: A model for studying the effect of sinusoidal cell damage on the liver. Hepatology 3, 105–111.

North, R.J. (1969) The mitotic potential of fixed phagocytes in the liver as revealed during the development of cellular immunity. J. Exp. Med. 130, 315–323.

Pereira, C.A., Steffan, A.M. and Kirn, A. (1984a) Interaction between mouse hepatitis viruses and primary cultures of Kupffer and endothelial liver cells from resistant and susceptible inbred mouse strains. J. Gen. Virol. (in press).

Pereira, C.A., Mercier, G., Oth, D. and Dupuy, J.M. (1984b) Interaction of natural killer cells and interferon during mouse hepatitis virus infection of inbred mouse strains. Immunobiology 166, 35–44.

Richman, L.K., Klingenstein, R.J., Richman, J.A., Strober, W. and Berzofsky (1979) The murine Kupffer cell. 1. Characterization of the cell serving accessory function in antigen specific T cell proliferation. J. Immunol. 123, 2602–2609.

Rogoff, T.M. and Lipsky, P.E. (1981) Role of the Kupffer cells in local and systemic immune responses. Progress article. Gastroenterol. Assoc., 854–860.

Steffan, A.M. and Kirn, A. (1979) Multiplication of vaccinia virus in the livers of mice after Frog Virus (FV3) induced damage to sinusoidal cells. J. Reticuloend. Soc. 26, 531–538.

Taguchi, F., Kanamura, S. and Fujiwara, K. (1983) Replication of mouse hepatitis virus with high and low virulence in cultured hepatocytes. Infect. Immun. 39, 955–959.

Virelizier, J.L. (1979) Pathogenesis of mouse hepatitis virus (MHV3) infection in various inbred strains of mice. In Mechanisms of viral pathogenesis and virulence (Bacmann, P.A., ed.), p. 251. WHO, Munich.

(Manuscript received 1 June 1984)