INBORN RESISTANCE OF MICE TO MOUSE HEPATITIS VIRUS TYPE 3 (MHV_3): LIVER PARENCHYMAL CELLS EXPRESS PHENOTYPE IN CULTURE

Heinz Arnheiter and Otto Haller

Institute for Immunology and Virology
University of Zürich, POB,
8028 Zürich, Switzerland

SUMMARY

Primary monolayer cultures of hepatocytes isolated from adult resistant A/J or partially resistant A/Sn or C3H/HeJ mice exhibited resistance to MHV_3 as the respective macrophages do: Compared to susceptible C57BL/6 hepatocyte cultures, cytopathic effect occurred later and was restricted to small foci, coinciding with areas specifically labelled by immunofluorescence. Production of infectious particles was delayed, titers being 100 to 1000 fold lower at the moment of maximal yields in susceptible cultures. Pretreatment with interferon could reduce the titers in susceptible cultures to a level as seen in resistant cultures not treated with interferon. Nevertheless, interferon was not responsible for the genetic resistance of hepatocytes: it reduced virus titers in susceptible and resistant cultures to the same extent and the addition of specific antibodies to interferon after infection did not augment susceptibility of resistant cultures. We assume that intrinsic resistance of liver parenchymal cells is an important facet of inborn resistance of mice in vivo.

INTRODUCTION

Inbred strains of mice can be ranked for their degree of innate resistance to MHV_3: A/J mice survive infection even with high virus doses. C57BL/6 mice, on the other hand, die with severe hepatitis soon after infection and are representative for several highly susceptible strains (1). Other strains, such as C3H/HeJ or A/Sn, show intermediate susceptibility: a certain percentage of animals survives

V. ter Meulen et al. (eds.), *Biochemistry and Biology of Coronaviruses*

infection, but survivors may become chronic virus carriers and show signs of a progressive neurologic disease (1,2,3). The fate of the adult mouse during the early phase of infection seems to be determined by 2 non H-2 linked genes (3); a coherent picture of how these genes may operate has not yet emerged.

Mononuclear phagocytes decisively determine pathogenicity of viruses for the liver: viruses undergoing productive replication in macrophages may cause viral hepatitis, whereas those incapable of growing in these cells in general do not express hepatotropism (4). Isolated macrophages of resistant animals show a certain degree of resistance to MHV_3 infection in vitro (5). They are considered to play an important role in mediating resistance in vivo.

Resistance at the cellular level may be restricted to cells of the mononuclear phagocyte system. Alternatively, macrophages could represent but one exemplary cell type displaying resistance by mechanisms common to all potential target cells. Our findings support the latter view. Hepatocytes, the main parenchymal target cells within the liver, isolated from resistant adult animals and kept in chemically defined media, exhibited resistance in vitro, whereas those from susceptible animals were fully permissive for MHV_3. This resistance was virus specific and appeared to be independent of the action of interferon.

MATERIALS AND METHODS

The method of isolating hepatocytes from adult mice by in situ collagenase perfusion of the liver and the procedures to establish primary hepatocyte monolayer cultures have been described in detail (6,7). Mice were purchased from the Jackson Laboratory, Bar Harbor, Maine, and were kept under conventional conditions.

Stock MHV_3 virus, originally obtained from Jean-Louis Virelizier, Hôpital Necker-Enfants-Malades, Paris, France, was prepared in C57BL/6 peritoneal macrophages. Virus was plaque purified and assayed in mouse DBT cells (kindly provided by A. Kirn, Groupe de Recherches sur la Pathogénie des Infections Virales, Strasbourg, France) exactly as described (8). Stock virus preparations and assays of the avian influenza A virus M-TUR (A/TUR/Engl/63, HavlNav3) adapted to grow in mouse liver cells, vesicular stomatitis virus (VSV) and herpes simplex virus type 1 (HSV-1) were as previously described (7).

For immunofluorescence, an antiserum obtained from A/Sn mice after infection with MHV_3 or a rabbit antiserum prepared against purified JHM virus (kindly supplied by Kathrin Holmes, Department of Pathology, USUHS, Bethesda, Md) were used. Indirect immunofluorescent

labelling was done by treating with antiserum, washing and adding protein A-FITC (a gift from Thomas Bächi from our Institute) to air dried and acetone fixed hepatocyte cultures established on glass cover slips coated with collagen (Calbiochem Corp, San Diego, Calif.).

Mouse β interferon (IFN-β), purified to 10^7 ref units/mg protein, and a sheep anti-mouse interferon globulin preparation (AIFN), both gifts of Ion Gresser, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France, were those previously described (7). AIFN was routinely used to neutralize interferon occurring spontaneously in hepatocyte cultures (7).

RESULTS

Influence of the host cell genotype on MHV_3 replication in hepatocytes in culture

Cultured hepatocytes were infected with MHV_3 at multiplicities of 0.1 to 0.001. With C57BL/6 hepatocytes, infection caused a marked cytopathic effect. Cell fusion, first detectable after 7 hours, finally resulted in formation of a giant syncytium over the whole culture plate. With hepatocytes from resistant A/J or semiresistant A/Sn or C3H/HeJ mice, syncytium formation was only detected after high multiplicity infection. It was delayed as compared to C57BL/6 cultures and was restricted to foci containing no more than 50 cell nuclei at the end of a 4 day incubation period (Fig. 1).

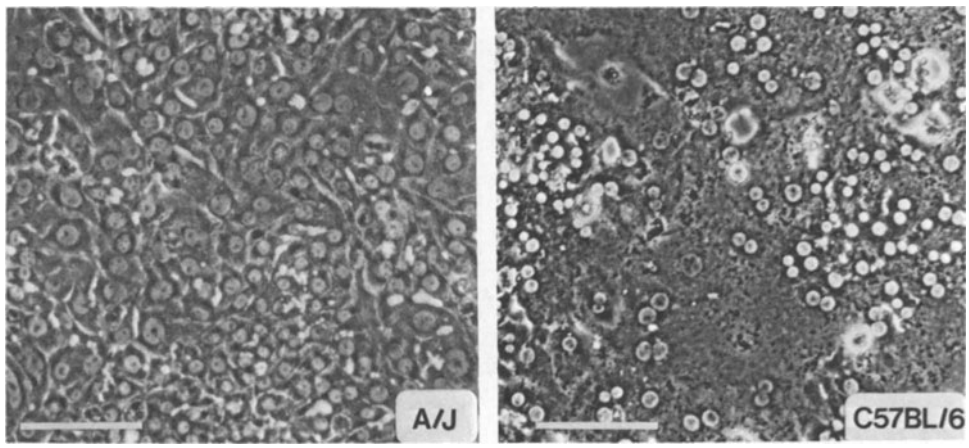

Fig. 1. Hepatocytes obtained from resistant A/J and susceptible C57BL/6 mice were cultured for 24 hrs and were then infected with MHV_3 at a multiplicity of 0.01. The micrographs were taken 24 hrs after infection. Phase contrast microscopy. Bars represent 50 µm.

Virus growth curves also revealed differences between the 2 types of hepatocyte cultures: at the moment of maximal virus production in C57BL/6 cells, the titers were 10^2 to 10^3 fold lower in resistant cells and never exceeded 10^5 plaque forming units (pfu)/ml thereafter (Fig. 2).

Replication of unrelated viruses in hepatocytes is independent of the host cell genotype influencing MHV_3 multiplication

A/J, C3H/HeJ and C57BL/6 mice are equally susceptible to influenza A virus infection (including the hepatotropic M-TUR variant) and to VSV (9, and unpublished observations), but differ with respect to MHV_3 (where A/J are resistant, C3H/HeJ are partially resistant, and C57BL/6 are susceptible) and HSV-1 (where C57BL/6 and C3H/HeJ are relatively resistant and A/J are susceptible) (10,11).

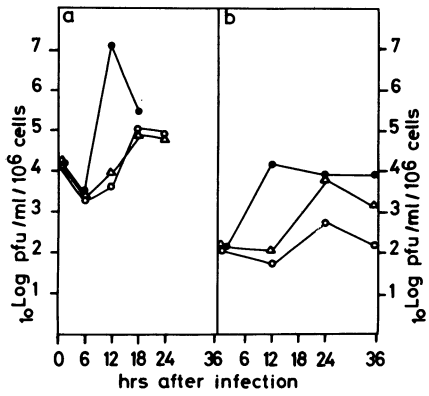

Fig. 2. Hepatocyte cultures established from adult A/J (o——o), C3H/HeJ (△——△) and C57BL/6 (●——●) mice were cultured for 48 hrs and were then infected with MHV_3 at a multiplicity of 0.1 (a) or 0.001 (b). Infectivity (plaque forming units,pfu) of cultures frozen and thawed 3 times at the end of the incubation period indicated was assayed in mouse DBT cells. In C57BL/6 hepatocytes, cytopathic effect was complete at 18 hrs (high multiplicity) and 36 hrs (low multiplicity) after infection.

A difference in permissiveness between hepatocytes prepared from these 3 strains was only seen with respect to MHV_3 (Table 1).

Study of MHV_3 replication of cultured liver cells by immunofluorescence and electron microscopy

Delayed production of infectious virus as seen in hepatocytes of resistant mice might be the result of improper morphogenesis or impaired excretion of viral particles, a phenomenon which would lead to intracellular accumulation of viral proteins. C57BL/6 cultures, labelled at 3 hour intervals after infection at a multiplicity of 0.1, showed a continuous increase of fluorescent cells from 5% at 6 hours to 100% at 12 hours. Spotty fluorescence was spread over the whole cytoplasma. Labelled nuclei could not be detected. In cultures from resistant A/J mice, a remarkebly different picture emerged: the percentage of fluorescent cells (themselves not distinguishable from those in the susceptible cultures) was 5% at 6 hours as in C57BL/6 cells, but even 48 hours after infection there were still large areas devoid of fluorescent cells. Labelled foci contained no more than 30 cells.

By transmission electron microscopy, cell fusion was first detected 7 hours after infection in C57BL/6 but not in A/J hepatocytes. At 12 hours, viral particles were found only in susceptible cultures.

Table 1. Susceptibility of hepatocytes from different mouse strains for various viruses

Mouse strain	MHV_3	M-TUR	VSV	HSV-1
A/J	$0.5x10^4$	$3.2x10^7$	$2.0x10^7$	$1.0x10^7$
C3H/HeJ	$1.0x10^4$	$4.0x10^7$	$1.0x10^7$	$6.5x10^7$
C57BL/6	$2.0x10^6$	$2.5x10^7$	$1.6x10^7$	$0.5x10^7$

Hepatocyte cultures were established from adult mice and were given AIFN from 0-24 hours. They were then infected with MHV_3 or herpes simplex type 1 virus (HSV-1) at a multiplicity of 0.1 or influenza A (M-TUR) or vesicular stomatitis virus (VSV) at a multiplicity of 10. 12 hour yields of MHV_3 are given as pfu/ml/10^6 cells. 24 hour yields of the other viruses are given as 50% tissue culture infective doses/ml, assayed in chicken embryo cells (M-TUR and VSV) or Vero cells (HSV-1).

Expression of the resistant phenotype in cultured hepatocytes: a role for interferon?

The striking observation that neutralization of endogenous interferon by specific antibodies enhanced the susceptibility of mice to MHV3 infection and could even abrogate the partial resistance of adult C3H/HeJ mice (12) suggested that interferon was at least partially responsible for resistance in vivo. But would interferon also account for resistance at the cellular level?

Hepatocytes spontaneously release interferon during the first 24 hours after being put in culture independently of their genotype (7, and unpublished observations). They were therefore exposed to anti-IFN-antibodies for a first culture period. These antibodies could be washed out thereafter, i.e. no detectable IFN neutralizing activity remained in the supernatant fluids. After MHV3 infection, small amounts of IFN became detectable but were difficult to interpret. To test indirectly whether IFN induced by the infection would have any effect on the course of virus growth, we added AIFN after infection. There was no enhancement of MHV_3 yield in either A/J or C57BL/6 hepatocytes, just as previously observed for VSV and HSV-1 (7, and unpublished observations).

We then tested hepatocyte cultures for their sensitivity to the anti-viral effect of IFN. Mouse IFN-β was added at graded doses to A/J and C57BL/6 hepatocyte cultures for 18 hours. The cells were then infected with either MHV_3, VSV or HSV-1. Yield reductions observed after exposure to 1000 ref units/ml of IFN are given in Table 2. The capacity of IFN to inhibit MHV_3 was similar in A/J and C57BL/6 cultures, as was its inhibitory activity against VSV or HSV-1. No effect of the resistance phenotype on interferon action was discernible.

DISCUSSION

Liver parenchymal cells isolated from mice with inborn resistance to MHV_3 infection were able to express a certain degree of virus specific resistance in vitro. Rather than being totally non-permissive for MHV_3, such cultures showed a delay in production of infectious virus as compared to their susceptible counterparts. This delay correlated well with the absence of widespread cell fusion, the scarcity of budding virions and the limitation of spread of viral antigens.

Macrophage cultures prepared from resistant A/J mice have been reported to be either totally resistant to MHV_3 (5) or to show a delay in onset of cytopathic effect (13). Hence, the course of MHV_3 infection in hepatocytes resembled that in macrophages of the same

Table 2. Inhibition of virus replication in hepatocytes of C57BL/6 and A/J mice by exogenous interferon

Virus	$_{10}$Log yield reduction after treatment with 1000 ref units/ml of mouse IFN-β	
	A/J	C57BL/6
MHV_3	1.3[a]	1.2
VSV	2.1	2.2
HSV-1	1.5	1.4

Legend to Table 2: Hepatocyte cultures, virus infections and titrations were as indicated in Table 1. Mouse IFN-β was given from 24-42 hours after cell harvest. Thereafter, the cultures were washed 3 times and were infected in presence of AIFN sufficient to neutralize 10,000 ref units of IFN. Yield reductions were calculated as $_{10}$log (yield without IFN/yield with IFN).

[a]The comparative value for A/Sn hepatocytes was 1.1.

genotype. It is therefore tempting to speculate that, at the molecular level, the same mechanisms act in the 2 cell types.

Of the various factors known to modulate acute infection, interferon appears to be very important (14). Its neutralization has been shown to abrogate the partial resistance of mice to MHV_3 (12). The growth curve of MHV_3 as seen in cells from resistant mice could be mimicked by treating susceptible cells with interferon, and this both in hepatocyte and macrophage cultures (data not shown). Furthermore, interferon is a prerequisite for expression of resistance against orthomyxoviruses, both in the living mouse and in its isolated cells (7,15,16). In contrast, IFN was obviously not required for expression of innate resistance to MHV_3 at the cellular level: its neutralization after infection was without effect and it protected susceptible and resistant cultures to the same extent. That other types of IFN or lymphokines might exert a differential effect is however still conceivable.

We assume that hepatocytes of resistant animals behave in vivo as they do in culture: they are intrinsically less permissive for MHV_3 than those from susceptible strains. A temporal delay in virus growth would be sufficient to enable the host specific and unspecific immune system to cope with the infection. The immune system of sus-

ceptible hosts, even if similarly effective in principle, would start too late to limit the rapid virus growth in the main target cells. When the immune system of a resistant host is substantially disturbed, delayed virus growth in its target cells cannot protect the animal from death. This would explain why various immunosuppressive treatments, impairment of macrophage function or the neutralization of endogenous interferon may fully or partly abrogate resistance in vivo. We conclude that cellular resistance, even of cells not usually involved in antiviral defence, is a necessary but by far not sufficient condition for protection in vivo.

ACKNOWLEDGEMENT

We are grateful to Drs. Ion Gresser for his generous gift of interferon and anti-interferon antibodies, Kathrin Holmes for her anti-JHMV antiserum, and A. Kirn for providing us the DBT cell line We thank J. Lindenmann for constant interest and critically reading the manuscript, C. Kaufmann and L. Schmutz for excellent technical and R. Leemann for expert secretarial assistance. This work was supported by the Swiss National Science Foundation, grant no. 3.393.078.

REFERENCES

1. C. Le Prévost, J.L. Virelizier, and J.M. Dupuy, Immunopathology of mouse hepatitis virus type 3 infection. III. Clinical and virologic observation of a persistent viral infection. J. Immunol. 115:640 (1975).
2. E. Lévy-Leblond, D. Oth and J.M. Dupuy, Genetic study of mouse sensitivity to MHV3 infection: influence of the H-2 complex. J. Immunol. 122:1359 (1979).
3. J.L. Virelizier, A.D. Dayan and A.C. Allison, Neuropathological effects of persistent infection of mice by mouse hepatitis virus. Infect. Immun. 12:1127 (1975).
4. S.M. Sabesin and R.S. Koff, Pathogenesis of experimental viral hepatitis. New Engl. J.Med. 290:944 (1974).
5. J.L. Virelizier and A.C. Allison, Correlation of persistent mouse hepatitis virus (MHV-3) infection with its effect on mouse macrophage cultures. Arch. Virol. 50:279 (1976).
6. H. Arnheiter, Primary monolayer culture of adult mouse hepatocytes - A model for the study of hepatotropic viruses. Arch. Virol. 63:11 (1980).
7. H. Arnheiter, O. Haller and J. Lindenmann, Host gene influence on interferon action in adult mouse hepatocytes: specificity for influenza virus. Virology 103:11 (1989).
8. N. Takayama and A. Kirn, An improved method for titration of mouse hepatitis virus type 3 in a mouse cell culture. Arch.

10. H. Kirchner, H.M. Hirt, D.L. Rosenstreich and S.E. Mergenhagen, Resistance of C3H/HeJ mice to lethal challenge with herpes simplex virus (39983). Proc. Soc. Exp. Biol. Med. 157:29 (1978).
11. C. Lopez, Genetics of natural resistance to herpesvirus infections in mice. Nature 258:152 (1975).
12. J.L. Virelizier and I. Gresser, Role of interferon in the pathogenesis of viral diseases of mice as demonstrated by the use of anti-interferon serum. V. Protective role in mouse hepatitis virus type 3 infection of susceptible and resistant strains of mice. J. Immunol. 120:1616 (1978).
13. M.R. Macnaughton and S. Patterson, Mouse hepatitis virus strain 3 infection of C57, A/Sn and A/J strain mice and their macrophages. Arch. Virol. 66:71 (1980).
14. I. Gresser, M.G. Tovey, C. Maury and M.T. Bandu, Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. II. Studies with herpes simplex, Moloney sarcoma, vesicular stomatitis, Newcastle disease, and influenza viruses. J. Exp. Med. 144:1316 (1976).
15. O. Haller, H. Arnheiter, I. Gresser and J.Lindenmann, Genetically determined, interferon-dependent resistance to influenza virus in mice. J. Exp. Med. 149:601 (1979).
16. O. Haller, H. Arnheiter, J. Lindenmann and I. Gresser, Host gene influences sensitivity to interferon action selectively for influenza virus. Nature 283:660 (1980).