The Severity of Hepatic Lesion after Intraperitoneal JHMV Infection in IFN-gamma Deficient Mice is Parallel to Viral Replication in Hepatocytes *in Vitro*

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1. INTRODUCTION

Several factors affect MHV infection in mice (Table 1). They can be classified into two groups, viral factors and host factors. Although JHMV induces a fatal encephalitis in mice after intracerebral infection, it does a mild hepatitis when it is inoculated intraperitoneally. On the other hand, host genetic factor is a critical determinant. Among MHV researchers, it is well known that SJL mice are relatively resistant to intracerebral infection with JHMV (Stohlman & Frelinger 1978). In addition, the immune system is one of the key players that determine MHV infection in mice.

Table 1. Factors that affect MHV infection

Viral factor	Host factor
Viral strain	Strain of mice (Genetical background)
Viral dose	Age
Route of infection	Immune status

The Nidoviruses (Coronaviruses and Arteriviruses).
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1.1 Subacute Peritonitis in IFN-gamma Deficient B6 Mice after Intraperitoneal JHMV Infection

Comparing wild type B6 mice with genetically engineered IFN-gamma (IFN-g) deficient B6 mice (B6-GKO), we demonstrated that IFN-gamma was involved in viral clearance after intraperitoneal JHMV infection (Kyuwa *et al.* 1998). Although the virus was cleared within 10 days from the liver in wild-type B6 mice, it persisted for 50 days in the liver in B6-GKO mice. B6-GKO mice survived in the acute phase, but some began to die from 2 weeks after infection.

We therefore examined the pathological changes in JHMV-infected B6-GKO mice in the subacute phase. In all the mice, a pseudomembrane was observed on the surface of the livers and organs in the abdominal cavity had adhered to each other and to the peritoneum. Approximately a half of the mice had an accumulation of a viscous fluid in the abdominal and thoracic cavities. Microscopically, the disease was characterized by disseminated granulomatous inflammation and exudative fibrinous serositis with plasma cells and eosinophils. The histopathological examination revealed that the liver lesion in B6-GKO mice was not progressive. This form of disease was not expected but reminded us a feline infectious peritonitis, another coronavirus-induced disease in cats (Olsen 1993).

1.2 Acute Hepatic Failure in IFN-g Deficient BALB/c Mice after Intraperitoneal JHMV Infection

To see further the role of IFN-g in intraperitoneal JHMV infection in mice, IFN-g deficient mice with BALB/c background (BALB-GKO) as well as B6-GKO mice were inoculated intraperitoneally with 10^6 PFU of JHMV (Kyuwa *et al.* in preparation). B6-GKO mice survived in the acute phase but suffered subacute peritonitis as described above. In contrast to B6-GKO mice, all the BALB-GKO mice died before 7 days postinfection.

The viral titer in the liver, serum alanine aminotranferase (ALT) activity and histopathological changes in the liver from B6-GKO and BALB-GKO mice at 5 days postinfection were examined. The viral titer in BALB-GKO mice was 100-fold higher than that in B6-GKO mice. Similarly, the ALT activity in BALB-GKO mice was 27-fold higher than that in B6-GKO mice. Histopathologically, the lesion in B6-GKO mice was larger than that in wild-type B6 mice, but restricted. In contrast, all the hepatocytes in BALB-GKO mice appeared to be necrotic. These data strongly suggest that BALB-GKO mice died as a result of acute hepatic failure caused by viral infection.

2. JHMV REPLICATION *IN VITRO* SYSTEM

Although the viral replication rate in hepatocytes is the most vital element that determines the viral growth in the liver, other extrinsic factor such as the immune response also influences the viral titer. In fact, we previously reported that T cells played a critical role in viral clearance in the liver (Kyuwa *et al.* 1996). To examine viral replication in the absence of the immune system, we set up JHMV replication systems with the primary hepatocytes and peritoneal macrophages *in vitro*.

2.1 JHMV Replication in the Primary Hepatocytes

The primary hepatocyte culture was prepared from B6-GKO mice, BALB-GKO mice and their wild type counterparts (Arnheiter 1980), with a minor modification. They were cultured in the presence or absence of recombinant IFN-g in 24-well plates overnight. After washing three times, they were inoculated with JHMV at an m.o.i. 0.1. The culture supernatants were collected 12 and 24 hrs postinfection and the viral titer was determined by plaque assay on DBT cells.

In the absence of IFN-g, the viral titer of the supernatant from BALB-GKO mice was 100-fold higher than that from B6-GKO mice. That from wild type BALB/c mice was almost equivalent to that from BALB-GKO mice and was also significantly higher than that from wild type B6 mice. These results suggest that the viral replication rate in BALB/c hepatocytes is significantly higher than in B6 hepatocytes and that the phenomenon is not dependent on IFN-g.

The pre-treatment of IFN-g clearly inhibited viral replication in dose dependent fashion and 100 U/ml of IFN-g completely inhibited the viral replication in the primary hepatocytes from both BALB-GKO and B6-GKO mice.

2.2 JHMV Replication in Macrophages

Since a significant difference was observed in JHMV replication in the primary hepatocytes from BALB-GKO and B6-GKO mice, the replication of JHMV in macrophages, another MHV-susceptible cell type in the mouse, was examined *in vitro*. Peritoneal exudates cells were collected from BALB-GKO and B6-GKO mice, and cultured in 24-well plates overnight. Non-adherent cells were removed and the adherent cells were inoculated with JHMV at an m.o.i. 0.1. Culture supernatants were harvested at 12 and 24 hrs postinfection and their viral titers were determined by plaque assay on DBT cells.

In contrast to the case of the primary hepatocytes, JHMV growth in macrophages from BALB-GKO was nearly equal to that from B6-GKO mice.

3. CONCLUSION

In this study, we demonstrated that unlike B6-GKO mice, BALB-GKO mice died within a week after intraperitoneal JHMV infection, due to acute hepatic failure. It is very interesting that JHMV induces a different type of disease in IFN-g deficient mice of different genetic backgrounds (Figure 1). Secondly, JHMV replication in the primary hepatocytes from BALB/c mice was significantly higher than that from B6 mice. Thirdly, JHMV replication in the primary hepatocytes was inhibited by IFN-g in dose-dependent manner. However, there was not a significant difference in JHMV replication in macrophages from both strains of mice. These results suggest that some intrinsic factors in hepatocytes are involved in the regulation of JHMV replication and a virus-induced disease in mice. The elucidation of the mechanism may offer the key to an understanding of the susceptibility to JHMV.

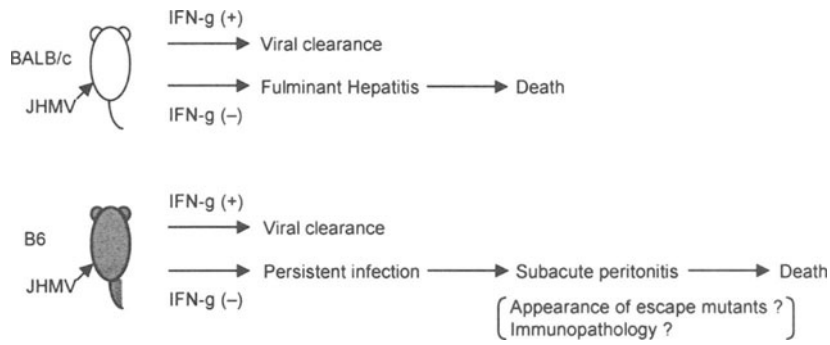

Figure 1. The clinical consequences of intraperitoneal JHMV infection in BALB-GKO mice, B6-GKO mice and their wild type counterparts. Whereas wild type BALB/c and B6 mice recover from a mild acute hepatitis, BALB-GKO and B6-GKO mice die after distinct consequences.

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