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MHV-INDUCED FATAL PERITONITIS IN MICE LACKING IFN-γ

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1. ABSTRACT

IFN-γ gene was disrupted by homologous recombination in A3–1 embryonic stem cells. Germinally transmitted chimeric mice were successfully obtained and backcrossed with C57BL/6 (B6) mice 5 or 6 times. Deficiency of IFN-γ in homozygous mice was confirmed by northern blot analysis of spleen cells stimulated with phorbor esther and calcium ionophore and also by IFN-γ production in the culture supernatant of spleen cells stimulated with the same reagents. B6 mice lacking IFN-γ were infected intraperitoneally (ip) with 10^6 PFU of JHMV and monitored for their survival. Approximately 90% of the mice died at 50 days post-infection (pi) and the mean survival time was 28 days. Mice sacrificed at 3 weeks pi showed severe peritonitis accompanying the accumulation of a viscous fluid in the abdominal and thoracic cavities. Microscopically, the disease was characterized by disseminated granulomatous inflammation and exudative fibrinous serositis in the abdominal cavity. Infectious virus was isolated in most tissues including the

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liver, spleen, kidney, pancreas and lung during the experimental periods. The disease was not observed in wild-type or heterozygous littermates infected ip with JHMV. These results suggest that IFN-γ plays a critical role in MHV infection in mice. This experimental model may provide a unique opportunity to address the pathogenesis of virus-induced peritonitis such as feline infectious peritonitis in cats.

2. INTRODUCTION

MHV induce a variety of diseases including hepatitis, enteritis, encephalitis in mice, dependent on virus strain, infectious route and strain, age and immune status of the hosts. In particular, T cell-mediated immunity has been suggested to play an important role in the protection against ip infection with JHM strain (JHMV) in B6 mice (Kyuwa et al., 1989, Kyuwa et al., 1996). IFN-γ is an antiviral cytokine, which is produced by type 1 helper T cells, cytotoxic T lymphocytes and NK cells. IFN-γ activates macrophage activity, induces MHC class I, class II and adhesion molecules, and enhances IgG2a and IgG3 production. However, there is a discrepancy in the role of IFN-γ among virus infections in vivo (Baumgarth and Kelso, 1996; Sarawar et al., 1997). In the present study, we attempted to evaluate the role of IFN-γ in ip infection with JHMV in mice and found that JHMV induced subacute fatal peritonitis in mice lacking IFN-γ.

3. MATERIALS AND METHODS

3.1. Mice

Production of mice lacking IFN-γ was described previously (Tagawa et al., in press). A 129/SvJ mouse with heterozygously disrupted IFN-γ gene (IFN-$\gamma^{+/-}$) was backcrossed with B6 mice 5 or 6 times. Genotype of mice was determined by PCR as described previously (Tagawa et al., in press), and 8- to 12-week-old female mice were used. Breeding mice were maintained in a laminar flow rack and routinely checked forserologically free of MHV, Sendai virus, Mycoplasma pulmonis, Clostridium piriformis (Tyzzer's organism).

3.2. Virus

The DL variant of JHMV was propagated and plaque assayed on DBT cells as described previously (Kyuwa et al., 1996). Mice were infected ip with 110^6 plaque-forming units (PFU) of JHMV in a volume of 0.2 ml.

3.3. Serological Tests

Serum neutralizing antibodies were assayed as described elsewhere (Lin et al. 1997). Total anti-JHMV antibodies were determined by enzyme-linked immunosorbent assay by a commercial kit (Denka Seiken, Tokyo).

3.4. Depletion of $CD4^+$ or $CD8^+$ T Cells in Vivo

Purified anti-CD4 (GK1.5) or anti-CD8 (2.43) monoclonal antibodies were injected ip to deplete $CD4^+$ or $CD8^+$ T cells in vivo as previously described (Kyuwa et al., 1996).

3.5. Histopathology

Samples were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Sections were prepared and stained with hematoxylin and eosin.

4. RESULTS

4.1. Production and Characterization of Mice Lacking IFN-γ

IFN-γ gene was disrupted by homologous recombination in A3–1 embryonic stem cells. The first exon was disrupted by inserting neomycine-resistant gene and cells homologously recombinated were selected in the presence of G418 (Tagawa et al., in press). Then, chimeric mice were produced and progenies were backcrossed with B6 mice 5 or 6 times.

The deficiency of IFN-γ in homozygous mice was confirmed by northern blot analysis of spleen cells stimulated with phorbor esther and calcium ionophore in vitro. Although spleen cells from wild type or heterozygous mice produced IFN-γ mRNA, those from homozygous mouse failed (Tagawa et al., in press). The lack of IFN-γ production in homozygous mice was also confirmed by protein level. IFN-γ protein was not detected in the culture supernatant of spleen cells from homozygous mouse stimulated with concanavalin A or phorbor esther and calcium ionophore (Tagawa et al., in press).

4.2. The Role of IFN-γ in the Acute Phase of JHMV Infection

IFN-$\gamma^{-/-}$, IFN-$\gamma^{+/-}$ and IFN-$\gamma^{+/+}$ mice were infected ip with 10^6 PFU of JHMV. After infection, survival of mice, viral growth and histopathological changes were examined. Although in heterozygous and wild type mice JHMV was cleared in the liver by 7 days after infection, the viral titers gradually decreased but persisted till 21 days after infection in homozygous mice. The result suggests that IFN-γ play a critical role in viral clearance during acute JHMV infection.

After ip infection with JHMV, a number of small lesions were observed in the liver in wild type mice 5 days after infection (Figure 1). In contrast, the liver lesions in IFN-$\gamma^{-/-}$ mice were larger than those in wild type mice, and infiltrated with abundant neutrophils (Figure 2).

4.3. Subacute Fatal Peritonitis in IFN-$\gamma^{-/-}$ mice

None of wild type and heterozygous mice died during the experimental periods. In contrast, some homozygous mice began to die from 16 days after infection, although they looked healthy till 10 days after infection. A 90% of the mice died at 50 days after infection. JHMV-infected homozygous mice were sacrificed at 21 days after infection. Most of mice showed severe peritonitis accompanying the accumulation of a viscous fluid in the abdominal and thoracic cavities. Microscopically, the disease was characterized by disseminated granulomatous inflammation and exudative fibrinous serositis in the abdominal and thoracic cavities. The deformation as well as adhesion of the abdominal organs was observed in affected animals (Figure 3). Although some neutrophils were infiltrated in the liver, hepatocyte injury was not observed and serum alanine aminotransferase (GPT) activity of the mice was normal. Lesions were observed not only in the abdominal cavity but also in the thoracic cavity. Infectious virus was recovered from most organs including the liver, spleen, mesenterium, kidney, pancreas and lung at 2 and 3 weeks after infection in IFN-$\gamma^{-/-}$ mice.

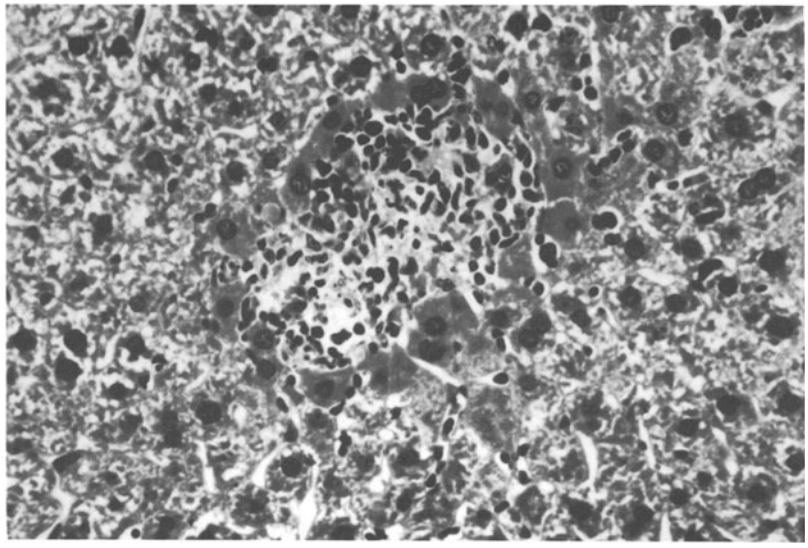

Figure 1. A representative lesion in the liver in JHMV-infected IFN-$\gamma^{+/+}$ mice. 5 days pi.

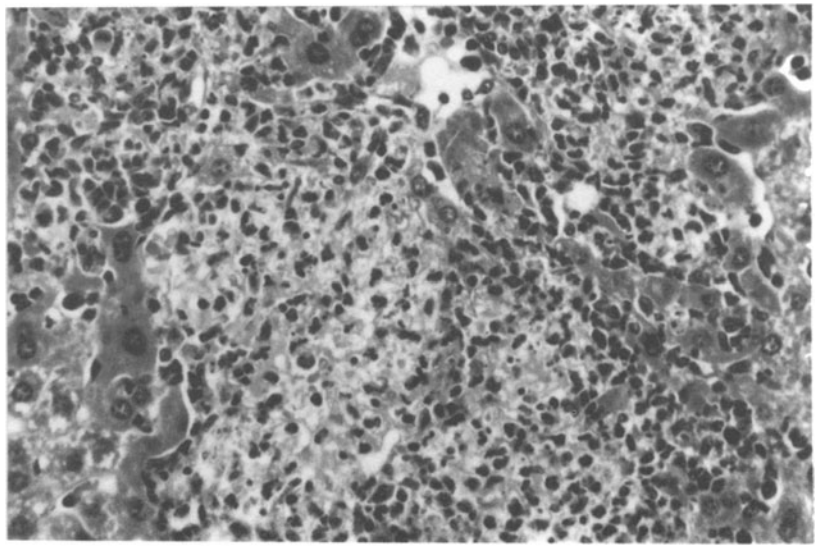

Figure 2. A representative lesion in the liver in JHMV-infected IFN-$\gamma^{-/-}$ mice. 5 days pi.

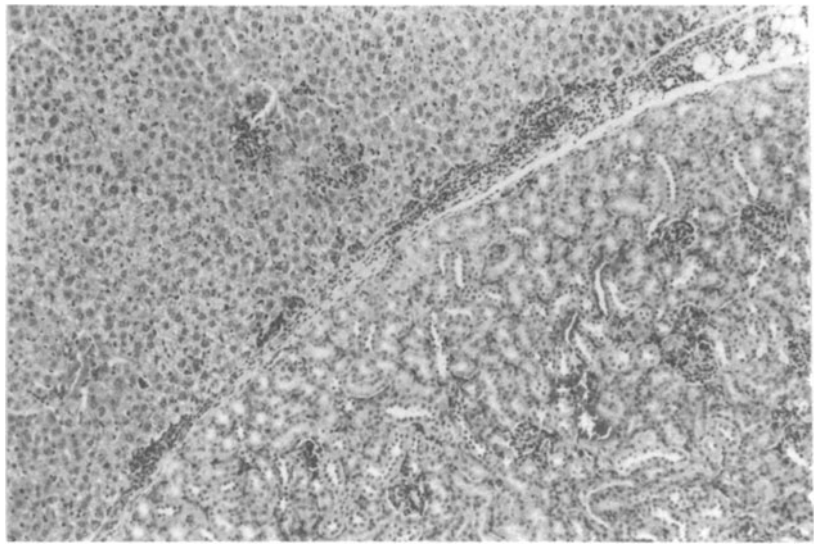

Figure 3. The adhesion of the liver and kidney in JHMV-infected IFN-$\gamma^{-/-}$ mice. 21 days pi.

Antiviral antibody responses in homozygous, heterozygous and wild type mice were examined. Sera were obtained from mice at 3 weeks after infection and total antiviral antibody titer was determined by a commercial ELISA kit. The antiviral antibody titers of homozygous mice were significantly higher (1:734) than those of heterozygous (1:165) or wild type (1:253) mice. Virus neutralization titer was also examined and the titers of homozygous mice were also higher than those of heterozygous or wild type mice.

4.4. The Role of $CD4^+$ and $CD8^+$ T Cells and Administration of Recombinant IFN-γ

Finally, effects of recombinant IFN-γ administration, depletion of either $CD4^+$T cells or $CD8^+$T cells on MHV-induced fatal peritonitis were examined. 3,000 units of recombinant IFN-γ or 200 μg of purified monoclonal antibodies were injected ip twice a week during the experimental periods. All of homozygous mice depleted of $CD8^+$T cells died within 10 days. Similarly, homozygous mice depleted of $CD4^+$T cells died within 2 weeks. These results suggest that protective effects of T cells are not solely mediated by IFN-γ. Recombinant IFN-γ administration partially inhibited MHV-induced fatal disease. At present, it remains obscure whether administration of higher dose of IFN-γ may completely prevent the disease or not.

5. DISCUSSION

In this study, we found some role of IFN-γ in MHV infection in mice using IFN-γ deficient mice. Complete viral clearance was not observed in IFN-γ deficient mice, suggesting

that IFN-γ plays a critical role in viral clearance in ip JHMV infection in mice. However, the survival time of homozygous mice depleted of each T cell subset after JHMV infection was shorter than that of PBS-treated homozygous mice, suggesting that T cell-mediated antiviral effects except IFN-γ production also play a key role in the protection.

Intraperitoneal JHMV infection induces subacute fatal peritonitis in IFN-γ deficient mice. Previously, Yanagisawa et al. reported that MHV-NuU induced a similar disease in ICR-nude mice at a lower rate (Yanagisawa et al., 1985; Yanagisawa et al., 1986). Although the pathgenesis of this disease remains obscure at present, this experimental model may provide a unique opportunity to address virus-induced peritonitis such as feline infectious peritonitis in cats.

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