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A Major Role of Macrophage Activation by Interferon-Gamma During Mouse Hepatitis Virus Type 3 Infection. II. Age-Dependent Resistance

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Abstract

In contrast to adult mice, young A/J mice, developed an acute hepatitis following infection with Mouse Hepatitis virus type 3. 100 % of the young animals died 4 to 5 days after the infection and high levels of virus were found in the liver and peritoneal exudate. Very low levels of IFN-γ were found in the serum and peritoneal exudate of infected young mice. This was in contrast to the levels observed in adult mice. Spleen cells and macrophage cultures from young A/J mice, again in contrast to adult A/J mice, were shown to be unable to synthesize IFN-γ and IFN-α/β respectively. Macrophages from either young or adult A/J mice were able to be activated with exogenous recombinant IFN-γ or IFN-α/β, enabling both sets of cells to restrict MHV3 replication. The results indicate that the ability of the immune system to synthesize IFN-γ and IFN-α/β may play a major role in the age-dependent resistance of A/J mice to MHV3.

Introduction

MHV3 constitutes a model of viral infection in which resistance depends on the age and the genetic background of the animal (1–5). A/J mice have been reported to be susceptible up to 3 weeks of age, developing an acute hepatitis and dying 4 to 5 days after infection.

Complete resistance develops after the 3rd week of life. After MHV3 infection, adult A/J mice show a mild disease which disappears 4 to 6 days later. Three types of mature cells has been shown to be required for transfering MHV3 resistance into young A/J mice: T lymphocytes, adherent spleen cells and a third population that shares several features with

Abbreviations: MHV3 = mouse hepatitis virus type 3; *IFN* = interferon; *LPS* = lipopolysaccharide; *Con A* = concanavalin A; *FCS* = fetal calf serum; *PFU* = plaque forming units; *ip* = intraperitoneally.

natural killer cells (3, 5). T lymphocytes and adherent cells such as peritoneal macrophages and Kupffer cells have been suggested by several authors to participate in the resistance against MHV3 (2, 4, 6–10), but natural killer cells have not been thought to be of overwhelming importance in the defense of mice against MHV3 (11, 12).

Depending on the genetic background of the adult animal, different patterns of susceptibility are observed such that, C3H mice are considered semi-susceptible while BALB/c are considered susceptible. C3H mice develop a chronic disease in a high percentage of mice following MHV3 infection whereas BALB/c mice develop an acute and fatal hepatitis in 100 % of the mice. It has been speculated that the crucial factors in determining resistance or susceptibility are the expression of a monocyte derived monokine that demonstrates procoagulant activity (13, 14), the antiviral state induced by IFN (4, 15, 16) and the virus replication in target cells (2, 17–19).

IFN and macrophages are considered to be important elements in resistance against different viral infections (4, 20–22). IFN-α/β is readily induced during the initial phase of infection, well before the generation of an immune response, and IFN-γ is rapidly induced following the generation of the immune response directed against the virus.

Several studies attempted to clarify the role of IFN and macrophages during viral infections, and those of experimental herpes virus infection provide examples of the importance of these two factors in host resistance (23, 24). Recently, Domke-Opitz et al. (25) have shown a role of IFN in persistent infection of macrophages with this virus, and Ellermann-Eriksen et al. (26) have demonstrated differential sensitivity of macrophages from herpes simplex virus-resistant and susceptible mice to respiratory burst priming by IFN-α/β.

Our previous studies, both *in vitro* and *in vivo* indicated that a T-cell dependent activation of macrophages in which IFN-γ plays a major role is required to confer resistance to adult A/J mice against MHV3 (27). These mice have macrophages that are very sensitive to IFN-γ and are also sensitive to IFN-α/β. During infection, IFN-γ is found in significant amounts in peritoneal exudate and serum and can be effective in restricting MHV3 multiplication in macrophages. On the other hand, mice from the susceptible BALB/c strain were shown to have macrophages that are not sensitive to IFN-α/β or IFN-γ. In spite of the high amounts of IFN-γ detected in the serum and peritoneal exudate during the first days of infection, the macrophages could not restrict virus multiplication. The animals died 5 to 6 days after infection with the high amounts of IFN-γ reflecting the virus replication and stimulation of the immune system (27).

In the present work, we investigate the involvement the macrophage-IFN-γ interaction in the age-dependent resistance showed by A/J mice after MHV3 infection.

Materials and Methods

Mice

15 and 60 days old A/J mice (originating from the Pasteur Institute, Paris, France, bred in our mouse colony and periodically controlled for the absence of Coronavirus or specific antibodies), here referred as young and adult A/J mice, respectively, were used to study the mortality, the virus growth and the IFN-α/β and -γ synthesis in peritoneal exudate and serum after MHV3 infection. We also investigated the MHV3 growth and IFN-α/β synthesis in cultured peritoneal macrophages as well as the IFN-γ synthesis in cultured spleen cells.

Virus

MHV3 was cultivated and titrated by plaque assay on L929 cells at 37 °C as previously described (12). Aliquots containing 2×10^5 plaque forming units per milliliter (PFU/ml) were stored at −80 °C and used in all experiments. The MHV3 titers in tissues of infected animals or supernatants of cell cultures, obtained as described below, were expressed as PFU per milliliter of peritoneal exudate or supernatants (PFU/ml), or PFU per gram of liver (PFU/g).

Cell cultures

The techniques used for preparation of the cell cultures have been described in detail elsewhere (12, 27). Briefly, peritoneal exudate cells were collected by peritoneal lavage and cultured in RPMI 1640 containing 10 % FCS on 96 well plates at a concentration of 2×10^5 cells per well. After 2 h incubation they were washed three times to remove the nonadherent cells. Spleen cell suspensions were cultured at 5×10^6 cells per well in RPMI 1640 medium containing 10 % FCS on 24-well plates.

Interferon assay

A cytopathic effect reduction test technique using monolayers of L929 cells and encephalomyocarditis virus, described in detail in a previous paper (27), was used as an IFN assay. For characterization of IFN-α/β and IFN-γ, antibodies to mouse IFN-α/β and monoclonal antibodies to recombinant mouse IFN-γ were always used. These antibodies showed no cross-reactivity (27).

Results

Mortality of A/J mice after MHV3 infection

After ip infection with 10^3 PFU of MHV3, all the young A/J mice were shown to be susceptible developing an acute hepatitis and dying 3 to 4 days after infection. On the other hand all the adult A/J mice were shown to be fully resistant, recovering from a mild disease 4 to 5 days after infection (data not shown).

Virus growth and IFN synthesis in infected A/J mice

The data shown in Figure 1 and 2 represent the kinetics of virus replication in the peritoneum and the liver, and the levels of IFN-α/β and IFN-γ in the serum and peritoneal exudate of young and adult A/J mice infected with MHV3. In spite of high titers of virus found in the liver and peritoneum of young A/J mice, very low levels of IFN-α/β and almost no
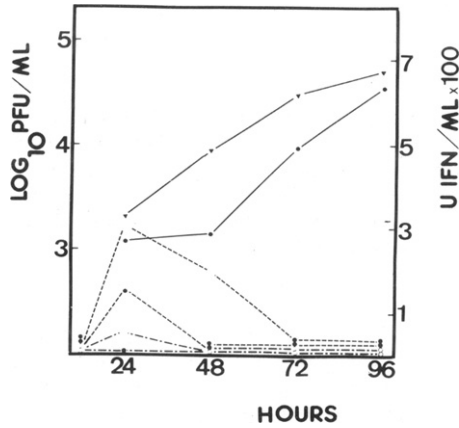
Figure 1. Virus titers (———) detected in the liver (▲) and peritoneal exudate (●), and IFN-α/β (– – – –) and IFN-γ (–·—·—·–) titers detected in serum (○) and peritoneal exudate (●) of MHV3 infected young A/J mice. Animals were ip inoculated with 10^3 PFU of MHV3 and after different times, groups of 5 mice were sacrificed, the tissues prepared and the virus or IFN titrated. The MHV3 titers, reported as Log_{10} PFU/g (for the liver) or Log_{10} PFU/ml (for the peritoneal exudate), and the IFN titers, reported as U IFN/ml × 100, are the average of 5 different determinations, which showed a small variation.

IFN-γ was found in the serum and peritoneal exudate, when compared with the high titers of virus and also IFN-α/β or -γ observed in susceptible adult animals from the BALB/c strain (27). These results are also in contrast to
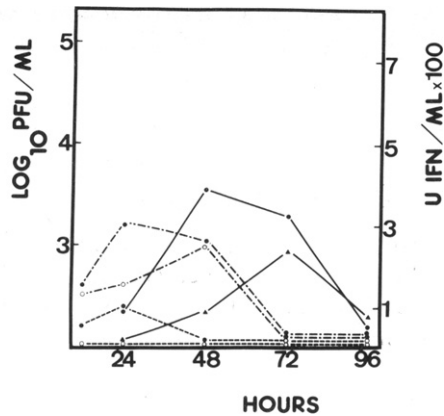
Figure 2. Virus titers (———) detected in the liver (▲) and peritoneal exudate (●), and IFN-α/β (– – – –) and IFN-γ (–·—·—·–) titers detected in serum (○) and peritoneal exudate (●) of MHV3 infected adult A/J mice. Animals were ip inoculated with 10^3 PFU of MHV3 and after different times, groups of 5 mice were sacrificed, the tissues prepared and the virus or IFN titrated. The MHV3 titers, reported as Log_{10} PFU/g (for the liver) or Log_{10} PFU/ml (for the peritoneal exudate), and the IFN titers, reported as U IFN/ml × 100, are the average of 5 different determinations, which showed a small variation.

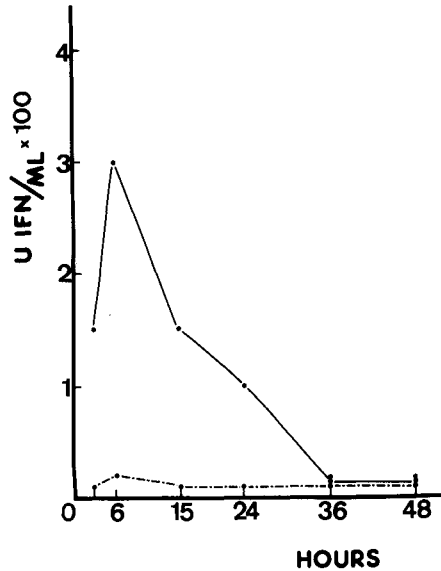Figure 3. Kinetics of the IFN-α/β synthesis by cultured peritoneal macrophages of adult (———) and young A/J mice (–·–·–·–), after stimulation with LPS. The macrophages prepared by peritoneal lavage were incubated for 24 h with 10 µg/ml of LPS. At defined time intervals, the supernatants were collected and the IFN titers determined. The titers are reported as U IFN/ml × 100 and are the average of 5 different determinations, which showed a small variation.

those obtained with adult A/J mice (Fig. 2), where low titers of virus were found in the tissues and higher levels of IFN-γ were found in the serum and peritoneal exudate.

IFN synthesis in macrophages and spleen cells of A/J mice

The *in vitro* production of IFN-α/β, by cultured macrophages from normal young and adult A/J mice, stimulated with LPS (Fig. 3), and the IFN-γ produced by cultured spleen cells from normal and MHV3 immunized young and adult A/J mice, stimulated with Con A or MHV3 (Table 1) were investigated. In contrast to the results obtained with the cells from adult A/J mice, no synthesis of IFN-α/β or IFN-γ was detected in cultured cells from young A/J mice, indicating that these cells were not capable of producing IFN after specific or non-specific stimulation.

Antiviral state in activated macrophages of A/J mice

The results shown in Table 2 indicate that macrophages from young or adult A/J mice were very sensitive to the induction of an anti-MHV3 state by IFN-α/β or IFN-γ. No MHV3 replication occurred in the cultures from young A/J mice and very low virus titers were obtained in those from adult

Table 1. Synthesis of IFN-γ in cultured spleen cells from A/J mice (a)

mice	immunization	stimulation	IFN-γ titer (U/ml)
	–	–	0
	–	ConA	50 ± 6.3
	–	MHV3	0
A/Jy	MHV3 uv	–	0
	MHV3 uv	ConA	20 ± 2.8
	MHV3 uv	MHV3	0
	–	–	10 ± 4.1
	–	ConA	1600 ± 80.4
	–	MHV3	50 ± 8.5
A/Ja	MHV3 uv	–	20 ± 5.3
	MHV3 uv	ConA	2200 ± 127.6
	MHV3 uv	MHV3	4600 ± 181.3

a. Young (A/Jy) and adult (A/Ja) A/J mice were ip immunized with 10^3 PFU of ultraviolet (uv)-inactivated MHV3. 10 days later, the mice were sacrificed and the spleen cells cultured. The cells were stimulated with ConA (10μg/ml) or MHV3 (10^3 PFU), and the IFN-γ titers determined in supernatants. The results are the mean values of 3 separate experiments ± S.D.

A/J mice. When the cells were activated with LPS (lipopolysaccharide from E. coli 0111:B4-Difco Laboratories) only a slight inhibition of virus replication was observed in cultured cells from young A/J mice when compared to the inhibition observed in cultured cells from adult A/J mice (Table 2). In a kinetic study, the macrophages were shown to be more sensitive to IFN-γ than to IFN-α/β (data not shown).

Table 2. MHV3 growth in activated macrophages from A/J mice (a)

mice	activation	MHV3 titer (PFU/ml)
	–	$1.7 ± 0.1 × 10^4$
	LPS	$1.2 ± 0.5 × 10^3$
A/Jy	IFN-γ	0
	IFN-α/β	0
	–	$3.2 ± 0.2 × 10^4$
	LPS	$1.4 ± 0.1 × 10^2$
A/Ja	IFN-γ	$2.0 ± 0.1 × 10^1$
	IFN-α/β	$8.0 ± 0.4 × 10^1$

a. Cultured macrophages from young (A/Jy) and adult (A/Ja) A/J mice were treated for 18 h with LPS (10μg/ml) and IFN-γ or IFN-α/β (100 U/ml) before infection with 0.1 moi of MHV3. Virus titers were determined in the supernatants collected 24 h after the infection. Results are the mean values of 3 separate experiments ± S.D.

Discussion

Previous work on MHV3 showed that both resistance gene(s) controlling the degree of viral replication in target cells such as macrophages, and an intact immune response are required for resistance of adult A/J mice to MHV3 infection (27). We have demonstrated that the genetic dependent resistance of these mice to MHV3 depends on the expression of a T-cell dependent mechanism in which the sensitivity of macrophages to IFN-γ plays a central role (27).

In further support of the role of IFN-macrophage interaction as a crucial step for the resistance to MHV3, we have shown that a nutritionally induced hypercholesterolemia in resistant adult A/J mice caused susceptibility to MHV3 infection. The inhibition of host resistance was a consequence of an impairment of Kupffer cell sensitivity to IFN (29).

Since resistance to MHV3 depends on the age and the genetic background of the animal, we decided to investigate whether or not the mechanism involved in the age-dependent resistance was of the same nature as that involved in the genetic-dependent resistance.

In contrast to the adult A/J mice, the data presented here clearly show that cultured spleen cells from young A/J mice were not capable of producing IFN-γ in vitro (Table 1). Nor did young A/J mice infected with MHV3 show significant amounts of IFN-γ in sera or peritoneal exudates, although the virus replicated to high titers, with the potential to elicit a strong stimulation of the immune system, as observed in the susceptible adult BALB/c mice (27). Also, IFN-α/β were produced in very low levels in the course of the MHV3 infection (Figs. 1 and 2).

The peritoneal macrophages from young A/J mice, in contrast to macrophages from adult A/J mice, failed to synthesize detectable levels of IFN-α/β after stimulation with LPS (Fig. 3) and consequently, only a slight inhibition of MHV3 replication, which was shown to be partially IFN-α/β-dependent (28), was observed (Table 2). On the other hand, the exogenous IFN sensitivity of the macrophages from young A/J mice (Table 2) was comparable to that observed in the macrophages from adult A/J mice (27). Both types of IFN induced an anti-MHV3 state in these cells, although, as in macrophages from adult A/J mice (27), lower doses of IFN-γ were necessary to induce the antiviral state (data not shown).

The data presented here led us to the conclusion that the interaction of IFN and macrophage (mainly IFN-γ) plays a central role in both age- and genetic-dependent resistance to MHV3 infection. In the case of age dependency, susceptibility of young A/J mice is caused by the immaturity of the immune system which is unable to synthesize IFN-γ, whereas the genetic dependency relates to the inability of the BALB/c macrophages to respond to IFN activation (27).

Our previous findings (27, 29) and these presented clearly indicate that the presence of IFN-γ and the integrity of the macrophage functions are essential for resistance to MHV3 infection. They are intimately linked since

the expression of the resistance is, to a large extent at least, a consequence of a T-cell-dependent mechanism of macrophage activation by IFN-γ.

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