

**A genetic analysis of macrophage activation and specific antibodies
in relation to the resistance of heterogeneous mouse populations
to MHV3 infection**

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Summary. The genetically selected high antibody responder mice (H_{III}) are susceptible and the low antibody responder mice (L_{III}) are resistant to the experimental infection with Mouse Hepatitis Virus 3 (MHV3). The mortality rates of the F₁ hybrids and of the F₂ segregants showed the codominance of the susceptible and resistant characters. The direct individual intrapopulation correlation between the induction of antiviral state in macrophages activated by IFN gamma and the resistance to the virus infection, showed that an antiviral state could be induced in resistant mouse macrophages, whereas in susceptible mouse macrophages no restriction of virus replication could be observed. A direct inter- and intrapopulation correlation of pre-existing antibody titres against MHV3 with the mortality and a direct interpopulation correlation of those titres with the mean survival time of susceptible animals was shown. The data indicate, among the mechanisms of resistance against the virus infection, a role of IFN gamma macrophage-activation and of antibodies against MHV3 which may delay the mean survival time in susceptible animals.

Introduction

The mouse hepatitis virus (MHV) strains of *Coronavirus* are responsible for well-known epizootics of enteritis that occur endemically in most mouse colonies. Most of the animals in these colonies were found to have antibodies against different types of MHV, such as MHV3, which have been isolated from a variety of mouse strains under diverse conditions [3, 6, 12, 19, 22]. The MHV3 was isolated by Dick et al. [3] and has been used as a model of viral infection in which resistance varies according to the genetic background of the mouse strain [1, 5, 8, 10, 11, 24]. The resistance pattern of mouse strains has been shown to be not directly linked to the presence of antibodies in sera of animals from contaminated colonies [12].

The virus replication in target cells, the antiviral state induced by interferon (IFN) and the expression of a monokine with procoagulant activity (PCA) have been implicated in the resistance/susceptibility of the genetic homogeneous or heterogeneous mouse populations to the MHV3 infection [1, 2, 4, 7, 17, 18, 23, 24]. We have shown that resistance to MHV3 infection can be a consequence of a T-cell-dependent mechanism, in which the production of IFN gamma and the sensitivity of macrophages to IFN gamma play an essential role [10, 12, 13].

The genetically defined high (H) and low (L) responder selected mice and their segregants and hybrids have been proved to be a useful tool for studying the intervention of multiple alleles in the polygenic control of infectious diseases such as *Salmonella typhimurium* and rabies virus infection [16, 20]. Moreover, the analysis performed in the F₂ heterogeneous population allows the elucidation of the major specific and nonspecific traits implicated in resistance/susceptibility to a given pathogen, as well as that of the environmental and genetic factors that play in these characters.

Mice selected for high (H) and low (L) responsiveness, were obtained by bidirectional selective breeding, and the effect of the polygenic regulation of responsiveness to selection antigens is essentially multi-specific, i.e., selected genes regulate the antibody response to many complex immunogens unrelated to those used during the selective breeding, the high or low states resulting from distinct mechanisms, including the regulatory role of macrophages [21].

The H_{III} and L_{III} mice, although showing no MHV in their tissues, were chronically infected by a MHV strain, and had in their sera distinct levels of antibodies against MHV3. H_{III} mice were shown to be fully susceptible and L_{III} mice fully resistant to the experimental infection with MHV3. One of the mechanisms suggested to be involved in the resistance against this virus infection was the ability of macrophages to develop an antiviral state following IFN gamma activation [2, 23].

The present study was undertaken in an attempt to investigate whether the sensitivity of macrophages to the induction of an antiviral state and the antibody responsiveness characters, correlated individually with the resistance to MHV3 and were influenced at least partially by the same genetic control. Therefore the investigation was directed towards a co-inherited expression of the two traits, aiming to elucidate the complexity of the biological factors that intervene in resistance to infection.

Materials and methods

Mice

High (H_{III}) and low (L_{III}) antibody responder mice from Selection III [21], from the Laboratório de Imunogenética, Instituto Butantan, were used in the experiments. Mice of both sexes were analysed at 2–3 months of age. Interline reciprocal crosses of (H_{III} × L_{III})F₁ hybrids, F₂ segregants and backcrosses (BcH_{III} and BcL_{III}), were analysed as well.

Virus

MHV3 originally obtained from Dr. J. L. Virelizier, Institut Pasteur, Paris, France, was cloned by limiting dilution. One plaque was selected and amplified on L929 cells to serve as the inoculum for future stocks [14] to limit spontaneous mutations. The stocks were always titrated by plaque assay on L929 cells as previously described [18]. Aliquots containing 2×10^5 plaque forming units per milliliter (pfu/ml) were stored at -80°C and used in all experiments. For the study of the resistance to virus infection, the animals were subcutaneously inoculated with 10^3 PFU of MHV3, and mortality was recorded daily for 30 days.

Macrophage cultures

The peritoneal exudates were collected by peritoneal lavage with 5 ml of RPMI, and centrifugated at 200 g for 10 minutes. The peritoneal exudate cells were re-suspended at a concentration of 1×10^6 cells/ml of RPMI containing 10% of fetal calf serum (FCS) and cultured on 96-well plates (100 μl /well). The cells were incubated for 2 h at 37°C in 5% CO_2 and washed three times with medium after vigorous shaking to remove nonadherent cells. Ninety percent of the cells were macrophages as determined by their ability to take up zymosan particles.

Virus replication assay

Peritoneal macrophages were treated with 100 U/ml of murine recombinant IFN gamma (Holland Biotechnology, Leiden, Holland). Twenty-four hours later, activated or non-activated cultures were infected with MHV3 at a multiplicity of infection (m.o.i.) of 0.01, in order to study the inhibition of MHV3 replication. The supernatants of cell cultures were collected at 24 h after infection and tested for the virus titre by plaque assay [18].

For the study of the individual correlation between *in vivo* resistance and *in vitro* inhibition of virus replication by IFN gamma, peritoneal macrophages from H_{III} , L_{III} and F_2 segregants were collected without sacrificing the animals, which were infected with MHV3 a week later. The macrophages were treated with IFN gamma, infected with MHV3 and the supernatants titrated, as described above.

Antibody assays

Mice from the colony were bled from the retro-orbital venous plexus and the individual anti-MHV3 antibody titres in their sera, reported as \log_2 , were expressed by the reciprocal of the highest serum dilution producing a 100% inhibition of cytopathic effect induced by MHV3 on L929 cells [18].

Results

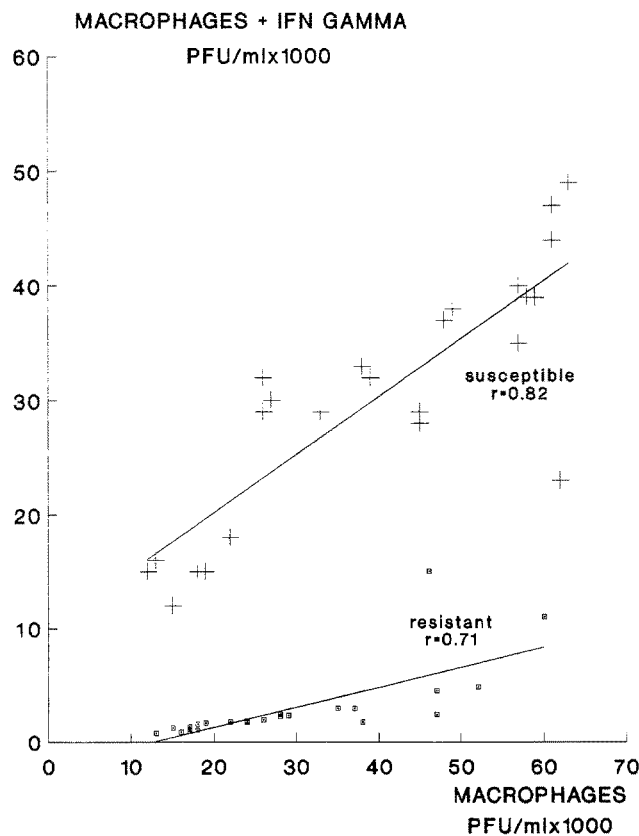
MHV3 replication in IFN gamma activated macrophages from H_{III} , L_{III} , F_1 and F_2 mice

The results in Table 1 show the resistance/susceptibility of mice to MHV3 infection, and indicate that the macrophages from L_{III} or resistant F_1 or F_2 mice were sensitive to the induction of an anti-MHV3 state by IFN gamma. However, under the same conditions, no anti-MHV3 effect could be induced in macrophages from the susceptible H_{III} parental line nor from the susceptible

Table 1. Resistance/susceptibility to MHV3 infection and partial restriction of MHV3 replication in IFN gamma activated macrophages from H_{III} and L_{III} mice, their hybrids (F₁) and segregants (F₂)

Resistance/susceptibility			MHV3 replication in macrophages	
mice			MHV3 titre (PFU/ml)	
lines	n	susceptible (S) resistant (R)	macrophage	macrophage +IFN gamma
H _{III}	13/13	100% S	$2.4 \pm 0.8 \times 10^4$	$1.3 \pm 0.4 \times 10^4$
L _{III}	0/18	100% R	$1.8 \pm 0.8 \times 10^4$	$7.8 \pm 1.5 \times 10^2$
F ₁	6/11	55% S	$3.0 \pm 1.3 \times 10^4$	$2.2 \pm 0.6 \times 10^4$
		45% R	$4.1 \pm 0.7 \times 10^4$	$3.3 \pm 0.7 \times 10^3$
F ₂	24/49	49% S	$4.0 \pm 1.7 \times 10^4$	$2.9 \pm 1.1 \times 10^4$
		51% R	$2.9 \pm 1.3 \times 10^4$	$2.8 \pm 3.2 \times 10^3$

Cultured macrophages were individually collected, treated for 18 h with IFN gamma (100 U/ml) before infection with 0.1 m.o.i. of MHV3. Virus titres were determined in the supernatants collected 24 h after the infection. One week later, the same animals were infected subcutaneously with 10^3 PFU of MHV3, observed for 30 days and the percent (%) of mortality recorded. The MHV3 titres are the average \pm standard deviation



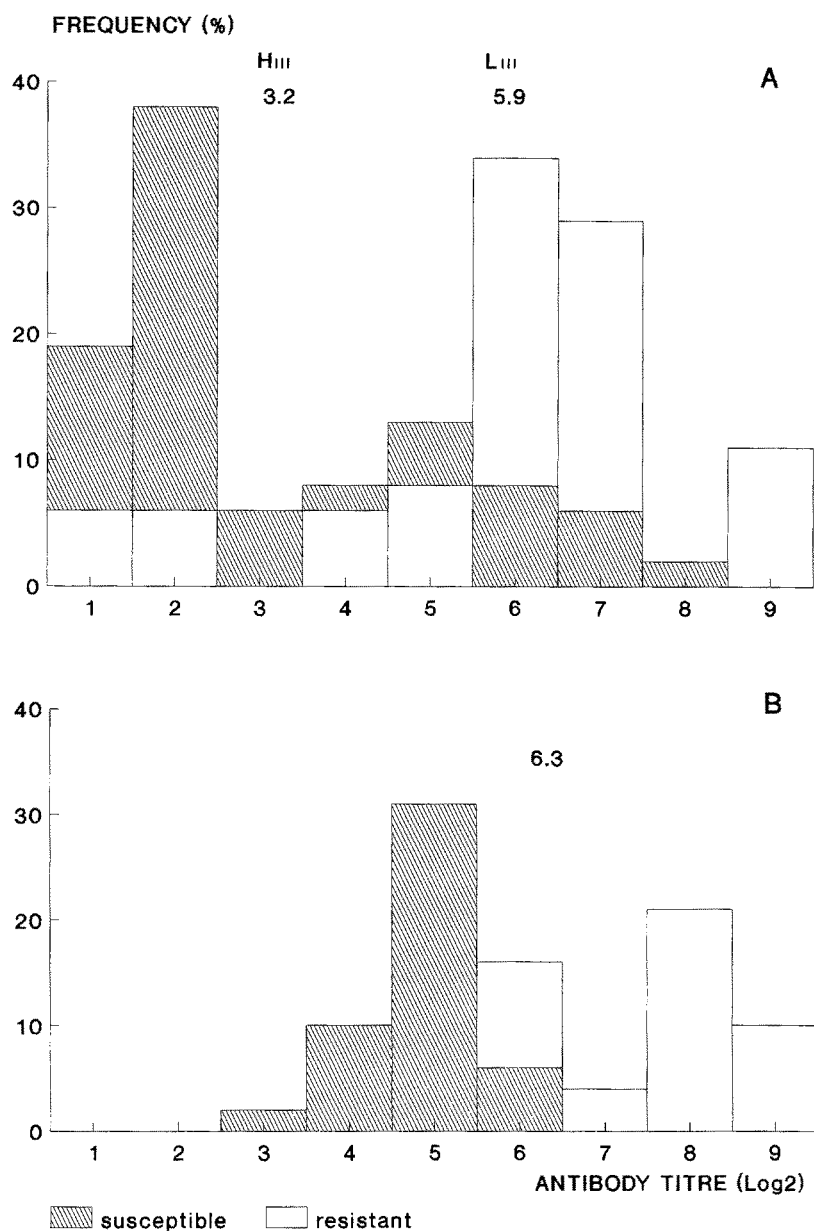


Fig. 2. Histograms of antibody titre against MHV3 in resistant and susceptible mice of the H_{III} and L_{III} lines (A) and F₂ segregants (B). Blank bars: mice resistant to MHV3 infection. Hatched bars: mice susceptible to MHV3 infection. Antibody titres were determined in sera of animals which were then infected subcutaneously with 10³ PFU of MHV3. Arrows indicate the mean log₂ antibody titre

Fig. 1. Correlation between the viral replication in IFN gamma activated and non-activated macrophages from resistant (·) and susceptible (+) F₂ segregants. Cultured macrophages were individually collected and treated for 18 h with IFN gamma (100 U/ml) before infection with 0.1 m.o.i. of MHV3. Virus titres were determined in the supernatants collected 24 h after infection. One week later, the same animals were infected subcutaneously with 10³ PFU of MHV3 and the resistance or susceptibility determined *r* Linear regression

F_1 or F_2 mice. The in vitro treatment with IFN gamma, which induced an anti-MHV3 effect only in resistant mouse macrophages, correlated with the in vivo resistance observed after MHV3 infection.

Figure 1 shows the linear regression analysis of individual values of the induction of antiviral state in macrophages of resistant and susceptible F_2 phenotypes, indicating a significant direct correlation between the resistance and the induction of an antiviral state in the macrophages.

Correlation between antibody responsiveness and resistance to MHV3 infection

The frequency of distribution of individual antibody titres against MHV3 before the experimental infection in resistant and susceptible mice, is shown in the histograms of the Fig. 2. A direct correlation was found from the interpopulation linear regression analysis of resistance and antibody responsiveness against MHV3 in the H and L parental lines and their hybrids, segregants and backcrosses ($r = -0.74$, $p < 0.05$). The survival of mice to MHV3 infection correlated directly with the antibody titres against MHV3 observed in their sera.

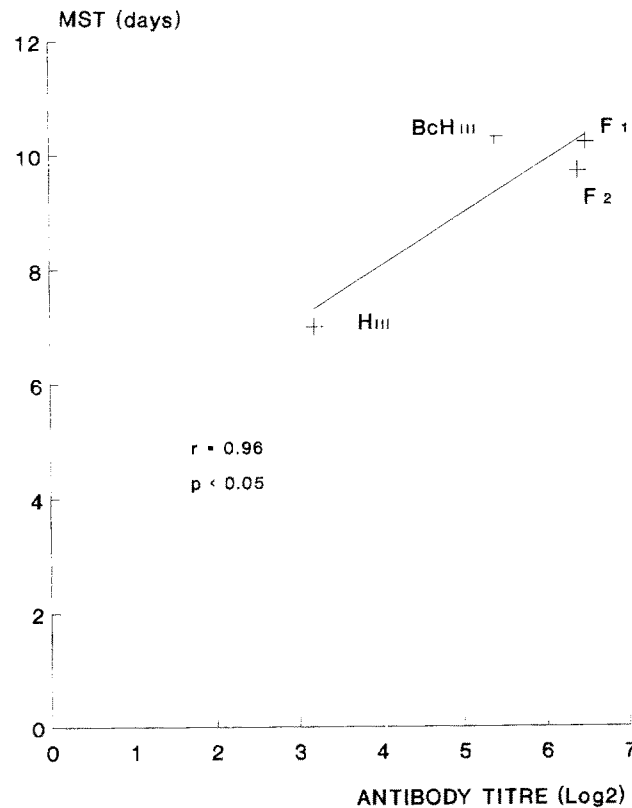


Fig. 3. Correlation between antibody titre against MHV3 and the mean survival time among H_{III}, their hybrids (F_1), backcrosses (BcH_{III}) and segregants (F_2). Antibody titres were determined in sera of animals which were then infected subcutaneously with 10^3 PFU of MHV3. Mean survival time (MST) is reported in days after infection and the mean antibody titre is reported as \log_2 . p Significance, r linear regression

Figure 3 shows the linear regression analysis of the correlation between the mean survival time (mst) and the antibody titres against MHV3 in groups which exhibited mortality. It indicates a significant direct correlation ($r = 0.96$, $p < 0.05$) between the mean survival time of susceptible mice infected with MHV3 and the anti-MHV3 antibody titres.

Discussion

The genetic studies based on the segregation analysis of resistant or susceptible inbred mouse lines to MHV3 infection showed that these characters are under the control of two major loci [4, 9].

More recently, a comparative study performed in genetically homogeneous and heterogeneous mouse populations confirmed these results, and demonstrated the codominance of the susceptibility and resistance characters. The same genes were shown to be present in the isogenic BALB/c (susceptible) and A/J (resistant) lines, as well as in genetically selected H_{III} (susceptible) and L_{III} (resistant) antibody responder mice [2].

The use of H_{III} and L_{III} mice made it possible to analyse the relationship between distinct traits in segregation studies, and demonstrated a direct interpopulation correlation between antibody production to unrelated antigen and mortality to MHV3 infection [2].

The present studies go deep into the intra- and interpopulation analysis of the correlation between resistance to infection and antiviral macrophage action induced by IFN gamma or specific anti-MHV3 antibody production. By studying, individually, the mortality of the parental mouse lines, their F₁ hybrids, F₂ segregants and backcrosses, the anti-MHV3 activity of their macrophages after IFN gamma activation, as well as the titre of antibodies against MHV3, we were able to show: i) a direct individual correlation between the resistance and the ability of macrophages to partially inhibit MHV3 replication after IFN gamma activation (Table 1 and Fig. 1), ii) an inter- and intrapopulation inverse correlation between the mortality and the antibody titres against MHV3 (Fig. 2), iii) an interpopulation direct correlation between the mean survival time of H_{III}, F₁, F₂ and BcH_{III} susceptible animals and the antibody titres against MHV3 (Fig. 3).

The genetic analysis performed in this study, demonstrates that the resistance to MHV3 experimental infection and the ability of IFN gamma-activated macrophages from resistant mice to display an antiviral activity, are submitted to at least partially common genetic control. This brings further support to our previous suggestion that one of the main mechanisms involved in the resistance of mice to the MHV3 infection is based on the ability of their macrophages to restrict the virus replication upon IFN gamma activation [10–13, 15].

Although the participation of antibodies in the resistance to MHV3 infection remains unclear, the data here shown indicate their possible role in resistance mechanisms and in prolongating the mean survival time of susceptible animals.

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