Mouse Hepatitis Virus 3 Binding to Macrophages Correlates with Resistance to Experimental Infection

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Abstract

Mouse hepatitis virus 3 (MHV3) infection of A/J and BALB/c mice has been used as a model of resistance/susceptibility. A/J mice recover from a mild disease after 4–6 days of infection and the BALB/c mice develop an acute hepatitis and die after 3–4 days of infection. In view of studying the MHV3 binding to cells or cell extracts, we performed an enzyme-linked immunosorbent assay-like virus-binding assay, preparing microplates with L929 cells, A/J or BALB/c mouse macrophages and also with proteins extracted from these cells. Higher MHV3 bindings were observed to proteins of BALB/c macrophages than to the A/J ones. The interferon-γ (IFN-γ) activation led to a reduction of MHV3 binding only to proteins of resistant A/J mouse macrophages. Our experiments contribute to the hypothesis that IFN-γ activation of macrophages plays an important role against MHV3 infection by downregulating the expression of viral receptors.

Introduction

Soon after I (Carlos A. Pereira) established my new viral immunology group at the Instituto Butantan, São Paulo, in 1987, I contacted Ivan Lefkovits at the Basel Institute for Immunology to ask him about the possibilities to identify macrophage proteins by 2D-gel electrophoresis. Since I was in Strasbourg and Freiburg, Ivan invited me to Basel to discuss the issue. I was especially pleased by the fact that the word 'macrophage' did not turn off Ivan's interest – the Institute was well known for the Jerneian neglect of macrophages. I was not only surprised by the friendly welcome in Ivan's laboratory, but was also impressed by Ivan's willingness to listen to our explanations – interrupted only by questions concerning the heterogeneity of macrophage populations (and also life span and protein synthetic capacity). We agreed to perform some exploratory experiments, and this initial good contact rapidly turned into an active collaboration. Ivan accepted my PhD student, Maria A. Lucchiari, to his laboratory, and in 1992, we published a paper showing that the pattern of protein synthesized in the liver was profoundly perturbed upon mouse hepatitis viruses (MHV) infection [1], and that some gene products related to the induction of antiviral state in macrophages resistant and sensitive to interferon-γ (IFN-γ) could be readily characterized [2]. These proteomic studies – done years before the term 'proteomics' was invented – represented a turning point in my approach to understanding the basis of anti-MHV state induced by IFN-γ. A few years later, supported by the Swiss National Science Foundation, we have found that IFN-γ was capable of inducing a down-regulation of the main viral receptor only in macrophages originated from resistant mice, explaining at least in part the cellular and molecular basis of mouse resistance to MHV infection [3, 4].

Besides scientific investigation, two other aspects of our interaction became of importance – Ivan's teaching in Brazil and my teaching at the Summer School in Czechoslovakia. Ivan is an enthusiastic and charming teacher and it was a great satisfaction to me and my colleagues in Brazil, when he accepted my invitation to come to São Paulo for an ICRO – UNESCO International Training Course on Virus Immunology in 1990. His lectures on quantitative immunology were of outstanding importance for science development in Brazil. I also had great pleasure in teaching in a Summer School Ivan organized in 1992 in Piestany, Czechoslovakia.

I became convinced during the 15 years of my friendship with Ivan that quantitative biology is indeed a clue to our understanding of science in general. But since *not everything that counts can be counted, and not everything that can be counted counts* (sign hanging in Albert Einstein's office at Princeton), I am sure that at least for one aspect of life the quantitative approach is not good, i.e. for the evaluation of the richness of the uncountable and truly enjoyable scientific or social moments of our friendship.

The MHV belong to the coronavirus family and are responsible for a great variety of murine diseases [5]. Among the MHV, several isolates although with a high degree of similarity regarding their structure and composition, show important differences of physiopathology. Hence, the MHV3 can induce an acute hepatitis depending on the genetic background of the host. Even low doses of MHV3 induce liver necrosis and high mortality among the BALB/c mice, but the A/J mice are capable of clearing the virus showing resistance to the infection [6–10]. The MHVA59 infection, on the other hand, induces encephalitis with chronic demyelination in several mouse strains [11]. Resistance/susceptibility to MHV3 is expressed in a cellular level by a partial restriction of MHV3 replication observed only in A/J mouse macrophages, mainly after IFN-γ activation. In a molecular level, we have indications that the expression of viral receptors plays an essential role in the restriction of replication. Only resistant A/J macrophages show a decreased expression of the gene encoding for the principal viral receptor (Bgp[1a]) [3]. Our quantitative biology studies [1, 4] showing upregulated and downregulated proteins and genes in resistant and susceptible macrophages opened a great perspective for investigations of the biological role of these cells and have also confirmed the downregulation of an MHV3 receptor gene by IFN-γ activation [4].

Since the viral infection normally requires the adhesion of viral particles to the cell surface and internalization of the viral genome, several studies were carried out to demonstrate and characterize viral receptors on target cells. Several viruses were shown to enter the cells by using more than one receptor and isolates of MHV are capable of infecting different cell lineages using different receptors [12] by fusion and leading to cell lysis. MHV binds to a glycoprotein on the cell membrane through the 180-kDa trimer of their S-glycoprotein resulting in a fusion of their envelope with the cell membrane [13]. The main MHV receptors were characterized as glycoproteins of the carcinoembrionic antigen – the biliary glycoproteins Bgp[1a] and Bgp[2] [14]. Hence, during the MHV infection, the receptor recognition by the viral S-glycoprotein confers the specificity and tissue tropism, although the subsequent steps like the membrane fusion and replication may also contribute to the degree of resistance/susceptibility [15]. Studies aiming to clarify the interaction between virus and cell-membrane proteins may represent an outstanding contribution to the understanding of resistance mechanisms.

Materials and methods

MHV3 and MHVA59 strains were cultivated and titrated by plaque assay on L929 cells and stored in aliquots with $1–5 \times 10^5$ plaque-forming units (PFU)/ml at $-80\,°C$. A/J and BALB/c spf mice, with age ranging from 8 to 12 weeks, were bred in the animal unit of the Instituto de Biociências II, Universidade de São Paulo. Animals were periodically sacrificed and the peritoneal exudate, serum and liver tissue samples obtained. No animal was found to have MHV in the liver or in the peritoneal exudate. Macrophages were prepared from peritoneal exudate cells collected by lavage with DMEM containing 10% fetal bovine serum and cultured in 96-well microplates at a concentration of 2×10^5 cells/100 μl. The cells were incubated for 2 h at $37\,°C$ in 5% CO_2, washed three times with DMEM after vigorous shaking to remove nonadherent cells and used in the experiments. For the binding assays, cells were cultivated in microplates, and macrophages from A/J and BALB/c mice were activated with 100 U/ml of IFN-γ for 18 h. Protein extracts were prepared by using TRI-Fast reagent (Gibco, BRL, Gaithersburg, MD, USA) as previously described [3]. The protein concentration was evaluated by the Protein Assay kit (Pierce, Rockford, IL, USA), and the preparations showed values ranging from 0.1 to 2.0 mg/ml. The enzyme-linked immunosorbent assay (ELISA) for determination of MHV3 binding was performed as previously described [3]. For the preparation of hyperimmune sera, MHV3 or MHVA59 suspensions were ultraviolet-inactivated for 30 min and used to infect groups of 10 mice with four weekly doses of 100 PFU. Animals were then bled and sera obtained and titrated for the presence of antibodies against MHV using a neutralization assay.

Results and discussion

The altered expression of a viral receptor in target cells constitutes a hypothesis to explain the genetic resistance mechanism of hosts to MHV infection [3]. It has also been shown that MHV3 replication attains higher titres in cells originated from susceptible mice than in those originated from resistant ones [8–10]. Also, only macrophages from resistant mice are capable of developing an antiviral state upon IFN-γ activation [9, 16].

Here we show data of a quantitative study in view of investigating the magnitude of MHV binding to proteins of A/J and BALB/c macrophages or L929 cells. We have observed that the MHV3 and MHVA59 binding to L929 cells was of the same magnitude and dependent on the amount of hyperimmune sera used. Even low amounts of virus used in the assay were enough for detection in the ELISA (Fig. 1).

Based on these assays, we establish that 100 PFU of virus and the hyperimmune sera diluted to 1/800 would be suitable for the ELISA. Indeed, when macrophages were used in the assay, good results of virus binding could be observed (Fig. 2). In this situation, low optical density (OD) values were obtained when normal sera or no virus were used. As expected, higher values of virus binding were detected when BALB/c macrophages were used, which are in direct correlation with the previous description of
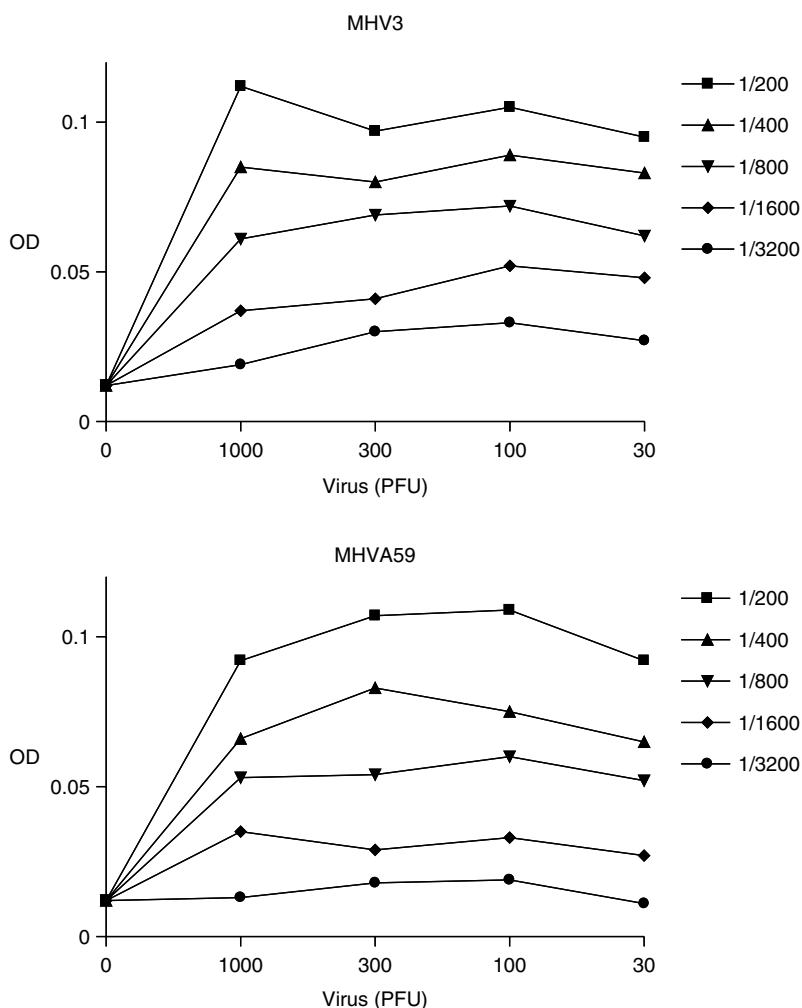
MHV3

MHVA59

Figure 1 Kinetics of MHV3- and MHVA59-binding assay to L929 cells using hyperimmune sera at different dilutions. The virus binding was assessed by enzyme-linked immunosorbent assay and the data expressed in optical density (OD). PFU, plaque-forming units.
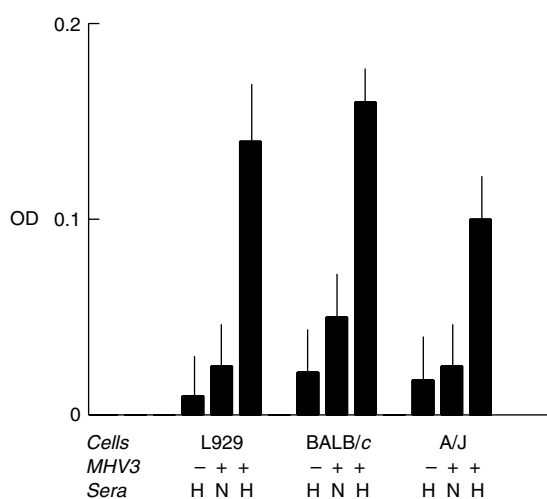

Figure 2 MHV3-binding assay to L929 and BALB/c or A/J macrophages using normal (N) or hyperimmune (H) sera diluted to 1/800. The virus (100 PFU) binding was assessed by enzyme-linked immunosorbent assay and the data expressed in optical density (OD) represent the average of three independent experiments. PFU, plaque-forming units.

higher ability of this virus to multiply in susceptible BALB/c macrophages [3, 8]. As expected also, the virus binding to L929 cells was of high magnitude correlating with the virus ability to grow in these cells that are commonly used for virus growth and titration.

OD obtained in the assays of virus binding to cells (Figs 1 and 2) show relatively low levels that can be explained by the low expression and recycling of receptors that are present on the cell membrane. In addition, macrophages are known to have large amounts of immunoglobulin Fc portion receptors, which lead to a higher degree of nonspecificity in this type of assay. Also, the physiological state of the cells may very much influence the binding results obtained.

In order to confirm the previous data and also circumvent the above-described difficulties, we further analysed the MHV binding by using, instead of whole cells, proteins extracted from the membrane of A/J and BALB/c macrophages. Also, in view of the central role of IFN-γ activation of macrophages that has been associated with virus resistance, we analysed the MHV binding to proteins extracted from macrophages activated by IFN-γ.
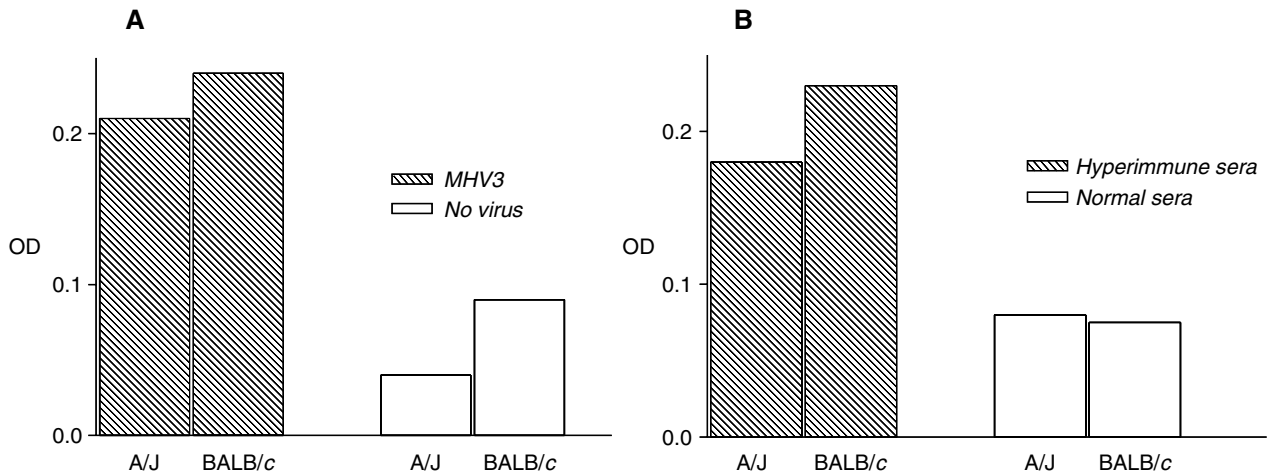
Figure 3 Control experiments of MHV3-binding assay to protein extracts (16 μg) from BALB/*c* or A/J macrophages using normal (N) or hyperimmune (H) sera diluted to 1/800 (A) or in the presence (100 PFU) or absence of MHV3 (B). The virus binding was assessed by enzyme-linked immunosorbent assay and the data expressed in optical density (OD) represent the average of three independent experiments. PFU, plaque-forming units.

Control experiments are shown in Fig. 3. For proteins extracted from BALB/*c* mouse macrophages, the virus-binding OD values were of 0.24 ± 0.02 and when no virus was used in the assay the values were of 0.09 ± 0.01. For proteins extracted from A/J mouse macrophages, these values were, respectively, of 0.21 ± 0.03 or 0.04 ± 0.02. When the hyperimmune and normal sera were compared, the virus-

binding OD values were, respectively, of 0.23 ± 0.02 and 0.075 ± 0.01 for BALB/*c* macrophage proteins and 0.18 ± 0.01 and 0.08 ± 0.02 for A/J macrophage proteins. These data gave us a reasonably good confidence to proceed with our binding assays.

As shown in Fig. 4, we have observed that only two proteins of A/J mouse macrophages, we observed a
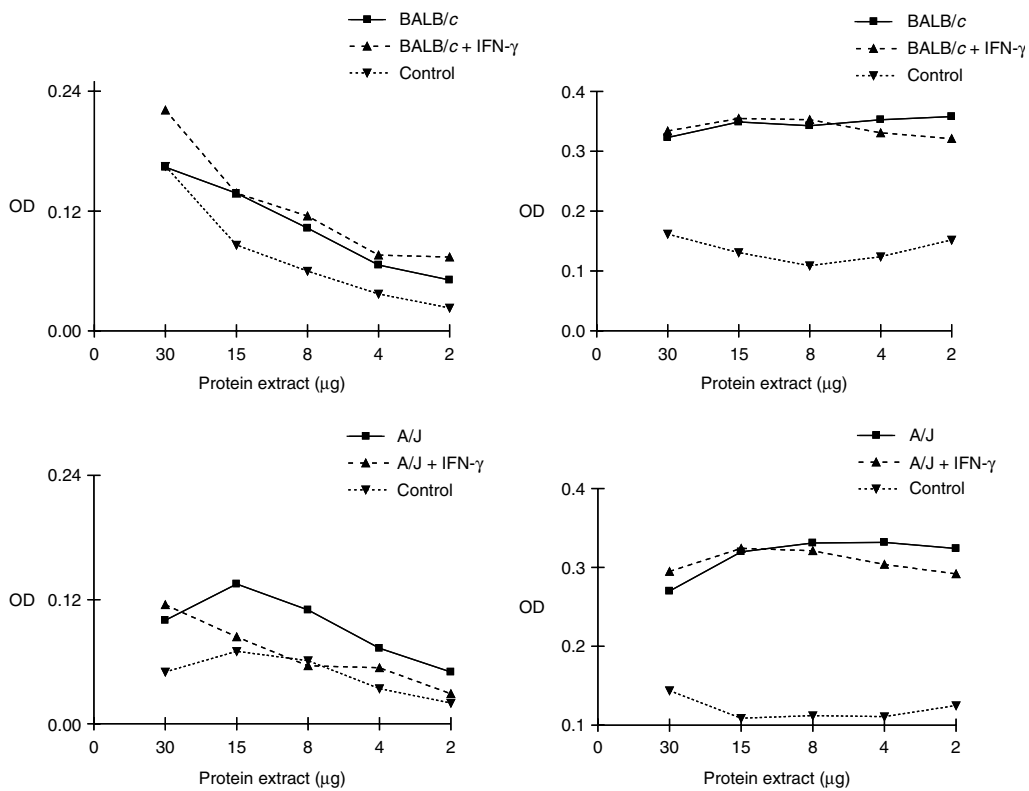

Figure 4 Kinetics of MHV3- and MHVA59-binding assay to different concentrations of proteins extracted from BALB/*c* and A/J macrophages activated or not by interferon-γ (IFN-γ) using hyperimmune sera diluted to 1/800. The virus (100 PFU) binding was assessed by enzyme-linked immunosorbent assay and the data expressed in optical density (OD). PFU, plaque-forming units.

decreased MHV3 binding when they were activated with IFN-γ. For the BALB/*c* macrophages, the IFN-γ activation did not lead to a decreased MHV3 binding. It correlates with previous data showing a partial restriction of MHV3 replication only in resistant A/J mouse macrophages upon IFN-γ activation [3, 9]. For the MHVA59, a coronavirus that induces a quite distinct pathological process, we do not observe the same pattern of resistance/susceptibility for A/J and BALB/*c* mice as we do for the MHV3. In this case, we also did not observe a difference in virus binding to protein extracts from macrophages, what reinforces our hypothesis of relationship between MHV3 binding, restriction of replication and resistance in A/J and BALB/*c* mice.

In conclusion, our data on MHV3 binding to proteins of resistant and susceptible mouse macrophages suggest that not only the virus binding to receptors on the cell membrane may have consequences for the magnitude of virus multiplication, but also that the expression of these viral receptors can be downregulated by IFN-γ activation. These observations are in accordance with our previous data on partial restriction of virus replication in IFN-γ-treated macrophages and to the pattern of resistance/susceptibility of mice to MHV3 infection.

Acknowledgments

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