Role of Macrophages and Interferon in Natural Resistance to Mouse Hepatitis Virus Infection

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1 Introduction

Among the many models of experimental viral infections, the mouse hepatitis virus (MHV) model offers a number of advantages for investigation of the host-virus relationship:

1. MHV infection is a naturally occurring infection in the mouse (*Rowe* et al. 1963); *Broderson* et al. 1976; *Ishida* et al. 1978) and thus is not an artificial model.

2. In the case of mouse hepatitis virus type 3 (MHV-3), the natural resistance is observed to various degrees according to the mouse strain considered (*Virelizier* and *Allison* 1976), with either full susceptibility, full resistance, or semiresistance being present. This provides the opportunity to make correlations with parameters of the immune response in a more precise manner than in other models where only two situations (susceptibility and resistance) are observed.

3. The mouse strains showing variable degrees of susceptibility to MHV-3 (namely, A/J, C3H/He, and C57/BL-6) are well-known inbred strains whose biologic parameters have been thoroughly investigated.

4. MHV infections have been among the first models in which the role of macrophages in host defense have been investigated after the pioneering studies of F. Bang and his colleagues (*Bang* and *Warwick* 1960; *Shif* and *Bang* 1970).

5. MHV-3 induces a very easily recognizable cytopathic effect in mouse macrophage cultures by fusing infected macrophages into multinucleated giant cells (*Malucci* 1965). The appearance of this cytopathic effect closely parallels the intensity of viral replication and thus provides a useful tool for in vitro studies in macrophages from different strains of mice (*Virelizier* and *Allison* 1976).

6. MHV-3 infection is so far the only coronavirus model in which the protective role of interferon in vivo has been demonstrated (*Virelizier* and *Gresser* 1978).

7. Finally, the modification of immune responsiveness during acute or persistent infections has been investigated (*Virelizier* et al. 1976).

These various aspects of MHV-3 infection make this model a useful tool to investigate the mechanisms underlying the natural resistance to this type of virus.

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2 The Pathogenesis of MHV-3 Infection in Various Strains of Mice

MHV-3 is a member of the coronavirus group (*McIntosh* 1974) and was first described in 1956 (*Dick* et al. 1956). Although oral administration is not followed by death, parenteral administration always leads to death in outbred and most inbred mouse strains, regardless of the route of injection (intravenous, intraperitoneal, subcutaneous, or intracerebral). MHV-3 causes a systemic infection, with very high virus titers being found in all organs, including liver, lymphoid organs, brain, kidney, and blood (*Piazza* et al. 1967). After the first cycles of replication within Kuppfer cells, the virus can be visualized by electron microscopy in hepatocytes and in the Disse space between Kuppfer cells and hepatocytes (*Miyai* et al. 1963). During acute infection, tissular lesions are mainly observed in the liver, where a massive necrosis, leading to death within 5 to 10 days, is observed. However, lymphoid organs also show profound modifications, especially the thymus where a rapidly progressing destruction of the cortical, but not the medullary, area is observed as early as 48 h after intraperitoneal virus infection (*Virelizier* et al. 1976). By day 4 after infection, the thymus cortex is completely depopulated. A typical lesion observed in all MHV-3-infected organs is giant cell formation, in which MHV-3 antigens can be found by immunofluorescence. By day 5, necrotic lesions are widely disseminated, especially in the liver (almost entirely destroyed), in lymphoid organs (particularly in the spleen where the marginal zone shows giant cells and necrosis), lymph nodes, and Peyer patches. This lethal, systemic infection is observed in most mouse strains, including Swiss, BALB/C, DBA2, and C57/BL 6 and 10, in which 100% of infected mice die.

In contrast, 100% of mice of the A/J strain survive after parenteral administration of MHV-3, whatever the amount or the route of inoculation (*Virelizier* et al. 1975). Instead of the regular increase observed in susceptible mice until death, MHV-3 replication is efficiently controlled in all A/J organs as early as day 2, and the virus cannot be recovered from any organ by day 7 (*Leprevost* et al. 1975a). The A/J thus proved to be a strain fully resistant to MHV-3.

In the course of our study on the in vivo susceptibility of various mouse strains to MHV-3, we soon discovered that old (10–18 weeks) C3H/He mice resist the acute phase of the disease but develop a chronic illness with progressive neurologic signs (*Virelizier* 1972). A correlation was observed between the clinical evolution and the titer of virus tested during the acute phase of the infection. C3H/He mice show intermediate virus titers in all organs tested by day 4 as compared to susceptible or fully resistant strains (*Leprevost* et al. 1975b). Since C3H/He and A2G mice both develop signs of a chronic neurologic disease, we have investigated the neuropathologic effects of persistent MHV-3 infection in these two strains (*Virelizier* et al. 1975). The majority (75%–95%) of mice from these strains appear normal untill 3–12 weeks after intraperitoneal injection. They then begin to look ill, showing failure to thrive, oily hair and loss of activity. Progressive neurologic signs appear as the mice show incoordination and paresis of one or more limbs. Organ suspensions from chronically sick mice, when injected into susceptible recipients, induce a fulminant hepatitis and a highly virulent virus can be recovered. This indicates that the chronic neurologic disease is associated with the persistence of MHV-3 in its virulent, hepatotropic state. Thus, intraperitoneal inoculation of a nonneurologically adapted strain of MHV-3 into semiresistant mice provides a new model of persistent infection in which persistence is due to a peculiar response of the host but not to an unusual adaptability of the virus. Whereas A2G mice develop mostly a chronic chorio-epen-

dymitis leading to hydromyelia and hydrocephalus, C3H mice suffer mostly from diffuse vasculitis found in kidney, liver, spleen, brain, and spinal cord. As described previously (*Virelizier* et al. 1975), vessel walls show polymorph infiltration, leukocytoclasis, and often fibrinoid necrosis. Both arteries and veins of large and small diameter are affected, but damage is more often seen in veins and venules than in arteries and arterioles. There is also perivascular infiltration of inflammatory cells, sometimes of an almost granulomatous character. Nearby, long ascending and descending tracts are frequently damaged. Both myelin and axis cylinders are destroyed – always in close association with proliferative, perivascular lesions. Viral antigens and immunoglobulins can be found in the walls of damaged vessels. Since no viral antigen is detected in neural cells, it is likely that neurologic damage is the consequence of the systemic vasculitis. Infective virus, found (although at very low titers) in most organs tested by transfer to susceptible recipients, possibly persists within vessel walls. This is clearly different from the situation observed during infection with a neurotropic strain of mouse hepatitis virus (JHM), in which demyelination has been shown to be the direct result of infection of oligodendrocytes (*Weiner* 1973; *Lambert* et al. 1973). Systemic amyloid is found in C3H mice chronically ill for more than 6 months and is a probable consequence of chronic immunologic stimulation associated with virus persistence in the face of an active host response (*Virelizier* et al. 1975).

Thus, various types of host-virus relationship can be observed during MHV-3 infection. As discussed earlier (*Virelizier* 1979b), genetic differences of host resistance are responsible for the different courses observed, ranging from acute disease (either mild or lethal) to persistent viral infection. It is thus important to know which host defense mechanism(s) underlie the genetically determined course of the disease in different inbred strains of mice.

3 Virus Restriction in Individual Macrophages as a Possible Obstacle to MHV-3 Spread: The Blood-Organ Barrier

Macrophages can play a vital, although nonspecific role in viral infections. An elegant way to investigate the antiviral role of macrophages in vivo is to inject mice with colloidal silica, which after phagocytosis induces intracellular breakdown of lysosomes leading to death of the macrophages (*Allison* et al. 1966). Using this technique, *Zisman* et al. (1970) have shown that impaired macrophage function is associated with increased spread of herpes simplex virus to the liver parenchyma, with hepatitis and early death resulting. Macrophages may act within virus-induced lesions, since mononuclear cells appear to be selectively recruited into sites of virus multiplication, as shown during Sindbis virus infection (*McFarland* et al. 1972). Macrophages, especially Kuppfer cells lining the sinusoid in the liver, constitute a functionally complete barrier between blood and hepatocytes. *Mims* (1964) has summarized evidence that viruses introduced into the blood stream are taken up by macrophages, where they can be visualized by immunofluorescence. If Kuppfer cells are able to restrict virus replication, the virus will not be able to reach adjacent hepatocytes. Macrophages would thus be ideally suited to form an efficient obstacle to virus spread from blood to organ tissues and could be key cells in natural resistance if their individual resistance to virus replication was genetically determined.

Evidence exists that indicates that natural resistance to various types of MHV is ex-

pressed at the level of individual macrophages. *Bang* and *Warwick* (1960) found that adult mice of the Princeton (PRI) strain are susceptible to lethal infection with the Nelson strain of MHV (MHV-2), whereas the C3H strain is resistant. Mating experiments show that susceptibility to infection segregates as a single Mendelian dominant genetic factor. Cultures of hepatic or peritoneal macrophages from mice of susceptible strains support multiplication of MHV-2, whereas macrophages from resistant mice do not. Thus inherited resistance is manifested in vitro in the absence of any specific immune response. This resistance, however, is not a general one to all types of viruses. For example, the PRI mice, which are resistant to arboviruses (*Sabin* 1954), are highly susceptible to MHV-2, and the two resistance factors segregate independently among the offspring of hybrids (*Kantoch* et al. 1963). At an early stage of infection with MHV-2, viral antigen is demonstrable by immunofluorescence in sinusoidal lining cells of the liver and is more prominent in susceptible than in resistant mice (*Taguchi* et al. 1976). In vitro interactions of MHV-2 and macrophages have been investigated by *Shif* and *Bang* (1970). The virus is absorbed equally well to resistant and susceptible cells, but it persists without multiplication in resistant cells, while it disappears into eclipse phase in the susceptible cells and subsequently replicates. Thus a true restriction of virus replication appears to operate. These original observations from *Bang* and his colleagues have been extended in other MHV models. *Allison* (1965) reported that macrophages taken later from neonatally thymectomized animals support the multiplication of the avirulent MHV-1, whereas the virus multiplies to a very limited extent in macrophages from intact adult animals. Using the highly virulent MHV-3, we have shown that there is a precise correlation between the in vitro ability of the virus to grow in macrophages from a given strain of mice and the in vivo course of the disease (*Virelizier* and *Allison* 1976) in the three types of host-virus relationships observed in this model. Thus very little or no virus replication is observed in macrophages from A/J mice in which MHV-3 induces a mild disease with 100% recovery. In cultures of macrophages from susceptible strains (C57/BL, DBA2, etc.), MHV-2 replicates freely, with giant cell formation (see Figure 1), in parallel with the fulminant hepatitis seen in vivo leading to death in 100% of animals. In contrast to this full susceptibility or resistance, macrophage cultures from a strain of mice in which persistent infections occur show intermediate susceptibility, as judged by the intensity of the cytopathic effect, the presence of viral antigens in the cytoplasm, and the levels of viral replication. Thus a genetically controlled "semiresistance" to MHV-3 is manifested in individual macrophages by an intermediate level of viral replication. That macrophages have an antiviral role in vivo during MHV infections has been further suggested by cell transfer experiments. *Stohlman* et al. (1980) have shown that young susceptible mice can be protected from the encephalitis induced by intracranial inoculation with the JHM strain of MHV after transfer of adherent spleen cells from adult resistant animals. Adherent cells were protective even after depletion of T cells before transfer. In contrast, in the MHV-3 model, transfer of resistance from adults to newborn is obtained only when both adherent and nonadherent spleen cells are transferred. Transfer of resistance to MHV-3 is obtained, however, when peritoneal cells are associated with adherent spleen cells (*Levy-Leblond* and *Dupuy* 1977). A third population of bone marrow cells enhances the protective ability of transferred spleen cells (*Tardieu* et al. 1980).

This array of evidence strongly implicates macrophages in natural resistance to MHV and suggests that these cells, being primary targets for the virus, may represent a first line of antiviral defense. However, the precise mechanism(s) through which

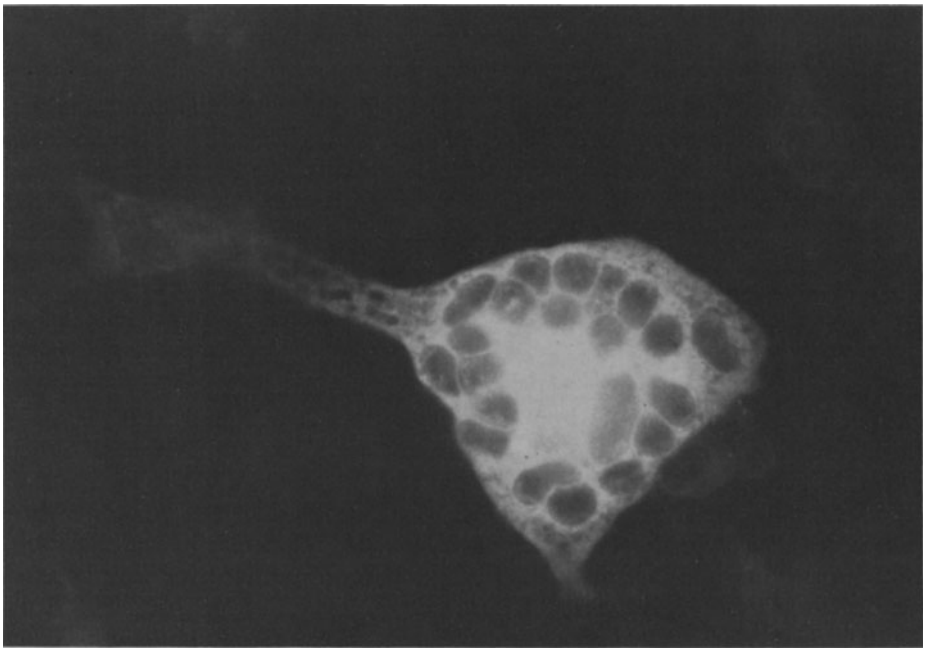

Fig. 1. Multinucleated giant cell observed in culture of adherent peritoneal cells of C57/BL mice infected 48 h previously with MHV-3 (Mill-Hill strain). Note the process of fusion of a single macrophage into a giant cell. Direct immunofluorescence shows viral antigens in cytoplasm, but not in nuclei

macrophages exert this antiviral role in vivo is not yet clear. Restriction of virus replication may not be the only, or even the main, macrophage role during infection. Apart from inactivating virus following endocytosis, mononuclear phagocytes may interfere with virus multiplication and spread through many mechanisms. Firstly, their ability to process and present antigens may facilitate the induction of the specific antiviral cell-mediated response (*Cowing* et al. 1978). Secondly, they may act as nonspecific cytotoxic effector cells able to destroy virus-infected cells, as is suggested in the Semliki Forest virus model (*Rodda* and *White* 1976). Thirdly, macrophages may be major producers of soluble mediators (*Allison* 1978) during viral infections, including interferon (*Glasgow* and *Habel* 1963). *Hirsch* et al. (1970) have shown that peritoneal macrophages transferred from adult CBA mice protect suckling syngeneic mice from intraperitoneal infection with herpes simplex virus. The enhanced resistance provided by stimulated macrophages was associated with more efficient intracellular destruction of virus and with greater production of interferon. Infected macrophages from suckling mice did not produce detectable interferon. Thus, the maturation of antiviral host defense during the first weeks of life correlates with a greater ability of individual macrophages to restrict herpes simplex virus and to produce interferon. Since macrophages are preferential host cells for the first cycle of MHV-3 replication, they could play a decisive antiviral part by producing an early interferon response during infection.

4 Protective Role of Early Interferon Production During MHV-3 Infection

Decisive evidence for a protective role of the production of endogenous interferon during viral infections has long been lacking, although administration of exogenous interferon had been shown to confer a marked protection of virus-infected animals under precise experimental conditions (*Finter* 1973; *Baron* 1973). Using an inverse approach, it has more recently been possible to neutralize in vivo the antiviral effects of interferon, a procedure which provides an elegant way to selectively abolish a single component of the host response, namely, endogenous production of interferon during viral infections. *Fauconnier* (1970) has shown that inoculation of sheep anti-mouse interferon serum of low potency was associated with a more rapid onset of disease and an increased mortality in mice infected with Semliki Forest virus. Using a potent antiserum to mouse fibroblast interferon, *Gresser* and his colleagues have provided decisive evidence that interferon plays a major antiviral part in many experimental models. Administration of this antiserum to mice infected with encephalomyocarditis virus resulted in the multiplication of virus to high titers in visceral organs, rapid onset of disease, and early death (*Gresser* et al. 1976a). Similar results were obtained in mice infected with Herpes simplex, Moloney sarcoma, vesicular stomatitis, and Newcastle disease, but not in mice infected with influenza viruses (*Gresser* et al. 1976b).

In the MHV-3 model we have shown that interferon is produced during infection in both resistant (A/J) or susceptible (C57/BL) mice, with peak production of about 1000 units observed 24–48 h after intraperitoneal administration of the virus (*Virelizier* et al. 1976). On the other hand, we have reported that "immune" interferon produced by stimulated leukocytes can inhibit MHV-3 replication in macrophages cultures from susceptible mouse strains (*Virelizier* et al. 1977), an observation which opens up the possibility that immune interferon, as a soluble mediator of specifically sensitized leukocytes, may strengthen the macrophage barrier by inducing an antiviral state in individual cells. *Virelizier* and *Gresser* (1978) have shown that injection of an antiserum to mouse fibroblast interferon before MHV-3 administration accelerates the onset of death in C57/BL mice and induces almost 100% acute mortality in C3H mice, which usually do not die of acute disease (see Fig. 2). Anti-interferon serum administration also causes death in 4- and 6-week-old mice of the resistant strain A/J. These observations demonstrate an important protective role of the endogenous production of "classical" interferon and also show that the efficiency of interferon is variable according to the mouse strain considered. In fully susceptible C57/BL mice, death is observed whether or not interferon has been neutralized, suggesting that interferon production is not sufficient by itself to protect mice and that other factors must be involved which are also critical for recovery. In fully resistant A/J mice, potentiation of infection is clearly noted in young animals (4- to 8-week-old). However, 10- to 22-week-old A/J mice resisted infection whether or not anti-interferon serum was administered. This suggests that endogenous interferon production is essential for protection during the maturation process which transforms susceptible suckling A/J into fully resistant adults during the first 2–3 weeks of life. From then on, another resistance mechanism seems to take over. The possibility exists that A/J macrophages (and/or other cells) have irreversibly acquired the ability to restrict virus replication by the 10^{th} week of life; this maturation process could itself be the consequence of previous production of interferon due to past exposure to bacterial infection in animals which

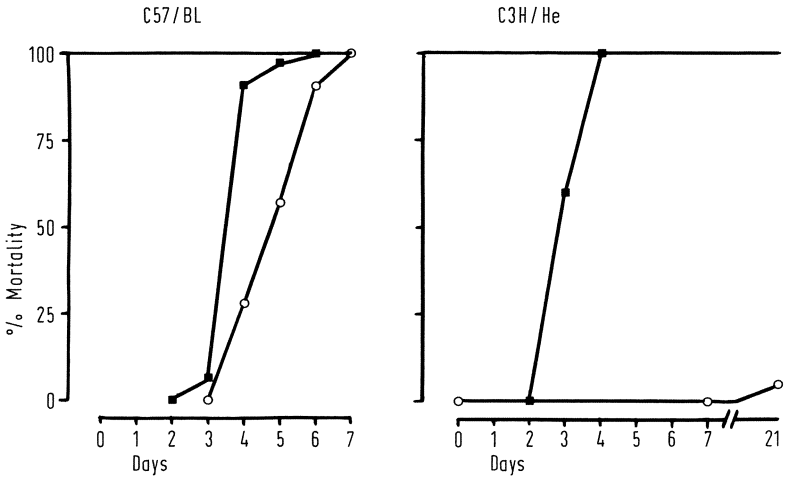

Fig. 2. Mortality observed in mice of the C57/BL (susceptible) or C3H/He (semiresistant) strain infected intraperitoneally with MHV-3 one h after administration of either normal serum (O) or sheep anti-mouse fibroblast interferon (■)

have not been raised under germ-free conditions. In semiresistant C3H mice, interferon production is clearly a critical host defense factor, since injection of anti-interferon serum leads to fulminant hepatitis, whereas control mice develop a chronic neurologic disease associated with virus persistence at very low titers as described above. It should be emphasized that neutralization of circulating interferon during the first 48 h of infection was enough to potentiate the infection, even when interferon was present in the serum at 72 h. This introduces the notion that the fate of MHV-3 infection (chronic disease versus acute hepatitis) is decided very early during infection at a stage when the specific immune response is unlikely to be already operative. Finally, we observed that injection of antiserum into C3H mice chronically infected (2–4 months after virus administration) did not appear to exacerbate the disease. It should be underlined, however, that our antiserum is directed against type I ("classical") virus-induced interferon, so that these results do not preclude a role of type II ("immune") interferon in the chronic disease. Indeed, we have shown that very low titers of interferon circulate in persistently infected C3H mice (*Virelizier* et al. 1976), but the antigenicity of this interferon has not been investigated.

The exact mechanism(s) through which interferon exerts its antiviral activity during MHV-3 or other virus infections is not known. Interferon may induce an antiviral state in every cell of the body or mostly in macrophages. Alternatively, interferon may act through activation of immunologic effectors of immunity (*Virelizier* 1980) such as NK cells (*Gidlund* et al. 1978), cytotoxic macrophages (*Schultz* and *Chirigos* 1979), or cytotoxic T lymphocytes (*Lindahl* et al. 1972).

5 Respective Roles of Various Parameters of the Anti-MHV Response

Although it is clear that both interferon and macrophages have a protective role during MHV infection, other factors are possibly involved in host defense mechanisms. The hu-

moral response, however, is unlikely to play a major part, since transfer of serum from immunized A/J mice is not effective in protecting susceptible DBA2 mice against challenge with MHV-3 (*Le Prevost* et al. 1975a). The role of cell-mediated immunity is probably important, in view of the potentiation of infection observed in mice subjected to immunosuppressive drugs. The antiviral role of specific cytotoxic T lymphocytes has not yet been investigated in any MHV model. The possible role of soluble mediators of specifically sensitized T lymphocytes deserves consideration. That viral antigens can induce the secretion of immune interferon has been established with other viruses (*Epstein* et al. 1972; *Valle* et al. 1975) but not yet with MHV antigens.

Various types of immunomanipulation of the host have been used in the hope of obtaining evidence on mechanisms of antiviral defense to MHV in vivo. MHV-2 infection is potentiated by treatment with cortisone (*Gallily* et al. 1964) or cyclophosphamide (*Willenborg* et al. 1973). MHV-3 infection is potentiated by X-ray irradiation or antilymphocyte serum treatment (*Dupuy* et al. 1975). The results of this type of experiment are usually taken as evidence that specific cell-mediated immunity is of major importance in resistance. Other interpretations are possible, however, since immunosuppressive agents do not affect only T lymphocyte functions. For example, we have shown that cortisone, antithymocyte serum, or X-ray irradiation profoundly decreases the production of Sendai virus-induced leukocyte interferon in normal or nude mice (*Virelizier* 1979b), indicating that a non-T-cell-dependent mechanism of host defense is impaired after immunosuppressive treatment. Interestingly, X-ray irradiation breaks resistance to MHV-3 in normal mice, but not in immune A/J mice (*Dupuy* et al. 1975). This is reminiscent of our observation that, whereas nonspecific induction of leukocyte interferon is radiosensitive, the secondary-type secretion of immune interferon by sensitized lymphocytes is radio resistant (*Virelizier* and *Guy-Grand* 1980).

Cell transfer experiments usually involve separation of "macrophages" from "lymphocytes" through cell adherence. Certain cells of the monocyte lineage, however, do not readily adhere to glass or plastic. Promonocytes are nonadherent, nonphagocytic cells, and usually contaminate suspensions of nonadherent lymphocytes. Promonocytes are good candidates for effectors of natural killing (*Lohmann-Mattes* et al. 1979) and leukocyte interferon production (*Virelizier* 1979b). Clearly, cell transfer experiments will benefit from future progress in cell separation and identification.

The immunosuppression observed during viral infections has often been considered as a possible way for a virus to escape the host's immune response. Immune responsiveness is indeed modified during MHV-3 infection (*Virelizier* et al. 1976). However, the role of timing was shown to be critical during acute infection: infecting mice before unrelated antigen (sheep erythrocytes) administration led to immunodepression, whereas simultaneous injection of virus and antigen led to immunostimulation. If this immunologic enhancement also applies to antigens of the infecting virus, the modifications induced by the infection may in fact enhance antiviral responses and thus be beneficial for the host.

6 Which Resistance Factor (S)$_1$ is Genetically Controlled?

As pointed out by *Allison* (1965), inherited differences in susceptibility are sometimes manifested in cell cultures and are thus a property of individual cells. Nevertheless, there

may be a synergistic effect with immune mechanisms of the host, so that impairment of the latter greatly increases susceptibility. This is clearly the case in the MHV models, where genetic resistance in individual macrophages is observed in vitro while in vivo resistance can be broken by various immunosuppressive agents, some of which are unlikely to directly affect macrophages. Resistance in the MHV-3 model is under complex, probably polygenic, control (*Levy-Leblond* et al. 1979).

Each effector of the anti-MHV-3 response could be under direct genetic control. The effect of the resistant gene(s) could be exerted on viral restriction by macrophages. Indeed, full susceptibility, full resistance or semiresistance is manifested in vitro in individual macrophages. However, this ability of macrophages to restrict viral replication could itself be under the influence of soluble mediators, the secretion of which would be genetically determined.

Macrophage-mediated cytotoxicity against virus-infected target cells would be an interesting possibility. However, the two mouse strains that resist MHV-3 infection, namely A/J and C3H, show a genetically controlled defect of macrophage tumoricidal capacity (*Boraschi* and *Meltzer* 1979). This hypothesis is thus unlikely if we assume that antiviral and anti-tumor cytotoxic activities of macrophages represent a common phenomenon.

Similarly, natural killer activity is under genetic control in mice and is known to be low in animals of the A/J strain (*Petranyi* et al. 1975).

Genetic differences in interferon production could be involved, since interferon has been shown to have an antiviral role in vivo during MHV-3 infection. However, we could not find significant differences in the titers of circulating interferon in susceptible C57/BL or resistant A/J mice during MHV-3 infection. Furthermore, *De Maeyer* et al. (1975) have reported that A/J mice are among the strains that produce a low circulating interferon response after in vivo induction with Newcastle disease virus, and we have found that A/J leukocytes are low producers of mitogen-induced immune interferon in vitro (unpublished). However, these observations cannot rule out the possibility that macrophages from resistant strains produce an early, but not necessarily intense, interferon response in close contact to neighboring susceptible cells during MHV-3 infection.

Finally, the interesting possibility remains that sensitivity to interferon rather than intensity of interferon production is higher in resistant than in susceptible mice. Such an hypothesis has experimental basis, since cells (including monocytes) from humans with trisomy 21 have an enhanced sensitivity to the antiviral effects of interferon (*Epstein* et al. 1980). A genetically determined difference between strains of mice in sensitivity to interferon has also been suggested by *Haller* et al. (1979). In their model, natural resistance of A2G mice to influenza is manifested in vitro in macrophages and nevertheless can be broken in vivo by administration of anti-interferon serum. In mice resistant to MHV, a high sensitivity to interferon could be shared by all cells of the body or mainly expressed at the level of the macrophage. Endogenous production of interferon, either spontaneous or triggered by immunologic (immune interferon) or infectious (classical interferon) experience, would induce an antiviral state in highly sensitive macrophages from resistant strains, but not in poorly receptive macrophages from susceptible strains. Resistant macrophages, able to resist the first cycle of virus replication, would in turn produce interferon more efficiently. If neighboring hepatocytes also were highly sensitive to interferon, protection would be amplified. Indeed, inborn resistance of mice to MHV 3 has been shown to be expressed in cultured liver parenchymal cells (*Arnheiter*

and *Haller*, to be published). Thus sensitivity to interferon produced before and during infection could be a critical factor of recovery. Whatever the exact mechanisms involved, it is probable that intimate interconnection between macrophages and interferon is a major aspect of natural resistance to MHV infection.

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