PATHOGENICITY AND PERSISTENCE OF MOUSE HEPATITIS VIRUS IN INBRED STRAINS OF MICE

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Typical virus infections are short in incubation, acute in course, and rapid in their progress to either death or recovery. Some viruses, however, are able to escape host defense mechanisms and persist for long periods of time, often indefinitely. The pathogenesis and mechanisms of virus persistency vary widely according to the type of model considered. With scrapie, mink encephalopathy, kuru and Creutzfeld-Jacob disease, the infection elicits no inflammatory response. Apparently, immunological factors neither control nor complicate the disease. In other models, the virus persists in the face of an active host immune response. Two situations may be distinguished : the virus may be actively replicated by host cells, and viremia persists until the death of the host. This is true, for example, of lactic dehydrogenase virus infection, Aleutian mink disease or equine infectious anemia. In these cases the virus circulates as infective immune complexes made of virus particles and specific antibody. Alternatively, like in herpes simplex and varicella-zoster infection, the virus remains latent and able to persist in cells of the nervous system. A permanent cell-mediated immune control is exerted on these infected cells, so that the virus is not actively replicated. Diminution of the effectiveness of T cell-mediated responses, for example during immunosuppressive therapy, allows reactivation of the infection.

An interesting aspect of mouse hepatitis virus type 3 (MHV-3) infection is that the severity and the type of infection produced depend entirely on the mouse strain experimentally used[1,2]. The susceptibility of animals to viruses is often genetically determined [3]. The early work of F.B. Bang and his colleagues[4,5] has shown that susceptibility to mouse hepatitis virus type 2 (MHV-2)

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is under genetic control at the cellular level. In the case of MHV-3, the natural resistance shows various degrees according to the mouse strain considered[6,7]. Most mouse strains show a full susceptibility leading to death within a few days. A unique strain, A/J, shows a full resistance with 100% survival of infected animals. In the course of an early study on the in vivo resistance to MHV-3, we found that old C3H/He mice resist the acute phase of the disease but develop a chronic illness with progressive neurological involvement[8]. Clearly, in this type of model, there are two variables : the virus and the host. The fate of the infection is not determined by the virus, which intrinsic virulence remains constant, but rather by host resistance factors which vary in efficiency in different mouse strains. The present communication intends to review the different pathogenetic aspects of MHV-3 infection and to discuss the possible mechanisms leading to virus persistence.

ACUTE MHV-3 INFECTION

MHV-3 is one of the most virulent strains of mouse hepatitis viruses. In the majority of mouse strains, including C57 BL/6 and 10, DBA/2, BALB/c and NZB, parenteral administration of as little as 10 LD 50 always leads to fulminant hepatitis and death. The first cycles of replication occur in macrophages, including Kuppfer cells of the liver. Infection is soon generalized and the virus can then be found in all organs tested, including blood, liver, spleen, lymph nodes, brain and kidney[9]. Giant cells actively replicating the virus are observed in the liver and lymphoid organs. Neighbouring cells, especially hepatocytes, are soon infected. Large, confluent areas of necrosis are seen in the liver. Lymphoid organs also show profound anatomical modifications, particularly in the thymus where the cortical, but not the medullary area is strikingly depleted of lymphocytes as early as 48 hours after intra-peritoneal virus inoculation[10]. Mice die in 5 to 10 days, the clinical picture suggesting acute hepatic failure.

In the A/J strain of mice, full resistance to MHV-3 is observed. Whatever the dose of virus used, all adult mice survive. The virus is cleared from liver, brain and serum within 7 days[6]. The virus titers found in all organs tested are less than 10^2 LD 50/0.1 ml, contrasting with titers of more than 10^4 found in susceptible animals at the time of death. Pathological sections show no tissue lesions. This full resistance of the A/J strain, however, is age-dependent. Up to 15 days of age, A/J animals regularly die even when injected with low doses of virus. Resistance develops suddenly during the third week of life[6]. Adult resistant mice always show, after recovery, a clear serum anti-MHV-3 antibody response.

PERSISTENT MHV-3 INFECTION

In contrast with the full susceptibility or the full resistance described above, mice of the C3H/He and the A2G strains show a genetically determined semi-resistance leading to virus persistence [11,12]. In these two strains, intraperitoneal administration is not followed by acute death, except in a minority of animals. The majority (75 to 95 %) of animals survive, but develop a chronic disease. Three to six weeks after infection, mice progressively begin to show loss of activity, failure to thrive, ruffled fur and neurological signs. Incoordination and paresis of one or more limbs, especially the hind limbs, are observed. When suspended by the tail, mice show circling movements. The date of appearance of paralysis and the intensity of the neurological disorder vary widely from one animal to another. The animals die between 1 and 12 months after infection. The semi-resistance of A2G mice appears to be less reproducible, however, since some batches show full susceptibility and early death in a majority of animals.

This chronic disease is associated with persistence of MHV-3 in its virulent, hepatotropic form. Indeed, most brain suspensions, and occasional liver, spleen and lymph node suspensions, induce a typical lethal hepatitis upon transfer into susceptible recipients. Thus parenteral inoculation of a non-neuro-adapted strain of MHV-3 leads to virus persistence and neurological signs. It should be noted that the virus titers are always very low (about 10^1 LD 50/g of tissue), and that the virus cannot be transferred from the blood.

An age-dependent resistance of a peculiar type is observed in C3H/He and in two other semi-resistant stains, CBA and AKR [12]. These animals are fully susceptible at 6 weeks of age, and it is only by the 12^{th} week of age that animals escape the lethal hepatitis and develop the chronic neurological disease. Since host factors, rather than intrinsic MHV-3 virulence, are clearly involved in this age-dependent phenomenon, our observations indicate that the maturation of anti-MHV-3 effectors is slower and incomplete in semi-resistant strains, whereas it is early and complete in the resistant A/J strain.

PATHOLOGICAL EFFECTS OF PERSISTENT MHV-3 INFECTION

Persistence of MHV-3 is associated with severe lesions of the central nervous system and with systemic amyloid, as reported previously [11]. The neuropathological lesions are partially different in A2G and C3H mice, and will now be described successively. The neuropathological findings in persistently infected A2G mice are dominated by severe hydrocephalus and hydromyelia associated with chronic ependymitis and inflammatory cell cuffing

of small blood vessels in the subependymal tissues and choroid plexus. C3H mice sacrified one to 8 months after infection had lesions both in the brain and the spinal cord. In the former, the lateral and third ventricles were moderately dilated. The predominant lesion was vasculitis, with polymorph infiltration, leukocytoclasis, and often fibrinoid necrosis. These vascular lesions were observed in both arteries and veins of large and small diameter. In most mice, nearby long ascending and descending tracts were damaged, both myelin and axis cylinders being destroyed by proliferative perivascular lesions. Such neural changes were seen more often around the root of the fifth cranial nerve, the choroid plexus in the third and fourth ventricles, and the basilar artery in the brain, widely throughout the leptomeninges of the cord and in many spinal nerve roots. It should be stressed that there was no extensive neuronal damage nor areas of selective demyelination other than the rims of spongy change around well-defined vascular lesions. This picture suggests that the neural damage was in fact the consequence of the vasculitis.

Amyloid was found in the kidneys, liver and spleen of C3H mice infected for more than 6 months. In the spleen, the amount of amyloid deposition was considerable, occupying up to 50 % of the organ. In the mouse, amyloidosis has been detected in survivors of acute reovirus type I and II infection, at a stage when infective virus is no longer recoverable [13]. In the MHV-3 model, it is likely that amyloidosis is the consequence of virus persistence in the face of an active immune response.

MECHANISMS OF NEUROLOGIC LESIONS

The mechanisms through which virus persistence leads to neurological lesions in the absence of direct cytopathic effects of the virus in neural cells has to be discussed. The severe hydrocephalus and hydromyelia observed in A2G are likely to be the consequence of the chronic inflammatory processes observed in ependyma and meninges. A role for chronic obstruction of the aqueduct by proliferating glial and ependymal cells, or disturbed cerebrospinal fluid absorption can be discussed, but has not been demonstrated. That acute viral ependymitis may be followed by hydrocephalus has also been demonstrated in the reovirus type I and the mumps model [14,15]. Lesions of the central nervous system in C3H mice appear to be secondary to the immunopathological, systemic vasculitis. Indeed, no evidence of primary demyelination was observed unlike what is seen with another coronavirus, JHM [16]. In chronically infected C3H mice, old fibrotic neurological lesions, or more recent destruction of neural structures, were almost always observed in close association with a perivascular infiltration of inflammatory cells, and these changes were predominant in the areas of the CNS with the richest vascularization.

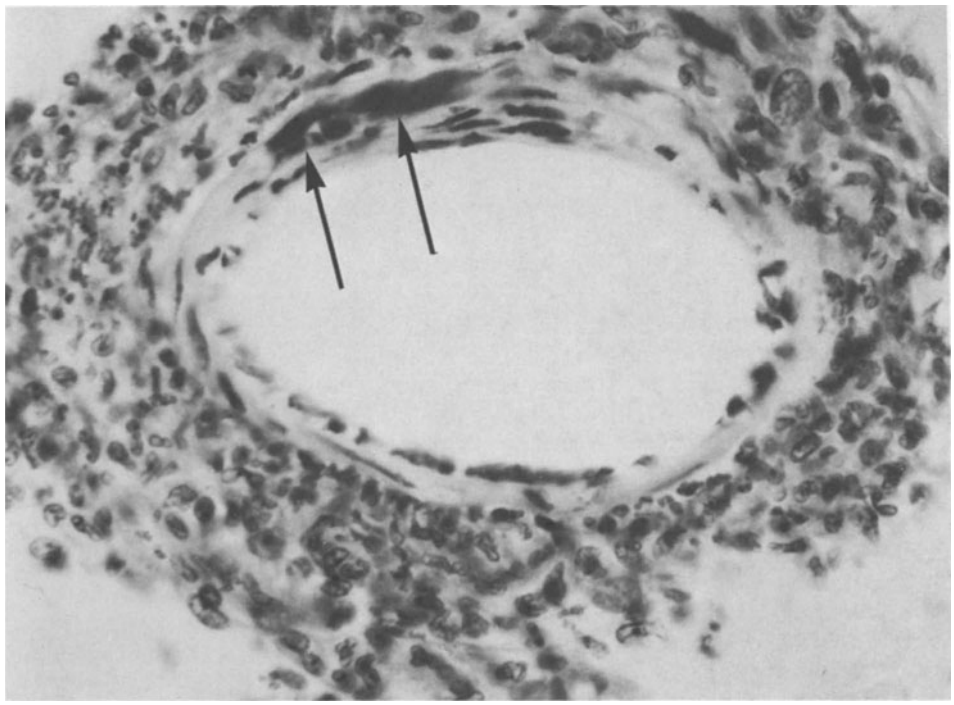

Fig. 1. Vasculitis observed in the basilar artery of a C3H/He mouse sacrified 150 days after intra-peritoneal injection of MHV-3. Note infiltration by polymorphs and areas of fibrinoid necrosis (arrows) in the wall of the vessel (Picro-Mallory staining, X 700)

SITES OF VIRUS PERSISTENCE

Direct and indirect immunofluorescence techniques have been used to detect MHV-3 antigens and immunoglobulin deposition in persistently infected animals [11]. In contrast to clinical and pathological evidence of neural damage, MHV-3 antigens could not be detected in any neural tissue. In A2G mice, a bright, patchy fluorescence of meningeal cells was found in large areas along the meninges. Many areas of the ependymal wall and choroid plexus contained groups of cells showing cytoplasmic fluorescence which indicated the presence of viral antigens. No fluorescence was seen in neurones. In C3H mice sacrificed 45 days to 8 months after infection, no viral antigens or immunoglobulin deposition could be found in neurones or meninges. However, in some parts of the ependyma and in all sections of the choroid plexus, bound immunoglobulins were detected, especially in choroid plexus vessels, suggesting the presence of immune complexes. In the

same areas, direct staining with a fluorescein-conjugated anti-MHV-3 serum detected the presence of viral antigens. Spleen sections from the same animals showed appearances typical of immune vasculitis with detection of both viral antigens and bound immunoglobulins in the walls of most arteries, veins and small vessels. Virus-carrier mice persistently infected but without major neurological signs showed also some degree of immune vasculitis. Three negative findings should be stressed : firstly, kidney sections of both control and infected C3H mice showed slight immunoglobulin deposits in the glomeruli, which were not detectably increased in infected animals. Secondly, no viral antigen could be detected in macrophages, either in Kuppfer cells of the liver or in the marginal zone of the spleen. Thirdly, hepatocytes from chronically ill mice were never shown to contain viral antigens by immunofluorescence.

Altogether, these findings suggest that MHV-3 persists mainly, and may be exclusively, in ependymal and meningeal cells (especially in A2G mice), and in vessel wall structures of C3H mice. No evidence could be found of viral persistence in parenchymal cells (hepatocytes or neurones) or in tissue macrophages. It should be underlined, however, that immunofluorescence techniques are not highly sensitive and may miss viral antigens present in very little amounts. Our observations thus do not exclude virus persistence in other tissues, but clearly indicate that persistency takes place essentially in meninges and in vessel walls, probably in endothelial cells. This is consistent with the findings that infective virus can be transferred more easily from brain and lymphoid organs than from liver or kidneys, and that the titers of virus in all organs tested are always very low. Viruses which persist by replicating freely in many cell types, including macrophages, such as lymphochoriomeningitis or lactic dehydrogenase virus, are always recoverable in high titers. Chronic MHV-3 infection with chronic vasculitis resembles another type of experimental model, equine viral arteritis, in which the virus has been shown by electron microscopy to replicate in the endothelial cells of the damaged vessels [17]. Indeed, the contrast between the absence of glomerular damage and the existence of a widespread vasculitis further favors the hypothesis that vascular damage is not due to deposition of circulating immune complexes but rather to in situ formation of immune complexes within vessel walls where MHV-3 antigens persist.

POSSIBLE MECHANISMS OF VIRAL PERSISTENCE

Viral persistence implies that the virus is able to escape, at least partially but indefinitely, the immune surveillance of the host. MHV-3 does not loose its virulence in chronically infected mice, since its transfer into susceptible recipients always leads to acute, lethal hepatitis. Clearly, persistence

depends on host factors, able to protect animals during the acute phase of the disease, but unable to clear completely the virus from the cells where it has found a refuge. Is this "semi-resistance" fundamentally different in its mechanisms from the full resistance observed in A/J mice ? The difference may only be a question of degree, since intermediate titers of virus are found in mice from semi-resistant strains during the first days of infection [12]. These titers are predictive, since virus titers in blood of C3H mice four days after infection show a clear correlation with the course of the disease. Most of the animals with high virus titers die of acute disease, whereas those that survive and subsequently develop a chronic disease show low virus titers. Thus early events appear to decide whether MHV-3 infection will be acute or chronic.

While antibody to MHV-3 seems to play very little role in resistance [6], two non specific early host factors appear to have a critical role in recovery : one is viral restriction in individual cells, including macrophages and hepatocytes. Indeed, macrophages are known to be the primary target cell of MHV-3 in vivo [9], and we have shown a precise correlation between in vivo resistance and a variable genetic ability of peritoneal macrophages to restrict virus growth, C3H macrophages being intermediate in this respect [7]. Moreover, primary monolayer cultures of hepatocytes isolated from adult, resistant A/J or partially resistant C3H/He mice exhibit resistance to MHV-3 as the respective macrophages do [18]. Thus, even if the virus were able to escape the macrophage blood-organ barrier, it would find resistance in individual hepatocytes. The second early host defense mechanism is interferon production, which neutralization in vivo with a potent anti-type I murine interferon serum potentiates strikingly MHV-3 infection in the C3H strain : all mice die of acute hepatitis [19]. Thus the fate of MHV-3 infection in C3H mice is decided at a stage (48 hours) when the specific immune response is unlikely to be operative. Classical (type I) interferon and macrophages are likely to collaborate in host defense against MHV-3, since macrophages are susceptible to the antiviral effect of interferon and also are able to produce interferon themselves. Furthermore, it is possible that lymphocytes and macrophages cooperate through immune (type II) interferon. Indeed, we have shown that the latter type of interferon, a product of activated leucocytes, can protect cultures of mouse peritoneal macrophages against MHV-3 [20]. In C3H mice, however, interferon production is not fully effective, since the virus persists in the face of permanent (although low) titers of circulating interferon [10]. As discussed previously [2], it should be envisaged that sensitivity to interferon action, rather than interferon production, is genetically deficient in C3H mice. According to this hypothesis, some body cells in this strain (especially meningeal and endothelial cells) would not be fully sensitive to the antiviral

effects of interferon and would serve as permanent refuge for the virus.

T lymphocytes also are likely to participate in host defense against MHV-3 infection, since it has been reported that the protective capacity of spleen cells transferred from adult, resistant to neonatal, susceptible A/J mice is abolished after treatment with anti-θ serum [21]. Finally, it should be remembered that persistent MHV-3 infection is associated with a profound, permanent state of immunodepression [10]. This may further diminish the ability of the host to eradicate the infection. Clearly, no single mechanism is likely to underlie the complex host-virus relationship leading to MHV-3 persistence.

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