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DEMYELINATION INDUCED BY MURINE CORONAVIRUS JHM INFECTION OF CONGENITALLY IMMUNODEFICIENT MICE

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ABSTRACT

Mouse hepatitis virus JHM (JHMV or MHV-4) induces demyelination in rodents and has been studied as a model for the human disease, multiple sclerosis (MS). As is proposed in MS, the mechanism of subacute demyelination induced by JHMV appears to be primarily immunopathological, since demyelination in JHMV-infected mice is abrogated by immunosuppressive doses of irradiation and restored by adoptive transfer of splenocytes. Thy-1^+ cells play a critical role in transmitting disease to these recipient mice. To further characterize cells which may mediate JHMV-induced immunopathology, we inoculated congenitally immunodeficient mice with JHMV. By 12 days post-inoculation, both immunocompetent C57BL/6J controls and athymic nude C57BL/6 mice had severe paralysis and demyelination. In marked contrast, C57BL/6 mice with the severe combined immune deficiency (SCID) mutation had little or no paralysis or demyelination. Adoptive transfer of immune spleen cells from nude mice to infected SCID mice produced paralysis and demyelination. These findings suggest that a cell population present in immunocompetent C57BL/6J and nude mice but absent or non-functional in irradiated and SCID mice is essential for JHMV-induced demyelination. Identification of cells which mediate demyelination in this experimental system may have implications for our understanding of coronavirus pathogenesis and human demyelinating diseases.

INTRODUCTION

Infection of rodents with the neurotropic murine coronavirus JHM (MHV-4) produces an acute, often lethal encephalitis. Survivors exhibit a subacute or chronic paralytic-

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demyelinating disease which has been proposed as a model for the human demyelinating disease, multiple sclerosis.

Two mechanisms for JHMV-induced demyelination have been proposed. Early studies suggested that myelin damage was due to cytolytic viral infection of the myelin-producing oligodendrocytes[1,2]. Support for the oligodendrocyte lysis hypothesis included the localization of virions within oligodendrocytes and the occurrence of some demyelination in immunosuppressed mice. More recently, however, evidence has accumulated supporting a mechanism whereby myelin damage is caused by the immune response to viral infection. Immunosuppressive irradiation up to six days post-inoculation (PI) can prevent demyelination and adoptive transfer of immune splenocytes restores demyelination to infected irradiated recipients[3]. In addition, depletion of cells bearing the Thy-1 marker from the adoptively transferred cell population prevents restoration of demyelination, suggesting a role for T lymphocytes in demyelination[4]. An immunopathological mechanism for JHMV-induced demyelination has also been demonstrated in rats, with both $CD4^+$ and $CD8^+$ T lymphocytes contributing to disease[5].

To characterize cells which may participate in immune-mediated demyelination, we infected congenitally immunodeficient mice with JHMV. Athymic nude mice developed paralysis and demyelination. In contrast, mice possessing the severe combined immune deficiency (SCID) mutation showed minimal paralysis and little or no demyelination. In addition, demyelination was adoptively transferred to infected SCID mice with immune splenocytes from nude mice. This supports an immunopathological mechanism for demyelination and suggests that a cell population present in immunocompetent and nude mice but absent or non-functional in SCID and irradiated mice is essential for JHMV-induced demyelination.

MATERIALS AND METHODS

Male C57BL/6J, C57BL/6J-*nu* (nude) and C57BL/6J-*scid*/SzJ (SCID) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and used at 5-7 weeks of age. Mice were housed in microisolators and handled in a biosafety hood. The neuroattenuated JHMV antigenic variant 2.2-V-1 has been described previously and produces demyelination in immunocompetent mice with little or no encephalitis[6,7]. Mice were infected with 10^3 plaque forming units (pfu) of virus in 30 µl of Dulbecco's modified essential medium (DMEM) by the intracerebral (i.c.) route. Selected mice were irradiated 3 days after intracerebral inoculation with 850 rads of gamma-irradiation from a Cobalt-60 source[4]. Donor nude mice were immunized with 10^6 PFU intraperitoneally 6 days prior to transfer. Recipient SCID mice were infected 3 days prior to transfer. Donor splenocytes (4×10^6 cells) were transferred by the intravenous route.

Mice were monitored for clinical signs of disease until 12 days PI, when they were sacrificed. Brains and spinal cords were removed and subjected to virus isolation on DBT cells[8] or histopathological analysis[9]. A combined hematoxylin and eosin/luxol fast blue stain was used to visualize myelin. Selected mice were perfused for electron microscopic analysis[6].

RESULTS

As has been demonstrated previously, immunocompetent C57BL/6J mice infected with 2.2-V-1 undergo severe paralysis accompanied by marked demyelination, which can be prevented by immunosuppressive irradiation 3 days post-inoculation (Table 1, Groups 1

Table 1. Outcome at 12 days post-inoculation of immunocompetent and immunodeficient mice, and after adoptive transfer

Group	Experiment[a]	Paralysis[b]	Demyelination[b]	Virus[c]
1	C57BL/6	+++	+++	–
2	Irradiated	–	–	+++
3	Nude	+++	+++	++
4	SCID	+	+/–	++
5	Nude to SCID Transfer	++	++	++

[a]Experimental groups of 6 to 8 mice. Mice were inoculated with 10^3 PFU of 2 2-V-1 by the intracerebral route "C57BL/6" refers to immunocompetent mice "Irradiated" refers to 850 rads at 3 days PI. "Nude to SCID transfer" refers to infected SCID recipients of adoptively transferred immune nude splenocytes.

[b]Paralysis and demyelination are indicated as severe (+++), moderate (++), minimal (+) or none (-) "+/-" indicates demyelination in one of six mice examined

[c]Recovery of infectious virus from brain homogenates at 12 days PI The sensitivity of the assay was 10^2 pfu per gram of brain

and 2)[4]. Nude mice infected with JHMV were also severely affected; most of these mice developed clinical paralysis, and both light microscopic and ultrastructural studies showed demyelination (Table 1, Group 3). In marked contrast, SCID mice showed only minimal clinical effects, mostly mild to moderate paraparesis (Table 1, Group 4). With the exception of one animal, no demyelination was evident by light microscopy or ultrastructural analysis in SCID mice studied. Whereas immunocompetent C57BL/6 mice were able to clear virus from the brain by day 12 PI, both nude and SCID mice had high titers of virus remaining in the brain at day 12 (Table 1).

Since the above results suggest that a cell population present in nude mice but absent in SCID mice is essential for JHMV-induced demyelination, immune splenocytes from nude mice were transferred into infected SCID mice. The results of this experiment are depicted in Table 1 (Group 5). Clinically, seven out of eight of these mice showed marked hindlimb weakness, and histopathologic analysis revealed plaques of demyelination. Disease in the SCID recipients of nude splenocytes was more severe than in normal SCID mice, but less severe than in nude or immunocompetent C57BL/6J mice.

DISCUSSION

Paralysis and demyelination have been previously reported in both nude mice and nude rats infected with JHMV[10,11]. These authors used non-neuroattenuated JHMV to infect athymic rodents and observed paralysis, demyelination and rapidly fatal encephalitis with destruction of neurons, making interpretation of these findings difficult. In the experiments reported here we used a neuroattenuated strain of JHM which allows us to study demyelination in immunodeficient mice with little or no confounding encephalitis.

The findings presented here support an immune-mediated mechanism for JHMV-induced demyelination rather than a viral cytolytic mechanism. Mice with the lowest level of immunocompetence (SCID and irradiated mice) showed the least demyelination. Thus, demyelination is correlated with the degree of immune function. The level of demyelination in SCID mice was augmented by the adoptive transfer of splenocytes from nude mice, suggesting that cells contributing to demyelination are present in the transferred splenocytes. SCID and irradiated mice showed little or no demyelination despite the presence of high

titers of infectious virus in the CNS at 12 days PI. This argues against a viral cytolytic mechanism for JHMV-induced demyelination.

At 12 days PI, we isolated infectious virus from the brains of irradiated C57BL/6 mice, nude and SCID mice, and SCID recipients of immune nude splenocytes. Only immunocompetent C57BL/6 mice were able to clear infectious virus by 12 days PI. This is consistent with reports demonstrating a requirement for $CD4^+$ and $CD8^+$ T lymphocytes for viral clearance[12,13]. Since nude mice developed severe paralysis and demyelination, yet were unable to clear the virus, distinct cell populations may be involved in demyelination and viral clearance. Although T lymphocytes are essential for demyelination[4], thymically educated T lymphocytes do not appear to be required, since athymic nude mice develop severe demyelination.

In conclusion, we have demonstrated paralysis and demyelination in JHMV-infected immunocompetent and nude mice. In contrast, little or no demyelination was evident in SCID and irradiated mice. Adoptive transfer of immune splenocytes from nude mice resulted in paralysis and demyelination in SCID recipients. Our findings support an immune-mediated mechanism for demyelination. and suggest that a cell population present in immunocompetent and nude mice, but deficient in SCID and irradiated mice, is essential for JHMV-induced demyelination. Identification of this cell population may lead to new insights into the pathogenesis of human demyelinating diseases.

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