IMMUNOPATHOGENESIS OF DEMYELINATION INDUCED BY MHV-4

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INTRODUCTION

Mouse hepatitis virus-4 (MHV-4 or JHM) is a murine coronavirus which causes central nervous system (CNS) demyelination in rodents (1). Experimental MHV-4 infection has been used as a model of human demyelinating diseases, such as multiple sclerosis. One of the major questions relating to MHV-4 pathogenesis is the degree to which demyelination caused by this virus is due either to 1) direct viral cytopathology, especially for oligodendrocytes, the cells which produce and maintain myelin, or 2) immunological responses elicited by viral infection.

We have examined this issue by using a neutralization-resistant variant of MHV-4, designated 2.2-V-1 (2). This variant, immunoselected by a monoclonal antibody to the major glycoprotein (E2), has little neurovirulence but causes marked paralytic-demyelinating disease in at least 70% of mice inoculated intracerebrally (3). The role of immunity in experimental demyelination of mice during MHV-4 infection was explored in two ways. First, immunity was abrogated by X-irradiation of MHV-4-infected mice. Second, immunity was reconstituted by adoptive transfer of spleen cells into irradiated, infected recipient mice. Both approaches indicated that immune responses play a critical, essential role during demyelination induced by this virus.

RESULTS

Six-week old C57BL/6J male mice (Jackson Laboratories) seronegative (4) for MHV, were inoculated intracerebrally (i.c.) with 10^3 plaque forming units (pfu) of MHV-4, variant 2.2-V-1, as previously described (2). These mice developed marked paralytic-demyelinating disease, and at day 12 post-inoculation (p.i.) viral antigen and infectious virus were not detectible in the CNS (Fig. 1; Group 1, Table). In a second experiment, infected mice were immunosuppressed with 850 rads of X-irradiation on day 3 p.i. and observed daily

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until sacrifice at day 12 p.i. Despite the presence of abundant CNS virus, most prominently in oligodendrocytes, these mice did not develop paralytic-demyelinating disease (Fig. 2; Group 2, Table). Irradiation of mice infected with another variant of MHV-4, JHMV-DS (5) also blocked demyelination (data not shown). Similarly, paralytic-demyelinating disease was abrogated after immunosuppression of 2.2-V-1-infected mice with cyclophosphamide (200 mg/kg intraperitoneally (i.p.) on day 3 p.i.) (data not shown). Thus, unrestrained viral replication in oligodendrocytes during immunosuppression is insufficient to cause demyelination.

To explore the role of immune cells, mice were inoculated with MHV-4 i.c. and irradiated at day 3 p.i. as before; subsequently, these mice were reconstituted by the adoptive transfer of approximately 5 x 10^7 donor spleen cells intravenously. In the first adoptive transfer, recipient mice were given splenocytes from 6-week old C57BL/6 mice which had been immunized with 3 x 10^6 pfu of MHV-4 i.p. six days previously, that is, MHV-4-immune donors. Disease was fully reconstituted by this transfer (Fig. 3; Group 3, Table). However, when the adoptive transfer was repeated with MHV-4-immune donor cells which had been depleted of T lymphocytes by prior treatment with complement and anti-Thy-1.2 monoclonal antibody (5a-8, Cedarlane), disease was not restored (Group 4, Table), indicating that MHV-4-immune T cells are necessary for demyelination. Preliminary studies indicate that the cell predominantly responsible for adoptive transfer of disease may have the $CD8^+$ phenotype (Fig. 4).

In following experiments, spleen cells from naive, unimmunized donor mice were transferred to MHV-4-infected, irradiated mice (Group 5, Table). The majority (75%) of these mice were normal clinically and histologically at d. 12 p.i.; the remaining mice showed signs of disease which developed less rapidly and with less intensity than in virally-infected (Group 1) or immune-reconstituted (Group 3) mice. Similar results were obtained with transfers in which keyhole limpet hemocyanin (KLH) (Sigma) was used to immunize donor mice (Group 6), indicating that robust, efficient transfer of disease requires cells which have been specifically sensitized to MHV-4. Transfer of MHV-4-immune cells into uninfected, irradiated recipients (Group 7) did not result in disease, indicating that immunopathological responses are primarily directed against virus or virus-altered determinants, not self. In other experiments, the transfer of allogenic immune spleen cells from BALB/c ($H\text{-}2^d$) mice into infected, irradiated C57BL/6J $H\text{-}2^b$) mice did not result in paralysis or demyelination, suggesting that adoptive transfer of disease is restricted by the major histocompatibility complex.

Low levels (approximately 1:100 end point titers) of anti-MHV-4 IgM antibody were detected in all groups of mice, except number 2 (infected, irradiated mice that were not reconstituted by splenocytes). The finding that group 4 mice, lacking T cells but positive for antibody, do not develop paralytic-demyelinating disease indicated that antibody alone is insufficient to cause disease.

Table 1. Outcome at 12 days post-inoculation, after Irradiation and Adoptive Transfers

Group	[a]Experiment	[b]Paralysis	[b]Demyelination	[b]Virus	Antibody
1	virus only	+	+	-	+
2	virus, irradiation	-	-	++	-
3	virus-immune→ virus recipient	+	+	-	+
4	virus-immune (T cell depleted)→ virus recipient	-	-	+	+
5	non-immune→ virus recipient	+/-	+/-	+	+
6	KLH-immune→ virus recipient	+/-	+/-	+	+/-
7	virus-immune→ uninfected recipient	-	-	-	+

[a]Experimental groups, as explained in text; "virus" refers to MHV-4, variant 2•2-V-1, "irradiation" to 850 rads given on day 3 p.i., and arrows indicate the adoptive transfer of approximately $5x10^7$ spleen cells intravenously into irradiated recipients on day 3 p.i. Donor mice were prepared by the intraperitoneal administration of 3×10^6 pfu of virus or 150 ug of KLH 6 days prior to adoptive transfer as indicated.

[b]Outcome of experiments, judged at 12 days p.i. Positive symbols indicate severe hindleg paralysis, marked demyelination on blinded histologic evaluations, infectious virus recovery from brain homogenates (2), and IgM antibody measured by enzyme-linked immunosorbent assay (4), respectively. Where positive results were obtained in occasional mice or were of low titer, a "+/-" symbol is used.

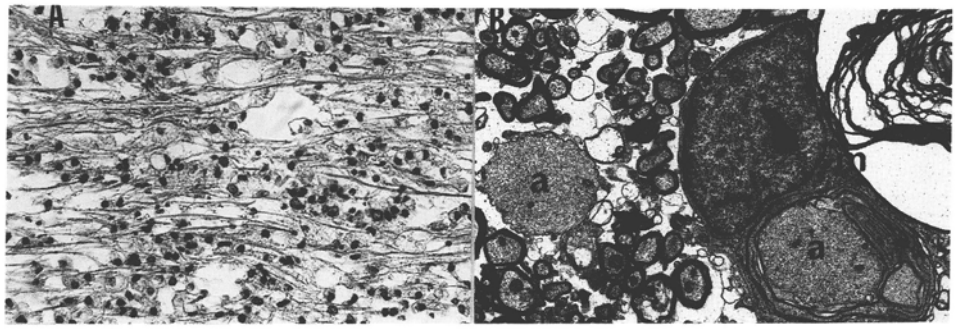

Figure 1. Mouse infected with virus and sacrificed at day 12 p.i. (Group 1, Table) A. Longitudinal section of spinal cord, stained immunohistochemically for MHV-4 antigen and counterstained with hematoxylin, as previously described (3), x 100. Note extensive white matter rarefaction, marked inflammatory infiltrate consisting primarily of lymphocytes and macrophages, and the absence of viral antigen. B. Epon-embedded transverse section of spinal cord, prepared as previously described (2). Note demyelinated axons (a) and macrophage stripping myelin (m). x 6,000.

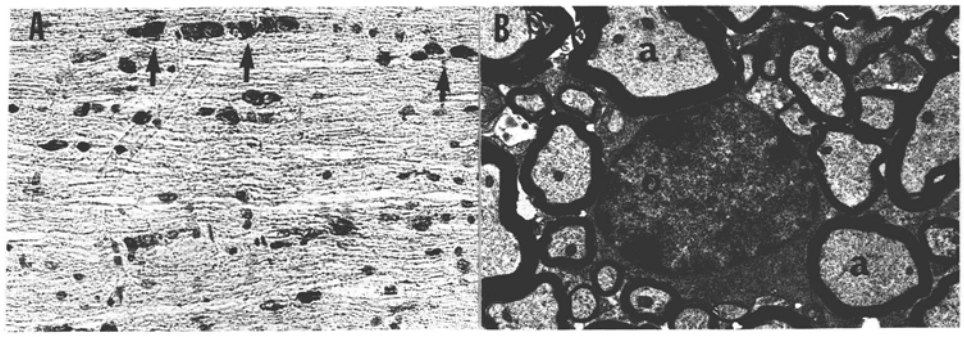

Figure 2. Mouse infected with virus, irradiated at day 3 pi., and sacrificed at day 12 p.i. (Group 2, Table). A. Spinal cord stained for viral antigen, as in Fig. 1A. Note abundant viral antigen in cells with the appearance of intrafascicular oligodendrocytes (arrows). x 200. B. epon-embedded spinal cord, prepared as in figure 1B. Note normal appearance of myelinated axons (a) and an oligodendrocyte (o) x 6,000.

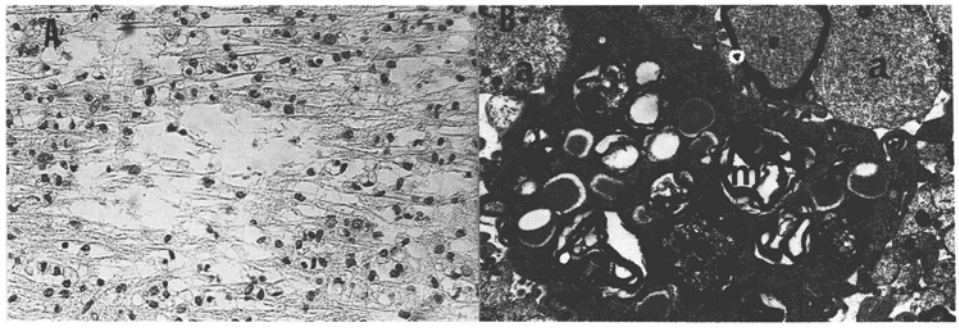

Figure 3. Adoptive transfer in which virus-immune donor splenocytes were given intravenously at day 3 p.i. to a virus-infected, irradiated recipient (Group 3, Table). A. spinal cord, prepared as in Fig. 1A. Note intense inflammation, similar to virus-only mouse shown in Fig. 1A x 200. B. Spinal cord, prepared as in 1B. Note demyelinated axons (a) and macrophage (m) with phagocytosed myelin debris. x 15,000.

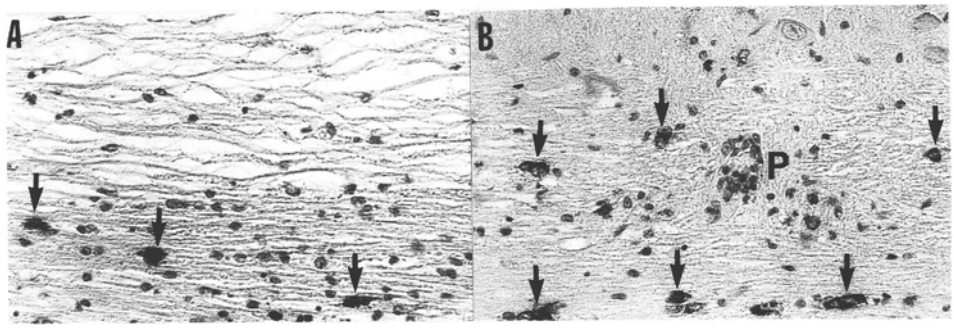

Figure 4. Longitudinal section of mouse spinal cords, stained immunohistochemically for viral antigen, as in Fig. 1A. Viral-immune donor splenocytes were depleted of different T cell subsets by incubation with complement and specific monoclonal antibodies prior to transfer into virus-infected, irradiated recipient mice. A. After depletion of donor $CD4^+$ cells (anti-L3T4 monoclonal antibody RL172.4 recognizing helper-inducer T cells (6)). Note demyelinated lesion at top of figure; although white matter rarefaction is severe, cellular infiltration is less intense than virus-only control mice (Fig. 1A). Scanty viral antigen is seen <u>outside</u> of the borders of this lesion (arrows) x 200. B. After depletion of donor $CD8^+$ cells (anti-Lyt-2 monoclonal antibody recognizing cytotoxic-suppressor T cells (7)). Note perivascular infiltrate (P) and abundant antigen (arrows). White matter rarefaction and demyelination are not present. x 200 Treatment of donor splenocytes with complement only had no effect on the transfer of disease (data not shown).

DISCUSSION

MHV-4-induced demyelination has often been considered to be a direct consequence of viral infection of oligodendrocytes. This hypothesis is supported by three lines of evidence. First, many in vivo and in vitro studies have established the tropism of MHV-4 for oligodendrocytes and other CNS cells of rodents (1,8,9,10). Second, in an early study, mice infected with MHV-4 and treated with cyclophosphamide showed "small areas of demyelination" despite the apparent ablation of humoral and cellular immunity (11). Similarly, treatment of MHV-4-infected rats with cyclophosphamide (12) or with cyclosporin A (13) resulted in a panencephalitis in which foci of necrosis were noted in white matter, although the status of myelin was not specifically addressed in these reports. Third, immunodeficient, athymic mice (12) and rats (14) also develop small demyelinated foci after MHV-4 infection. It is important to note, however, that in the studies cited immunosuppression or immunodeficiency converted the normally non-fatal MHV-4 infection into an acute fatal panencephalitis. It is not clear that occasional demyelinating lesions in the setting of an overwhelming panencephalitis accurately reflect the natural disease as originally described by Bailey et al (15); in this case, MHV-4 produces a paralytic disease in which pathology is centered in the white matter and large demyelinating lesions are readily apparent.

We have studied MHV-4-induced demyelination under conditions in which acute encephalitis is minimized and does not confound the analysis of subacute white matter pathology. This was achieved by two means: 1) a relatively avirulent, less encephalitic, MHV-4 strain, MHV-4 2.2-V-1 (2), was used, and 2) immunosuppression was applied at day 3 p.i., that is, after the initial phase of viral replication, but before severe white matter pathology develops. Using this protocol, we were able to ablate demyelination by means of immunosuppression and reconstitute demyelination by the adoptive transfer of MHV-4-specific immune T cells. As noted, preliminary results indicate that T cells with the $CD8^+$, or cytotoxic-suppressor cell, phenotype may be the critical element which clears virus (J. Williamson et al, this volume) and contributes to the development of typical foci of primary demyelination (fig 4). Together with previous investigations, these results suggest two contrasting roles for the immune system during MHV-4 pathogenesis. First, as shown by studies of immunodeficient rodents (12,14) or animals immunosuppressed simultaneously with viral inoculation (11,12,13), immunocompetence is necessary early in MHV-4 infection in order to prevent an overwhelming, fulminant CNS infection. In other words, the primary role of the immune system is to protect mice from acute encephalitis and to clear virus from the CNS (16). Second, the results above indicate that participation of the immune system, especially T cells, is essential during subacute demyelination elicited by MHV-4. In this sense, the role of the immune system is a negative one, resulting in disease. Learning in detail how MHV-4 elicits this response may be an important step in understanding human diseases such

as multiple sclerosis in which a viral etiology and an immunopathological basis for demyelination are suspected.

CONCLUSIONS

1. MHV-4-induced demyelination is not a direct, cytolytic consequence of virus infection of oligodendrocytes.

2. Immunosuppression abrogates MHV-4-induced demyelination.

3. MHV-4-specific T cells play a critical role in MHV-4-induced demyelination.

4. Preliminary evidence indicates that $CD8^+$ T cells may be the predominant cell in adoptive transfer of demyelination; transfer of disease also appears to be restricted by the major histocompatibility complex.

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