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Short Communication

Neither B cells nor T cells are required for CNS demyelination in mice persistently infected with MHV-A59

AE Matthews,[1] E Lavi,[2] SR Weiss,[1] and Y Paterson[1]

[1]*Department of Microbiology and* [2]*Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA*

Murine hepatitis virus A59 infection of the central nervous system (CNS) results in CNS demyelination in susceptible strains of mice. In infected B-cell-deficient mice, demyelination not only occurred but was also more severe than in parental C57BL/6 animals. This increase may be due to the persistence of virus in the CNS in the absence of B cells. In mice lacking antibody receptors or complement pathway activity, virus did not persist yet demyelination was similar to parental mice. In infected RAG1$^{-/-}$ mice, moderately sized, typical demyelinating lesions were identified. Therefore, demyelination can occur in the absence of B and T cells. *Journal of NeuroVirology* (2002) **8,** 257–264.

Keywords: MHV-A59; demyelination; B cells; Fc receptor KO; complement KO

Introduction

Murine hepatitis virus (MHV) A59 is a coronavirus, which can cause acute hepatitis and encephalitis. After viral clearance, the encephalitis develops into central nervous system (CNS) demyelination (Lavi *et al*, 1984a; Sutherland *et al*, 1997). Demyelination can first be detected 6 days postinfection (d.p.i.) as very small lesions in the presence of an active infection. Large demyelinating lesions develop by 20 d.p.i. and peak around 30 d.p.i., at which time points infectious virus is undetectable although viral RNA is still present (Lavi *et al*, 1984b; Das Sarma *et al*, 2000). The demyelination is histologically similar to that seen in humans with multiple sclerosis (MS) (Lampert *et al*, 1973; Herndon *et al*, 1977; Jordan *et al*, 1989; Lucchinetti *et al*, 1996; Barac-Latas *et al*, 1997; Lucchinetti *et al*, 2000).

Address correspondence to Yvonne Paterson, University of Pennsylvania, 323 Johnson Pavilion, 3610 Hamilton Walk, Philadelphia, PA 19104-6076, USA. E-mail: yvonne@mail.med.upenn.edu

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The mechanism of demyelination after MHV infection is currently unclear, although both macrophages and T cells have been implicated as mediators of pathology (Wu and Perlman, 1999; Wu *et al*, 2000). Lymphocyte-deficient SCID, RAG knockout, and irradiated mice infected with MHV-JHM rarely have even small lesions 7 to 15 d.p.i., despite high levels of virus (Wang *et al*, 1990; Fleming *et al*, 1993; Houtman and Fleming, 1996; Wu *et al*, 2000). These data suggest that lymphocytes are required for robust acute demyelination. A recent reconstitution study suggests that CD4$^+$ or CD8$^+$ T cells can mediate MHV-JHM-induced demyelination (Wu *et al*, 2000). However, in nude mice that lack CD4$^+$ and CD8$^+$ T cells, demyelination still occurs (Houtman and Fleming, 1996). If T cells are not absolutely required for demyelination, the question remains whether B cells, the other major lymphocyte population, are important effectors in the mechanism of demyelination. Antibody production by B cells is implicated in CNS demyelination in other models of demyelination (Schluesener *et al*, 1987; Mastaglia *et al*, 1989; Ozawa *et al*, 1989; Lucchinetti *et al*, 2000).

To determine whether B cells are required for MHV-A59 induced CNS demyelination, we infected 4-week-old, female B-cell-deficient mice (muMT, Jackson Laboratories) on a C57BL/6 genetic background with 50 p.f.u. MHV-A59 intracerebrally (i.c.) in 20 μl. Demyelination was assessed in luxol fast

blue-stained transverse sections by quantifying either the percent of spinal cord quadrants containing lesions or by a visual assessment of the area of white matter involved in demyelinating lesions (the scale was as follows: 1—only very small lesions detected, no larger than areas of perivascular cuffing; 2—obvious discrete lesions involving more area than in 1 and less than in 3; 3—total lesions occupy 25%–50% of the white matter in a transverse section; 4—total lesions occupy more than 50% of the white matter). By either assessment, demyelination occurs robustly in the absence of B cells (Figure 1). Initial demyelination was very similar to that observed in parental C57BL/6 mice. At 30 and 60 days postinfection (d.p.i.) CNS demyelination was actually significantly more severe in B-cell-deficient mice than in parental mice (Figure 1). However, we have shown that infectious virus persists until at least 60 d.p.i. in the CNS of the muMT mice (Matthews *et al*, 2001). Therefore, while the increased severity and prolonged presence of demyelinating lesions could be due to the absence of some protective mediating effect of B cells, it could also be due to direct pathologic effects of the persistent virus.

To answer the question of whether MHV-A59 can directly mediate CNS demyelination, we infected RAG-$1^{-/-}$ mice on the C57BL/6 background (B6.129S7-Rag1^{tm1Mom} from Jackson Laboratory, Bar Harbor, Maine). These mice are unable to develop mature B cells and T cells. Normally, MHV-A59 infection of RAG-$1^{-/-}$ mice results in death within 15 days of infection ($n = 5$). In one RAG-$1^{-/-}$ mouse, which was evaluated after death at 11 d.p.i., 25% quadrant demyelination was observed, although the lesions were small as would also be the case at 11 d.p.i. in a parental C57BL/6 mouse (data not shown). To enable the mice to survive to the point of maximum demyelination (20–30 d.p.i.), we treated them at the time of infection with a single dose of mouse hyperimmune serum (HIS) injected intraperitoneally (i.p.). HIS was raised in C57BL/6 mice against MHV-A59 and was partially purified by 45% ammonium sulfate precipitation and dialysis against PBS. The neutralizing titer (NT) of HIS was determined by incubating serial dilutions of serum antibody with 500 p.f.u./ml A59 in DME-10 for 30 minutes at 37°C. NT50 was calculated as the dilution at which plaque numbers were reduced by 50%. For protection, 1 ml of HIS (NT50 of 1:5000, equivalent to the neutralizing activity found in 1 mouse 3 weeks after i.p. infection) was injected i.p. at the time of infection. Treatment with HIS enabled the mice to survive to 25 d.p.i. or later without actually clearing the virus. Antibody treatment at the time of infection has been previously shown to improve survival (Buchmeier *et al*, 1984).

Antibody protected, MHV-A59-infected female RAG-$1^{-/-}$ mice had high titers of virus in the CNS at 22–33 d.p.i. (Table 1). When sections were stained with luxol fast blue to detect demyelinating lesions, discrete areas of white matter lacking blue stain were detected (Figure 2A–D). Quantification of these demyelinating lesions demonstrated that antibody protected RAG$1^{-/-}$, C57BL/6, and splenocyte-reconstituted RAG$1^{-/-}$ mice had similar levels of demyelination (Table 1). To determine more precisely the nature of these lesions, spinal cords were embedded in Epon and sections were stained with toluidine blue. Compared to normal tissue (Figure 3A), lesions revealed active primary demyelination characterized by ballooning myelin sheaths around intact axons (Figure 3B, C). The presence of naked axons in

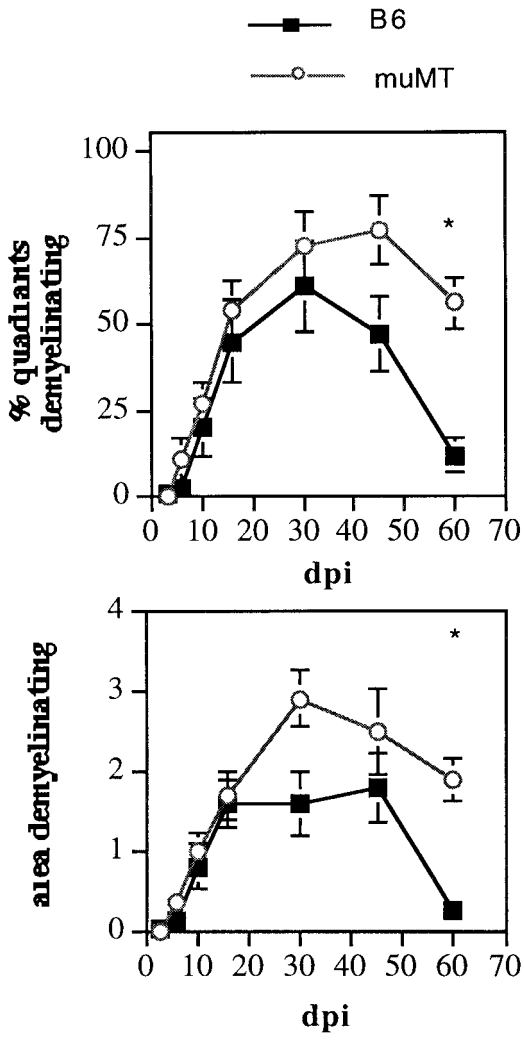

Figure 1 CNS demyelination in the absence of B lymphocytes. 4-week-old parental C57BL/6 and B-cell-deficient muMT mice were infected with 50 p.f.u. MHV-A59 i.c. At various time points after infection, mice were killed, and their spinal cords fixed in formalin. Each spinal cord was cut into six transverse sections, and these sections were assessed for demyelinating lesions using luxol fast blue. In (**A**), demyelinating lesions were quantified as the percentage of spinal cord quadrants of the total number containing lesions. In (**B**), the proportion of the white matter which consisted of lesion was determined using a visual assessment scale. These data were compiled from three separate experiments. Astericks mark time points at which a significant difference was detected between the two populations by *t*-test analysis. Error bars represent SEM.

Table 1 Viral titers and demyelination in MHV-A59 infected RAG1$^{-/-}$ mice

Mouse strain and treatment[a]	*n*	*Average brain viral titer in pfu/g (log 10)*	*Average percent of spinal cord quadrants demyelinating*
RAG1 KO[b]	7	5.27 ± 0.65[d]	54.3% ± 11.7
RAG1 KO + splenocytes[c]	4	0	56.5% ± 21.7
C57BL/6	5	0	49.6% ± 20.0

[a]Female mice were infected i.c. with 500 pfu MHV-A59 at 5.5–8.5 weeks of age.
[b]This group was injected with hyperimmune serum at infection.
[c]This group was reconstituted at 3–4 dpi with 500 μl i.p. containing ~1.5 × 10^6 splenocytes from C57BL/6 mice 6–7 dpi post-infection with MHV-A59.
[d]Averages ± SEM are shown.

some of these lesions (Figure 3D, E) is also indicative of primary demyelination. In addition to the lesions characterized by primary demyelination, there were also multiple small round lesions characterized by necrosis that were found predominantly in the white matter but also less frequently in the grey matter (average of 8.71 ± 8.06 necrotic lesions in white matter in six of seven mice, 2.14 ± 2.91 necrotic lesions in the grey matter in three of seven mice). Acute necrosis has been reported in mice infected with MHV-JHM early after infection (Jordan *et al*, 1989), but is very rare in chronic demyelination in parental C57BL/6 mice.

To determine whether the demyelination was associated with viral replication and to address the question of whether antibody exacerbates lesion formation, we controlled viral loads by injecting 200 μl of HIS (NT50 of 1:5000), age-matched naive serum, or sterile PBS i.p. at 13, 15, 17, and 19 days postinfection. Such treatment was previously used successfully to clear or control infectious virus in parental C57BL/6 mice (Matthews *et al*, 2001). However, although viral titers were significantly reduced ($P = 0.005$ by *t*-test), virus was still present at fairly high titers ($10^{3.6}$ pfu/g in antibody treated RAG1$^{-/-}$ mice versus $10^{5.3}$ in untreated RAG1$^{-/-}$ mice). The severity of demyelination was not significantly affected as detected by percent quadrants demyelinating (48.0% ± 21.6 in antibody treated RAG1$^{-/-}$ mice ($n = 4$) versus 60.7% ± 8.1 in untreated RAG1$^{-/-}$ mice ($n = 3$)) or surface area involved (1.6 ± 0.42 in antibody treated RAG1$^{-/-}$ mice versus 1.6 ± 0.29 in untreated RAG1$^{-/-}$ mice). Although these data did not allow us to confirm that the demyelinating lesions in RAG1$^{-/-}$ mice are due to persistent infectious virus, they did demonstrate that additional antibody does not exacerbate lesion formation.

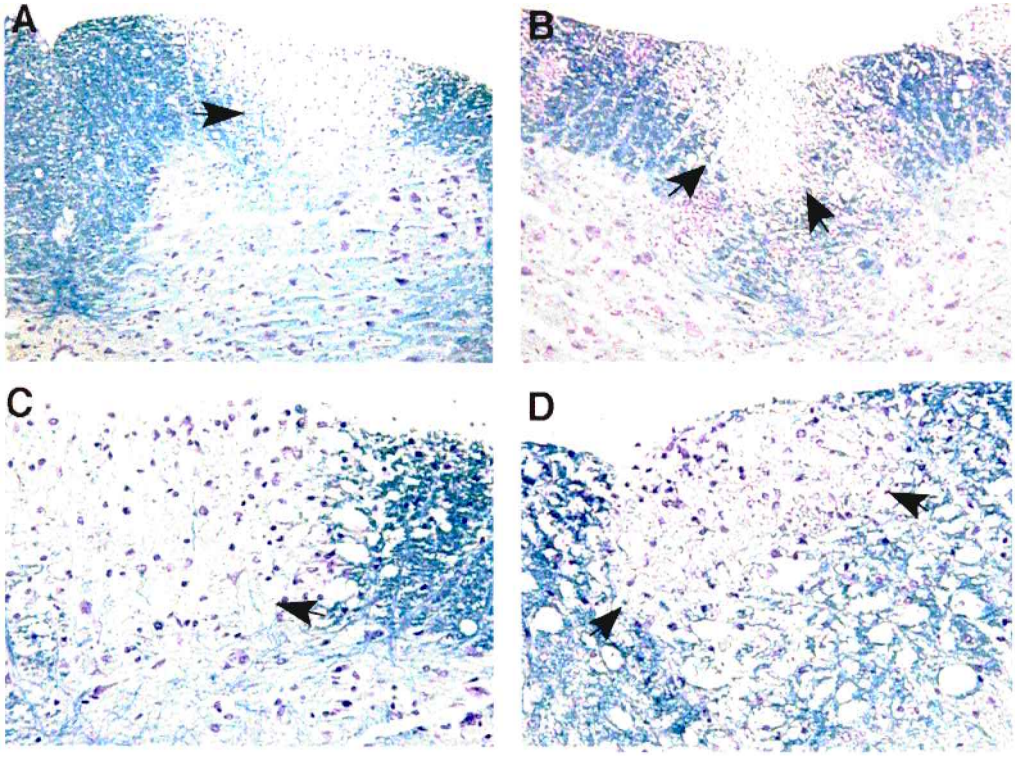

Figure 2 Luxol fast blue staining of MHV-A59-infected RAG1$^{-/-}$ mouse spinal cord sections. RAG1$^{-/-}$ mice were infected i.c. with 500 p.f.u. MHV-A59 at the same time as a protective dose of hyperimmune serum raised against MHV-A59 was injected i.p. Mice were killed and spinal cords were collected at 22 to 33 d.p.i. After fixation in formalin, spinal cords were stained with luxol fast blue which stains normally myelinated white matter blue. Examples of demyelinating lesions, indicated by arrows, in C57BL/6 (**A**, **C**) and RAG1$^{-/-}$ (**B**, **D**) mice are shown at original magnification of 100× (**A**, **B**) and 400× (**C**, **D**).

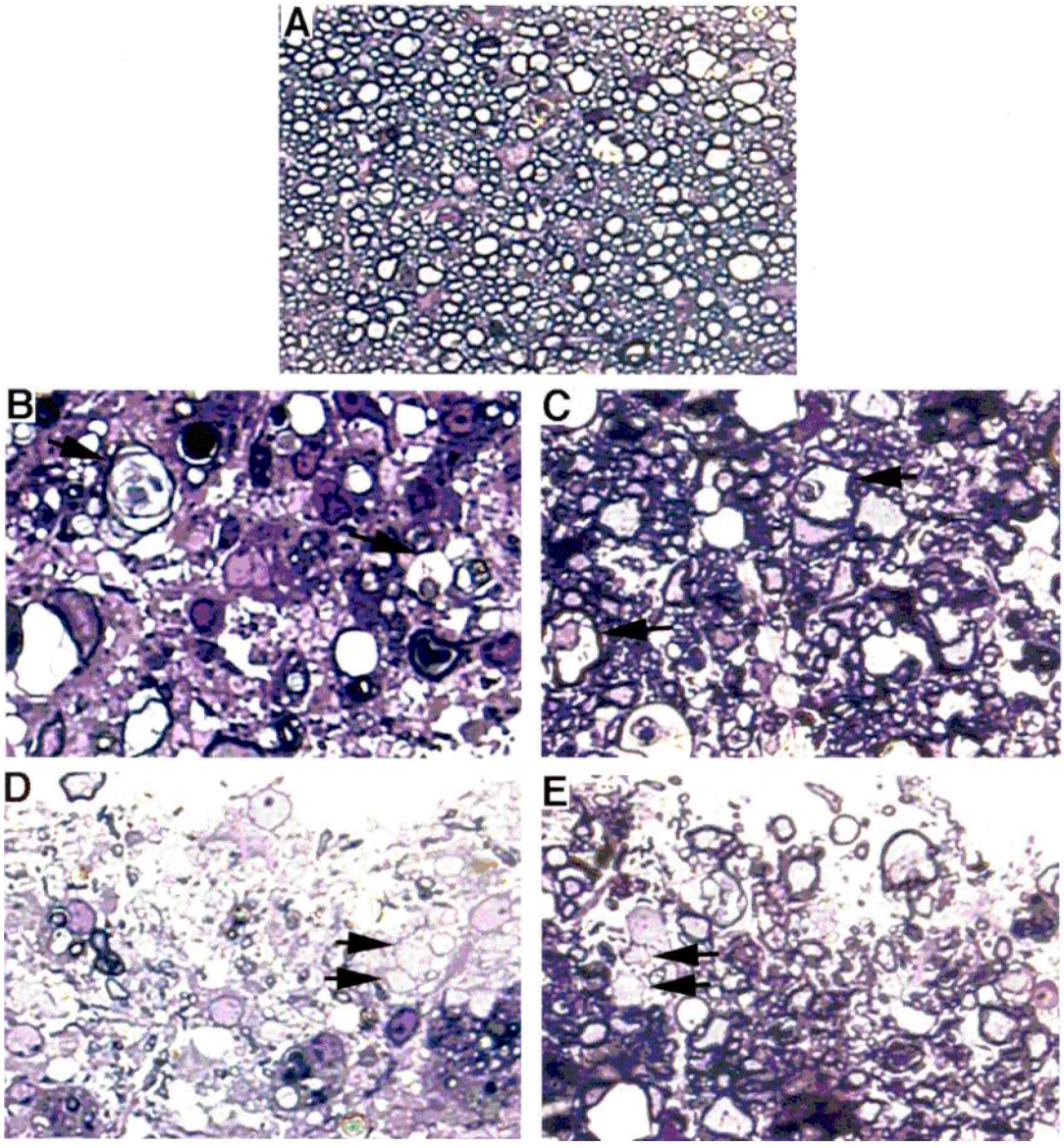

Figure 3 Toluidine blue staining of plastic embedded MHV-A59-infected parental C57BL/6 and RAG1$^{-/-}$ mouse spinal cord sections. RAG1$^{-/-}$ and C57BL/6 mouse spinal cords were collected 27 days postinfection and embedded in plastic. Toluidine blue was utilized to stain the myelin sheaths. A normal region of myelinated axons in the spinal cord of a C57BL/6 mouse is shown in (**A**). Myelin sheaths can be observed ballooning away from the axon in a C57BL/6 mouse (**B**) and in a RAG1$^{-/-}$ mouse (**C**). Naked axons, the sign of a successful primary demyelination, are present in both C57BL/6 (**D**) and RAG1$^{-/-}$ mice (**E**) and are indicated by arrows. In (**B–E**), the absence of significant numbers of normal myelinated axons, the presence of ballooning myelin sheaths, and the invasion of large macrophages creates the impression that a higher magnification was used than in (**A**). The original magnification for all five images was 1400×.

In contrast to our results in RAG1$^{-/-}$ mice, studies in which RAG1$^{-/-}$ (Wu and Perlman, 1999) or SCID (Houtman and Fleming, 1996) immunodeficient mice were infected with MHV-JHM revealed little to no demyelination even when adoptive transfer of neutralizing monoclonal antibodies was used to ensure survival to 30–40 days p.i. (Wu and Perlman, 1999). In our studies, polyclonal antibodies from MHV-A59 immune serum were used to control the initial viral infection and thus it is possible that this could contribute to the following disease. However, the half-life of MHV-specific monoclonal antibodies in uninfected mice has been determined to be between 8 and 13 days (Talbot and Buchmeier, 1987). Antibody in the presence of its antigen should have an even higher turnover rate. Therefore, it is unlikely that much antibody persists after acute infection. Also, transferring additional antibody during days 13–20 after infection of RAG1$^{-/-}$ mice did not result in any increase in demyelination, suggesting that antibody does not exacerbate lesions as it does in some models of EAE (Linington and Lassmann, 1987; Schluesener *et al*, 1987; Litzenburger *et al*, 1998). In MHV-JHM-infected mice, antibody treatment at injection

resulted in mice that developed symptoms but not detectable demyelination 23–47 d.p.i. (Wu and Perlman, 1999). Therefore, we suggest that the presence of antibody at the time of infection was not responsible for the presence of demyelination seen in our experiments with RAG1$^{-/-}$ mice.

Another possibility is that MHV strain A59 stimulates much more robust CNS demyelination in the absence of T and B cells than does MHV strain JHM. The presence of occasional demyelinating lesions in MHV-JHM-infected immunodeficient mice, though rare, suggests that this difference is quantitative rather than qualitative (Houtman and Fleming, 1996; Wu *et al*, 2000).

In B-cell-deficient mice, the persistence of infectious virus that can directly cause demyelination, makes it difficult to attribute the demyelinating effect observed to a lack of antibodies in these mice. We attempted to circumvent this problem by testing downstream mechanisms of antibody activity using transgenic mice lacking some of the pathways through which antibody normally mediates its function. Specifically, antibody can lyse infected cells or destroy virus by (1) activating the complement pathyway; (2) inducing antibody-dependent cell cytotoxitiy (ADCC); or (3) serving as a signal for phagocytosis. Complement activation has been implicated as the mechanism through which antibodies mediate demyelination in other models of demyelination (Linington *et al*, 1989; Ozawa *et al*, 1989; Piddlesden *et al*, 1993) and in MS (Lucchinetti *et al*, 2000), and therefore was a pathway of particular interest. In addition, recent work has demonstrated that antibody can enhance macrophage phagocytosis of myelin *in vitro* (Van der Goes *et al*, 1999), suggesting that FcR and opsonization could be crucial for macrophage mediated demyelination.

The C3-deficient mouse (backcrossed 10 generations onto C57BL/6 and kindly donated by Dr John Lambris with the permission of Harvey Colten (Circolo *et al*, 1999) does not express complement 3 protein and therefore cannot activate its complement pathway. The FcγR subchain-deficient mouse (C57BL/6 background; C57BL/6JMTac-[KO]Fcεr1γ,Taconic) lacks FcγR-I and III and therefore lacks ADCC. This strain also lacks opsonization *in vitro*, even that mediated by FcγR-II. The FcR double knockout (DKO) (only available on C57BL/6 $\times$ 129 background; (C57BL/6 $\times$ 129)-[KO]Fcεr1γ-[KO]FcγR2, Taconic) also lacks the other IgG receptor, FcγR-II, and therefore is incapable of opsonizing antibody coated cells or virus. We first tested these mice for viral clearance and determined that at 30 d.p.i. virus is cleared from brain and liver in all 3 strains ($n = 6$–10 mice per group). Therefore, unlike the situation in the B-cell-deficient mice, there is no confounding factor of viral persistence in the C3- and FcR-deficient mice. Demyelination was assessed by luxol fast blue staining. All three strains of mice exhibited obvious demyelinating lesions. When demyelination was quantified, there was no statistically significant difference in demyelination in any group compared to parental C57BL/6 mice (Table 2). Even though demyelination in the FcR DKO mice appears lower than in wild-type mice this is not a statistically significant difference ($P = 0.1113$ for percent quadrants demyelinating, $P = 0.056$ for surface area involved). Therefore, neither complement activation, ADCC, nor opsonization are required for MHV-A59-induced demyelination.

Table 2 Demyelination in mice lacking antibody effector pathways due to the absence of FcR or complement

Mouse strain	*Number of mice*	*% Quadrants demyelinating*[b]	*Area score for demyelination*
FcRγ KO	5	28.8 ± 13.9[c]	0.82 ± 0.39
C57BL/6	5	18.4 ± 8.16	0.63 ± 0.27
FcRγ DKO[a]	5	32 ± 13.5	1.04 ± 0.33
B6/129	5	57 ± 3.3	1.82 ± 0.11
C3 KO	6	21.4 ± 27.8	0.80 ± 0.43
C57BL/6	10	25.6 ± 18.8	0.96 ± 0.21

[a]Background of this strain is (C57BL/6 $\times$ 129). All other strains are on the C57BL/6 background.
[b]Animals were infected with 50 pfu of MHV-A59 i.c. C3 deficient mice and their 6 C57BL/6 controls were infected at 7–8 weeks of age. All other mice were infected at 4 weeks of age.
[c]Averages ± SEM are shown.

The prolonged persistence of demyelinating lesions in B-cell-deficient mice (Figure 1) suggests that healing by remyelination may be impaired. Studies in infectious, autoimmune, and toxic models of CNS demyelination have demonstrated the intriguing finding that passive transfer of IgM antibodies specific for various antibodies can promote remyelination (Rodriguez, 1991; Miller *et al*, 1994; van Engelen *et al*, 1994; Miller *et al*, 1997; Pavelko *et al*, 1998). To directly assess whether remyelination occurs in the absence of antibody, we embedded spinal cords from B-cell-deficient mice in Epon to preserve myelin sheath structure and stained with toluidine blue at various times following infection with MHV-A59. Remyelination was defined as regions where the axons have thin myelin sheaths as compared to the predominant myelin sheath thickness of other axons of similar diameter.

Of two B-cell-deficient mice tested at each time point, both mice demonstrated remyelination 60 d.p.i., yet no mice demonstrated remyelination 30 d.p.i. or 94–103 d.p.i. (Figure 4). C57BL/6 mice demonstrated remyelination at all time points. These data show that antibody is not absolutely required for remyelination. In the absence of antibody, remyelination is less frequently observed, but the persistence of infectious virus may also be affecting the development of remyelination.

We assessed a larger number of mice 60 d.p.i. and specifically chose mice with high viral titers to determine whether remyelination occurred in the

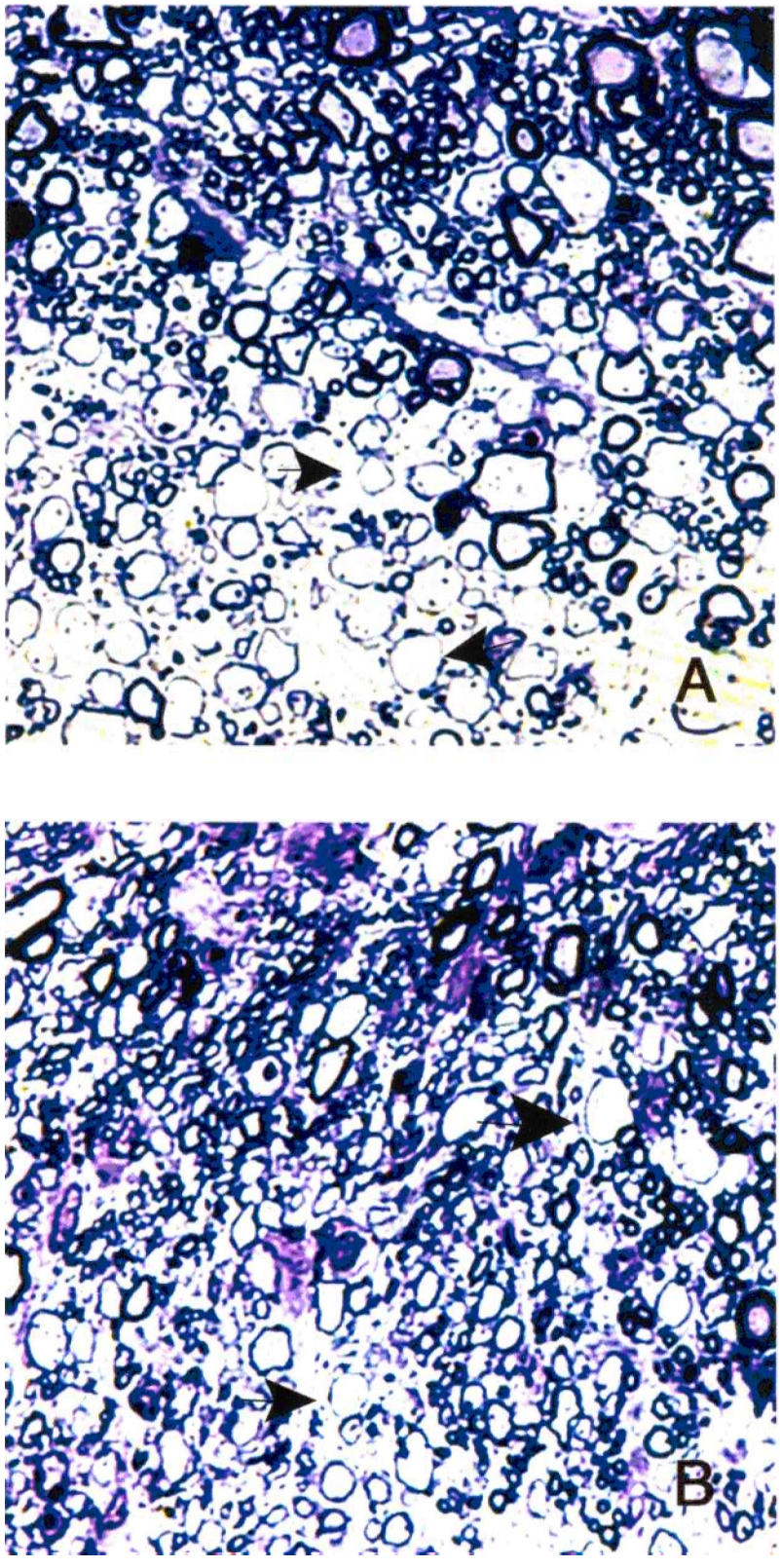

Figure 4 Remyelination in C57BL/6 and B-cell-deficient mice. Parental C57BL/6 and B-cell-deficient muMT mice were infected with 50 p.f.u. i.c. at 4 weeks of age. At 60 days postinfection, spinal cords were collected and embedded in plastic. Remyelination, which is characterized by myelin sheaths that are thin relative to axonal diameter as indicated by the arrows, is detectable in both C57BL/6 mice (**A**) and in muMT mice (**B**). Magnification is ×1200.

presence of concurrent viral replication. In muMT mice with viral titers between $10^{3.1}$ and $10^{5.6}$, remyelination was detected in five of five mice. Similarly, in C57BL/6 mice, none of whom had detectable viral titers 60 d.p.i., remyelination was detected in four of four mice. When the total number of remyelinating lesions was quantitated, no significant difference was detected (an average of 3.8 remyelinating lesions in muMT mice, 5.5 in C57BL/6 mice, $P = 0.2148$). Although it would be ideal to carefully measure and compare the areas of white matter involved in remyelination versus demyelination, muMT mice maintain active demyelination at 60 d.p.i. and have markedly more demyelination than C57BL/6 mice at this time point, frustrating such a comparison. We conclude from the previous data that persistent infectious MHV is not sufficient to inhibit CNS remyelination, nor does remyelination decrease markedly in the absence of antibody, although the onset of remyelination may be delayed.

If, as our data and the data of others suggest, neither $\alpha\beta$ T cells nor B cells alone are required for demyelination, the question arises as to whether the small percentage of lymphocytes that are $\gamma\delta$T cells are involved in this process. We infected $\gamma\delta$T cell-deficient mice with 50 pfu MHV-A59 i.c. ($n = 4$) and determined that these cells are also not required for demyelination (48% spinal cord quadrant demyelination).

Our data fit with an increasingly complex model for demyelination after MHV infection. No specific cell compartment [blood-born macrophages (Xue *et al*, 1999), $CD4^+$ T cells (Talbot and Buchmeier, 1987; Houtman and Fleming, 1996; Sutherland *et al*, 1997; Wu *et al*, 2000) , $CD8^+$ T cells (Talbot and Buchmeier, 1987; Gombold *et al*, 1995; Houtman and Fleming, 1996; Sutherland *et al*, 1997; Wu *et al*, 2000), $\gamma\delta$T cells (this study)] yet examined appears to be required for demyelination. In this study we determine that B cells are also not required for demyelination, nor do antibodies detectably enhance demyelination in persistently infected $RAG1^{-/-}$ mice. In complementary data, we determined that neither complement nor FcRs are necessary for robust demyelination. We have also demonstrated here the novel finding that MHV-A59 in the absence of lymphocytes can also cause demyelination, an observation that not only may account for the enhanced demyelination in B cell deficient mice but also adds another layer of complexity to the mechanism of demyelination in MHV-A59-induced disease. Therefore, although acquired immune responses and/or innate immune responses may play a role in early demyelination, it appears that in the absence of an intact immune response competent to control virus replication, persistent virus in the CNS is directly responsible for demyelination probably through the destruction of oligodendrocytes.

References

Barac-Latas V, Suchanek G, Breitschopf H, Stuehler A, Wege H, Lassmann H (1997). Patterns of oligodendrocyte pathology in coronavirus-induced subacute demyelinating encephalomyelitis in the Lewis rat. *Glia* **19:** 1–12.

Buchmeier MJ, Lewicki HA, Talbot PJ, Knobler RL (1984). Murine hepatitis virus-4 (strain JHM)-induced neurologic disease is modulated *in vivo* by monoclonal antibody. *Virology* **132:** 261–270.

Circolo A, Garnier G, Fukuda W, Wang X, Hidvegi T, Szalai AJ, Briles DE, Volanakis JE, Wetsel RA, Colten HR (1999). Genetic disruption of the murine complement C3 promoter region generates deficient mice with extrahepatic expression of C3 mRNA. *Immunopharmacology* **42:** 135–149.

Das Sarma J, Fu L, Tsai JC, Weiss SR, Lavi E (2000). Demyelination determinants map to the spike glycoprotein gene of coronavirus mouse hepatitis virus. *J Virol* **74:** 9206–9213.

Fleming JO, Wang FI, Trousdale MD, Hinton DR, Stohlman SA (1993). Interaction of immune and central nervous systems: contribution of anti-viral Thy-1+ cells to demyelination induced by coronavirus JHM. *Reg Immunol* **5:** 37–43.

Gombold JL, Sutherland RM, Lavi E, Paterson Y, Weiss SR (1995). Mouse hepatitis virus A59-induced demyelination in mice can occur in the absence of $CD8^+$ T cells. *Microb Pathogen* **18:** 211–221.

Herndon RM, Price DL, Weiner LP (1977). Regeneration of oligodendroglia during recovery from demyelinating disease. *Science* **195:** 693–694.

Houtman JJ, Fleming JO (1996). Dissociation of demyelination and viral clearance in congenitally immunodeficient mice infected with murine coronavirus JHM. *J NeuroVirol* **2:** 101–110.

Jordan CA, Friedrich VL Jr, Godfraind C, Cardellechio CB, Holmes KV, Dubois-Dalcq M (1989). Expression of viral and myelin gene transcripts in a murine CNS demyelinating disease caused by a coronavirus. *Glia* **2:** 318–329.

Lampert PW, Sims JK, Kniazeff AJ (1973). Mechanism of demyelination in JHM virus encephalomyelitis. Electron microscopic studies. *Acta Neuropathol (Berl)* **24:** 76–85.

Lavi E, Gilden DH, Wroblewska Z, Rorke LB, Weiss SR (1984a). Experimental demyelination produced by the A59 strain of mouse hepatitis virus. *Neurology* **34:** 597–603.

Lavi E, Gilden DH, Highkin MK, Weiss SR (1984b). Persistence of mouse hepatitis virus A59 RNA in a slow virus demyelinating infection in mice as detected by *in situ* hybridization. *J Virol* **51:** 563–566.

Linington C, Lassmann H (1987). Antibody responses in chronic relapsing experimental allergic encephalomyelitis: correlation of serum demyelinating activity with antibody titre to the myelin/oligodendrocyte glycoprotein (MOG). *J Neuroimmunol* **17:** 61–69.

Linington C, Morgan BP, Scolding NJ, Wilkins P, Piddlesden S, Compston DA (1989). The role of complement in the pathogenesis of experimental allergic encephalomyelitis. *Brain* **112:** 895–911.

Litzenburger T, Fassler R, Bauer J, Lassmann H, Linington C, Wekerle H, Iglesias A (1998). B lymphocytes producing demyelinating autoantibodies: development and function in gene-targeted transgenic mice. *J Exp Med* **188:** 169–180.

Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H (2000). Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination [see comments]. *Ann Neurol* **47:** 707–717.

Lucchinetti CF, Bruck W, Rodriguez M, Lassmann H (1996). Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. *Brain Pathol* **6:** 259–274.

Mastaglia FL, Carroll WM, Jennings AR (1989). Spinal cord lesions induced by antigalactocerebroside serum. *Clin Exp Neurol* **26:** 33–44.

Matthews AE, Weiss SR, Shlomchik M, Hannum LG, Gombold J, Paterson Y (2001). Antibody is required for clearance of infectious murine hepatitis virus A59 from the CNS but not the liver. *J Immunol* **167:** 5254–5263.

Miller DJ, Bright JJ, Sriram S, Rodriguez M (1997). Successful treatment of established relapsing experimental autoimmune encephalomyelitis in mice with a monoclonal natural autoantibody. *J Neuroimmunol* **75:** 204–209.

Miller DJ, Sanborn KS, Katzmann JA, Rodriguez M (1994). Monoclonal autoantibodies promote central nervous system repair in an animal model of multiple sclerosis. *J Neurosci* **14:** 6230–6238.

Ozawa K, Saida T, Saida K, Nishitani H, Kameyama M (1989). *In vivo* CNS demyelination mediated by antigalactocerebroside antibody. *Acta Neuropathol* **77:** 621–628.

Pavelko KD, van Engelen BG, Rodriguez M (1998). Acceleration in the rate of CNS remyelination in lysolecithin-induced demyelination. *J Neurosci* **18:** 2498–2505.

Piddlesden SJ, Lassmann H, Zimprich F, Morgan BP, Linington C (1993). The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement. *Am J Pathol* **143:** 555–564.

Rodriguez M (1991). Immunoglobulins stimulate central nervous system remyelination: electron microscopic and morphometric analysis of proliferating cells. *Lab Invest* **64:** 358–370.

Schluesener HJ, Sobel RA, Linington C, Weiner HL (1987). A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease. *J Immunol* **139:** 4016–4021.

Sutherland RM, Chua MM, Lavi E, Weiss SR, Paterson Y (1997). $CD4^+$ and $CD8^+$ T cells are not major effectors of mouse hepatitis virus A59-induced demyelinating disease. *J NeuroVirol* **3:** 225–228.

Talbot PJ, Buchmeier MJ (1987). Catabolism of homologous murine monoclonal hybridoma IgG antibodies in mice. *Immunology* **60:** 485–489.

Van der Goes A, Kortekaas M, Hoekstra K, Dijkstra CD, Amor S (1999). The role of anti-myelin (auto)-antibodies in the phagocytosis of myelin by macrophages. *J Neuroimmunol* **101:** 61–67.

van Engelen BG, Miller DJ, Pavelko KD, Hommes OR, Rodriguez M (1994). Promotion of remyelination by polyclonal immunoglobulin in Theiler's virus-induced demyelination and in multiple sclerosis. *J Neurol Neurosurg Psych* **57** (Suppl)**:** 65–68.

Wang FI, Stohlman SA, Fleming JO (1990). Demyelination induced by murine hepatitis virus JHM strain (MHV-4) is immunologically mediated. *J Neuroimmunol* **30:** 31–41.

Wu GF, Dandekar AA, Pewe L, Perlman S (2000). CD4 and CD8 T cells have redundant but not identical roles in virus-induced demyelination. *J Immunol* **165:** 2278–2286.

Wu GF, Perlman S (1999). Macrophage infiltration, but not apoptosis, is correlated with immune-mediated demyelination following murine infection with a neurotropic coronavirus. *J Virol* **73:** 8771–8780.

Xue S, Sun N, Van Rooijen N, Perlman S (1999). Depletion of blood-borne macrophages does not reduce demyelination in mice infected with a neurotropic coronavirus. *J Virol* **73:** 6327–6334.