

Chapter C8

CORONAVIRUS-INDUCED DEMYELINATION AND SPONTANEOUS REMYELINATION

Growth factor expression and function

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Abstract: MHV-A59 coronavirus infection produces a transient episode of demyelination that is followed by spontaneous remyelination. This paradigm provides a complex lesion environment to examine cellular and molecular mechanisms involved in successful CNS remyelination. Our work in this model has focused on the roles of platelet-derived growth factor and fibroblast growth factor 2 in regulating oligodendrocyte progenitor responses required for remyelination.

Key words: platelet-derived growth factor, fibroblast growth factor, estrous cycle, gender, demyelinating disease, remyelination, coronavirus, cuprizone

1. INTRODUCTION

Insufficient remyelination results in prolonged neurological impairment in demyelinating disease states, such as multiple sclerosis. A critical determinant of remyelination is regulation of oligodendrocyte lineage responses. Surviving and/or newly generated oligodendrocyte lineage cells must be recruited to appropriate sites within demyelinated tissues and induced to differentiate and form myelin. Each of these oligodendrocyte lineage cell responses appears to be regulated by signals within the lesion environment, such as growth factors, cytokines, and cell-cell interactions.

The pathology of multiple sclerosis (MS) lesions is heterogeneous between patients, with at least four fundamentally different patterns of

demyelination (12). Therefore, analysis of experimental models of demyelination with distinct mechanisms of pathogenesis is warranted. In addition, different experimental models have advantages for examining specific aspects within the course of demyelinating diseases. The mouse model of murine hepatitis strain A59 (MHV-A59) coronavirus infection serves as a relevant model for analyzing the cellular and molecular components involved in spontaneous remyelination. The complexity of MHV-A59 lesions includes infiltration of CD8+ and CD4+ T cells, B lymphocytes producing immunoglobulins, macrophages, and reactive glial cells (15,21). These lesion components are variably exhibited among categories of MS lesions.

The potential function of molecules that can promote remyelination in MS lesions, such as growth factors, is ideally analyzed in the context of a complex lesion environment due to contributing effects of cytokines, chemokines, infiltrating lymphocytes, and reactive cells. However, this complex lesion milieu can also make it difficult to delineate effects that are specific to the remyelination process. For this purpose, analysis of oligodendrocyte lineage responses is facilitated by comparison with a simpler lesion model, such as ingestion of cuprizone (14). Growth factor effects common to experimental lesions of diverse pathogenesis, such as MHV-A59 and cuprizone models, are most likely to be applicable more generally to demyelinating diseases.

This chapter will review recent findings of the expression and function of specific growth factors in MHV-A59 and cuprizone models of spontaneous remyelination. In addition, the complexity of the MHV-A59 model will be exemplified by discussion of the modulation of the disease course in correlation with gender and estrous cycle status. This modulation of the MHV-A59 disease course is also relevant to modulation of MS disease activity.

2. DISEASE SEVERITY IN THE MHV-A59 MODEL

Intracranial infection of female C57Bl/6 mice at 28 days of age with 1000 plaque forming units (PFU) of MHV-A59 virus produces a characteristic progression of demyelinating disease. Demyelination begins within the first week post-injection (wpi), with more extensive areas of myelin degeneration by 2 wpi (1,9). During this progression of demyelination, mice exhibit loss of motor function, and virus is present in cells of the white matter (9). In subsequent weeks, clearance of virus and myelin debris occurs, remyelination is initiated, and recovery of motor function proceeds (9,21). However, among mice similarly infected with MHV-A59, there is variability in the proportion of mice that exhibit distinct results from asymptomatic,

mild paresis, severe paralysis, to mortality. Early studies reported mortality rates that ranged from 11% (1) to 70% (11).

To take advantage of knockout mice for the analysis of growth factors and cellular components involved in spontaneous remyelination in this model, we needed to optimize the reproducibility of the MHV-A59 infection outcome. This objective led us to explore more quantitative methods to monitor disease progression and identify determinants of variation in disease severity, including mortality.

Intracranial MHV-A59 injection of 1000 PFU at 28 days of age has been used to maximize demyelination and minimize mortality compared to infection of younger mice (1,2,21). However, in addition to an age factor, a correlation was also observed between weight and mortality. Female 28-day old mice that eventually died during the acute infection weighed 12 ± 0.34 g (mean \pm s.e.; $n = 25$) while survivors weighed 13.5 ± 0.22 (mean \pm s.e.; $n = 51$) ($p < 0.0005$, unpaired t -test). Accordingly, subsequent experiments used only mice weighing 13-15 g at 28 days of age.

Variability of infection outcome still occurred among female mice within these weight and age restrictions. Measures were already in place to minimize experimental variability from animal husbandry and experimental treatment. Additional tests were conducted to quantify the neurological effects of MHV-A59 lesions and determine whether the infection outcomes were clearly different, which would suggest an additional factor contributing to infection outcome. A “hang time” test of limb motor function and grasping was chosen to assess the result of MHV-A59 infection because the lesion predilection sites include the spinal cord white matter adjacent to the ventral root exit zones throughout all spinal cord levels (8,10,21).

To perform the hang time test, mice were placed onto a wire cage top, and once the mice had gripped the bars the top was turned upside-down and held horizontally above a table (similar to ref. 23). The length of time that each mouse held onto the bars was recorded, up to a maximum of 60 seconds. Control mice easily grasped the cage top bars with paws, often curled tails around the bars, and moved around to explore while upside-down (Figure 1A). In contrast, mice with impaired limb motor function could not grasp cage top bars with paws, rarely curled tails around bars, and dropped off prior to the 60 seconds maximum time limit (Figure 1B). Inability to hold on was associated with demyelination, as evident in previous reports of the histopathology of MHV-A59 infected mice (1,21).

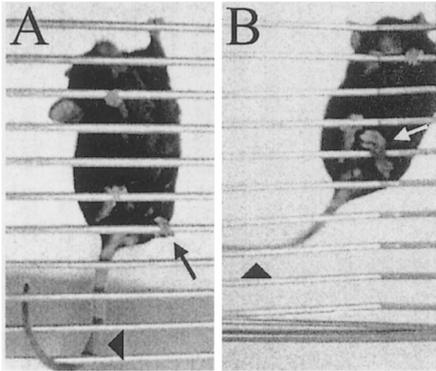


Figure 1. Control (A) and MHV-A59 infected (B) mice shown hanging from a cage top during hang time tests. The control mouse can easily grasp the bars with paws (black arrow) and tail (black arrowhead). The MHV-A59 infected mouse has hind limb and tail impairment so that the paws cannot normally grasp the bars (white arrow) and the tail is limp (black arrowhead).

The time course of individual mouse hang time performance over 4 wpi showed that some mice had only mild motor dysfunction while others had severe motor dysfunction (Figure 2). Operational definitions for mild and severe groups can be made several ways. One method is to sum hang times over the disease course, through 4 wpi when many mice have fully recovered. Clustering of subgroups of mice is evident as either a low summed hang time (severe motor dysfunction) or a high summed hang time (mild motor dysfunction). However, to allow mice to be used prior to the recovery phase, a practical set of criteria was needed by 2 wpi. After testing the hang time of mice at 7, 10, and 14 days post-infection (dpi), a mouse that had 2 or 3 hang time values of > 30 seconds or recovered to a 60 second hang time by 14 dpi was categorized as only mildly affected. A mouse that had 2 or 3 hang times ≤ 30 seconds and did not recover to 60 seconds by 14 dpi was categorized as severely affected.

In many models of experimental demyelination involving the spinal cord, disease severity is commonly quantified by a clinical scoring system. An adapted clinical rating has been applied in the MHV-A59 model (21). The clinical score scale is a measure of impairment based upon observed paresis or paralysis of limbs, so that 0 = no observable paresis/paralysis, 1-5 = paresis/paralysis in 1-5 limbs, respectively (such as limp tail, abnormal limb contracture or extension, dragging of limb, abnormal lateral movement of limb), and 6 = morbidity. The clinical scores of mildly affected mice over time are significantly less than the clinical scores of severely affected mice (Figure 2). However, the limited unit range of the clinical score scale does not detect individual variation as well as the hang time test in the MHV-A59 model.

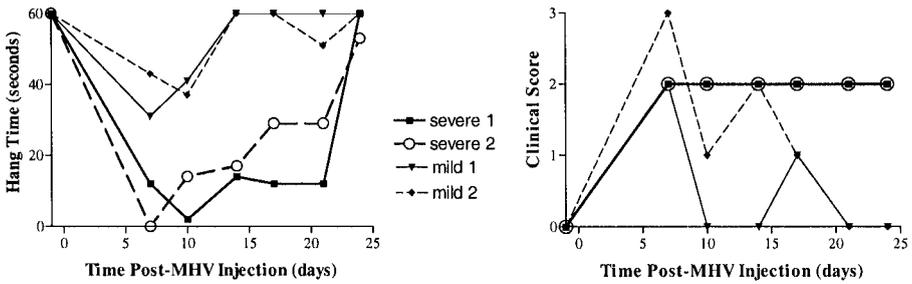


Figure 2. Examples of hang time and clinical score results. Individual records are shown for 2 mildly affected mice and 2 severely affected mice as representative examples of the variability in disease severity. The same mice are shown in both graphs. The hang times shown on each day are the averages of 3 trials.

3. MHV-A59 DISEASE SEVERITY RELATIVE TO GENDER AND ESTROUS CYCLE

Sex hormones have a role in disease susceptibility and severity in MS, which has been replicated in experimental autoimmune encephalomyelitis (EAE) in SJL mice (25). While females are more susceptible to MS than males (26), a worse survival curve is associated with male gender in primary progressive MS (6). In females with relapsing-remitting MS, worsening disease activity has been reported to vary with estrous cycle stage, but results attempting to correlate further to hormone levels have produced conflicting results (4,19,24,29).

To identify an underlying factor in the variability of disease severity in our MHV-A59 model, estrous cycle stage was monitored using vaginal smears (7)(Figure 3). Severely affected female mice were more likely to be in diestrus or metestrus during the early effector stage of the pro-inflammatory response (4 dpi). Estradiol and progesterone are relatively low at these stages of the estrous cycle. Female mice in proestrus or estrus (at 4 dpi), when estradiol and progesterone levels are elevated, more frequently experienced a milder outcome after MHV-A59 infection. Interestingly, EAE was ameliorated in SJL mice treated with estradiol at a sustained high dose, regardless of the dose of progesterone used in combination (25). However, the dose of estradiol needed to significantly decrease EAE severity was above levels that occur naturally during the estrous cycle (25). Thus, the MHV-A59 pathogenesis may be very sensitive to variation of hormone levels during the estrous cycle.

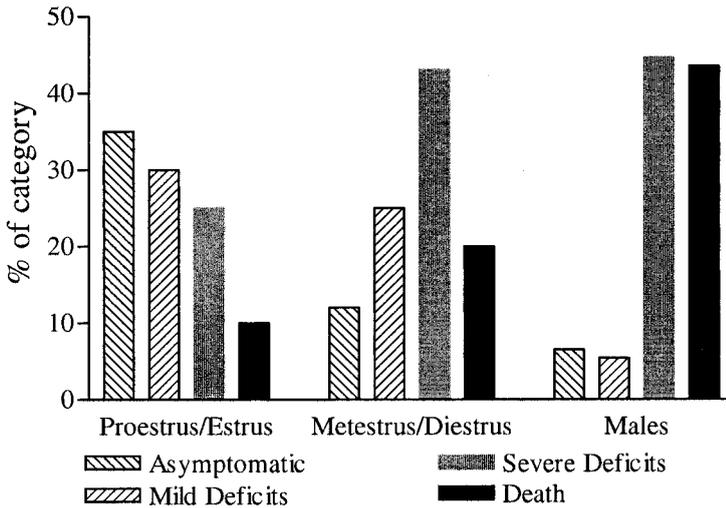


Figure 3. Outcome of MHV-A59 infection in female mice, at different stages of the estrous cycle, and male mice. The outcome of infection in each mouse was based upon hang time score using the classification described in the text. Each value represents mice combined from at least 3 separate infection experiments. Female mice, Proestrus/Estrus $n = 20$, Metestrus/Diestrus, $n = 129$; combined from 6 separate sets of infections; sampling difference reflects the proportional difference in the duration of the stages within the 4 day mouse menstrual cycle. Male mice, $n = 92$; combined from 3 separate sets of infections.

Male mice in this MHV-A59 model clearly had a more severe disease course and higher rate of mortality than female mice. A Th1 cytokine response, specifically interferon gamma, is required for clearing of the MHV-A59 virus from oligodendrocytes and resolution of the disease as a model of transient demyelination (18). Male hormonal conditions favor a bias towards Th2 cytokine production rather than a Th1 response (28), which may correlate with a lack of interferon gamma production in lesions of males and explain the increased rates of mortality compared to females.

4. GROWTH FACTOR EXPRESSION FOLLOWING DEMYELINATION

Discussion of growth factor pathways in MHV-A59 lesions will focus on oligodendrocyte lineage responses during remyelination. Given the variability of MHV-A59 disease severity noted above, studies of growth factors in this model utilize only the mice that are classified as “severe” to provide a reproducible status. Also, when possible, findings in the MHV-A59 model will be compared with the cuprizone model to establish common mechanisms.

Experimental models with different pathogenesis indicate that, when remyelination occurs, oligodendroglial repopulation of demyelinated lesions may result from a common mechanism: local proliferation and recruitment of oligodendrocyte progenitor cells. *In vitro* studies predict that specific growth factors regulate the oligodendrocyte progenitor responses needed for myelination during development and for remyelination (*i.e.* proliferation, migration, differentiation, and survival). Among the growth factors known to regulate the oligodendrocyte lineage, platelet-derived growth factor (AA homodimer, PDGF) and fibroblast growth factor 2 (FGF2) warrant high priority for analysis.

In vitro studies have documented the ability of PDGF and FGF2 to regulate responses of oligodendrocyte lineage cells isolated from normal neonatal and adult CNS. During development, neonatal oligodendrocyte progenitors proliferate in response to PDGF or FGF2, and grow as a self-renewing cell line in the combination of PDGF and FGF2 (5,16,22). PDGF and FGF2 can also enhance the proliferation of oligodendrocyte progenitor cells isolated from normal adult rodent CNS (13,27). FGF2 treatment of more mature stages of the oligodendrocyte lineage inhibits terminal differentiation (3).

In vivo studies have supported the hypothesis that growth factors may regulate oligodendrocyte progenitor responses involved in myelin repair. At the onset of remyelination (4 wpi) in spinal cords of MHV-A59 lesioned mice, increased expression of PDGF-A and FGF2 ligands, as well as the corresponding receptors, indicated the potential for each to act upon oligodendrocyte lineage cells involved in remyelination. Immunostaining for PDGF-A ligand demonstrated increased expression in reactive astrocytes in remyelinating lesions (21). *In situ* hybridization to detect PDGF-A mRNA revealed a 2.9 fold increase in the number of labeled cells in sections from MHV-A59 infected mice vs. PBS-injected control mice (21). Similar *in situ* hybridization of FGF2 demonstrated markedly increased signal in reactive astrocytes and microglia localized within and near white matter lesions (17). Over the course of MHV-A59 disease progression, FGF2 mRNA transcript abundance peaked at 4 wpi as greater than 5-fold over non-lesioned levels (17). At a corresponding stage in cuprizone demyelination, a similar increase was observed for FGF2 (2) and PDGF-A mRNA (unpublished observation). Interestingly, in both models the increased signal for FGF2 is clearly localized to the lesion while the increase in cells with detectable PDGF-A is much more widely distributed in the tissue. These distributions may indicate differences in the influence of these growth factors on the oligodendrocyte lineage cell response.

In addition to demonstrating the increased expression of PDGF-A and FGF2 mRNA, expression of corresponding receptors on oligodendrocyte lineage cells has been examined *in vivo*. In lesioned white matter undergoing remyelination after MHV-A59 infection, the number of cells expressing FGF receptors increased as much as 5-fold (17). Oligodendrocyte progenitors expressed receptors for both FGF2 and PDGF (specifically PDGF α R, the alpha form of PDGF receptor) (7,20,21). Oligodendrocytes also expressed FGF receptors (19,20).

In both the MHV-A59 model and the cuprizone model, the oligodendrocyte progenitor proliferative response corresponds with expression of PDGF α R (2,21). During early remyelination, there is a dramatic increase in the number of cells that are expressing PDGF α R mRNA transcripts and have incorporated bromodeoxyuridine (BrdU; indicative of DNA synthesis) during a 4-hour terminal *in vivo* pulse. In contrast, mature oligodendrocytes did not exhibit substantial proliferation. Taken together, these studies support the prediction that PDGF and FGF2 may play a role in the successful generation of oligodendrocyte lineage cells and subsequent remyelination observed in this mouse model; the ligands and receptors are expressed at an appropriate stage of the disease process and with the appropriate cellular localization, and association with a proliferation indicator.

A specific role of PDGF and FGF2 in regulating proliferation during remyelination has been demonstrated by culturing oligodendrocyte lineage cells from spinal cords of MHV-A59 infected mice. Approximately 20% of the white matter is demyelinated throughout the rostrocaudal levels of the spinal cord as a result of MHV-A59 infection (8). This extent of tissue involvement is sufficient for isolation of a population of oligodendrocyte progenitor cells that is significantly more proliferative than the population derived from normal adult mouse spinal cord (1). PDGF and FGF2, produced by reactive astrocytes and microglia within the cultures, are significant mitogens regulating this proliferative response (8). Interestingly, in cultures derived from different time points after MHV-A59 infection, the oligodendrocyte progenitor response varies with the disease stage; proliferation is more robust during the demyelination phase while differentiation is more extensive during remyelination (8).

Knockout and transgenic mice can test the function of growth factors throughout the progression of demyelination and remyelination, as has been done for FGF2 knockout mice (2). In both MHV-A59 and cuprizone models, oligodendroglial repopulation of lesions was enhanced in the absence of FGF2. During early remyelination, oligodendrocyte lineage cell proliferation and survival were not altered by the FGF2 genotype – possibly because other growth factors sufficiently supported these lineage responses (see ref. 2).

Thus, during remyelination, absence of FGF2 may promote oligodendroglial regeneration by enhancing oligodendrocyte progenitor maturation. This *in vivo* distinction among potential roles of FGF2 in differentiation versus proliferation demonstrates that transgenic and knockout mouse models are important for testing growth factor roles in the context of the lesion environment throughout the disease progression. The expression and activity of growth factors, and other regulatory molecules, change throughout the disease course. In addition, the oligodendrocyte lineage population changes with progressive stages of the disease course. Therefore, the effect of a given growth factor on the oligodendrocyte lineage population may vary *in vivo* during the disease course.

5. CONCLUSION

Future therapeutic developments may make it possible to intervene to promote remyelination for MS patients in which the disease progression can be arrested. Effective treatments to promote repair of demyelinated lesions will need to be tailored to the specific pathology of the patient and adapted for the disease stage. Animal models are clearly an important component for identifying and testing the potential roles of growth factors as candidate interventions to promote remyelination.

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REFERENCES

1. Armstrong R, Friedrich VL, Jr., Holmes KV, Dubois-Dalcq M. In vitro analysis of the oligodendrocyte lineage in mice during demyelination and remyelination. *J Cell Biol.* 1990;111:1183-1195.
2. Armstrong RC, Le TQ, Frost EE et al. Absence of fibroblast growth factor 2 promotes oligodendroglial repopulation of demyelinated white matter. *J Neurosci.* 2002;22:8574-8585
3. Bansal R, Pfeiffer SE. Regulation of oligodendrocyte differentiation by fibroblast growth factors. *Adv Exp Med Biol.* 1997;429:69-77

4. Bansil S, Lee HJ, Jindal S et al. Correlation between sex hormones and magnetic resonance imaging lesions in multiple sclerosis. *Acta Neurol Scand.* 1999;99:91-94
5. Bogler O, Wren D, Barnett SC et al. Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells. *Proc Natl Acad Sci U S A.* 1990;87:6368-6372
6. Cottrell DA, Kremenchutzky M, Rice GP et al. The natural history of multiple sclerosis: a geographically based study. 5. The clinical features and natural history of primary progressive multiple sclerosis. *Brain.* 1999;122 (Pt 4):625-639
7. Freeman ME. The neuroendocrine control of the ovarian cycle in the rat. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction.* 2nd ed. New York, NY: Raven Press, 1994:613-658
8. Frost EE, Nielsen JA, Le TQ, Armstrong RC. PDGF and FGF2 regulate oligodendrocyte progenitor responses to demyelination. *Journal of Neurobiology.* 2003; 54:457-472.
9. Jordan CA, Friedrich VL, Jr., Godfraind C et al. Expression of viral and myelin gene transcripts in a murine CNS demyelinating disease caused by a coronavirus. *Glia.* 1989;2:318-329
10. Jordan CA, Friedrich VL, Jr., de Ferra F et al. Differential exon expression in myelin basic protein transcripts during central nervous system (CNS) remyelination. *Cell Mol Neurobiol.* 1990;10:3-18
11. Kristensson K, Holmes KV, Duchala CS et al. Increased levels of myelin basic protein transcripts in virus-induced demyelination. *Nature.* 1986;322:544-547
12. Lucchinetti C, Bruck W, Parisi J et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol.* 2000;47:707-717
13. Mason JL, Goldman JE. A2B5+ and O4+ Cycling progenitors in the adult forebrain white matter respond differentially to PDGF-AA, FGF-2, and IGF-1. *Mol Cell Neurosci.* 2002;20:30-42
14. Matsushima GK, Morell P. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. *Brain Pathol.* 2001;11:107-116.
15. Matthews AE, Weiss SR, Paterson Y. Murine hepatitis virus--a model for virus-induced CNS demyelination. *J Neurovirol.* 2002;8:76-85
16. McKinnon RD, Matsui T, Dubois-Dalcq M, Aaronson SA. FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron.* 1990;5:603-614.
17. Messersmith DJ, Murtie JC, Le TQ et al. Fibroblast growth factor 2 (FGF2) and FGF receptor expression in an experimental demyelinating disease with extensive remyelination. *J Neurosci Res.* 2000;62:241-256.

18. Parra B, Hinton DR, Marten NW et al. IFN-gamma is required for viral clearance from central nervous system oligodendroglia. *J Immunol.* 1999;162:1641-1647
19. Pozzilli C, Falaschi P, Mainero C et al. MRI in multiple sclerosis during the menstrual cycle: relationship with sex hormone patterns. *Neurology.* 1999;53:622-624
20. Redwine JM, Blinder KL, Armstrong RC. In situ expression of fibroblast growth factor receptors by oligodendrocyte progenitors and oligodendrocytes in adult mouse central nervous system. *J Neurosci Res.* 1997;50:229-237.
21. Redwine JM, Armstrong RC. In vivo proliferation of oligodendrocyte progenitors expressing PDGFalphaR during early remyelination. *J Neurobiol.* 1998;37:413-428.
22. Richardson WD, Pringle N, Mosley MJ et al. A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell.* 1988;53:309-319
23. Sango K, McDonald MP, Crawley JN et al. Mice lacking both subunits of lysosomal beta-hexosaminidase display gangliosidosis and mucopolysaccharidosis. *Nat Genet.* 1996;14:348-352
24. Smith R, Studd JW. A pilot study of the effect upon multiple sclerosis of the menopause, hormone replacement therapy and the menstrual cycle. *J R Soc Med.* 1992;85:612-613
25. Voskuhl RR, Palaszynski K. Sex hormones in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. *Neuroscientist.* 2001;7:258-270
26. Whitacre CC, Reingold SC, O'Looney PA. A gender gap in autoimmunity. *Science.* 1999;283:1277-1278
27. Wolswijk G, Noble M. Cooperation between PDGF and FGF converts slowly dividing O-2A adult progenitor cells to rapidly dividing cells with characteristics of O-2A perinatal progenitor cells. *J Cell Biol.* 1992;118:889-900.
28. Zaffaroni M, Ghezzi A. The prognostic value of age, gender, pregnancy and endocrine factors in multiple sclerosis. *Neurol Sci.* 2000;21:S857-860
29. Zorgdrager A, De Keyser J. Menstrually related worsening of symptoms in multiple sclerosis. *J Neurol Sci.* 1997;149:95-97