Brazilian Journal of Medical and Biological Research (2001) 34: 603-609
IISSN 0100-879X Review

The extracellular matrix in multiple sclerosis: an update

R.A. Sobel

Pathology and Laboratory Services, Veterans Affairs Health Care System, Palo Alto and Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA

Abstract

Extracellular matrix (ECM) molecules play important roles in the pathobiology of the major human central nervous system (CNS) inflammatory/demyelinating disease multiple sclerosis (MS). This mini-review highlights some recent work on CNS endothelial cell interactions with vascular basement membrane ECM as part of the cellular immune response, and roles for white matter ECM molecules in demyelination and remyelination in MS lesions. Recent basic and clinical investigations of MS emphasize axonal injury, not only in chronic MS plaques, but also in acute lesions; progressive axonal degeneration in normal-appearing white matter also may contribute to brain and spinal cord atrophy in MS patients. Remodeling of the interstitial white matter ECM molecules that affect axon regeneration, however, is incompletely characterized. Our ongoing immunohistochemical studies demonstrate enhanced ECM versican, a neurite and axon growth-inhibiting white matter ECM proteoglycan, and dermatan sulfate proteoglycans at the edges of inflammatory MS lesions. This suggests that enhanced proteoglycan deposition in the ECM and axonal growth inhibition may occur early and are involved in expansion of active lesions. Decreased ECM proteoglycans and their phagocytosis by macrophages along with myelin in plaque centers imply that there is "injury" to the ECM itself. These results indicate that white matter ECM proteoglycan alterations are integral to MS pathology at all disease stages and that they contribute to a CNS ECM that is inhospitable to axon regrowth/regeneration.

Correspondence
R.A. Sobel
Department of Pathology, L-235
Stanford University School
of Medicine
300 Pasteur Drive
Stanford, CA 94305
USA
Fax: +1-650-852-3205
E-mail: raysobel@stanford.edu

Presented at
SIMEC 2000 - International
Symposium on Extracellular
Matrix, Angra dos Reis, RJ,
Brazil, September 24-27, 2000.

Research supported by the National Multiple Sclerosis Society (No. RG 3055).

Received October 19, 2000
Accepted February 6, 2001

Key words
- Axon regeneration
- Central nervous system
- Extracellular matrix
- Proteoglycans
- Multiple sclerosis
- Versican

Introduction

In 1998, I reviewed the potential roles of extracellular matrix (ECM) molecules in the immunopathology of multiple sclerosis (MS). Mechanisms of ECM alterations, their contribution to the distinct neuropathological features of this major human central nervous system (CNS) demyelinating disease and the failure of reparative mechanisms in chronic lesions were discussed (1). In the interim, several advances from basic and clinical investigations further implicate ECM alterations in MS. Here, I will highlight some recent developments and emerging insights into MS pathogenesis in which ECM molecules are likely to have important roles. I will also present some of our recent data on white matter proteoglycans in the CNS of MS patients.

The classic MS lesion - cellular and humoral pathogenesis

MS has long been described as a disease with multiple discrete inflammatory/demyelinating lesions or plaques scattered throughout normal-appearing, i.e., fully myelinated and intact, white matter. Based on extensive studies of affected MS tissues and on data from various animal models, the earliest lesions are generally regarded to be immune responses in which there is localized damage to the blood-brain barrier and T and other mononuclear cells infiltrate the CNS and mediate tissue injury. The major target of injury is white matter myelin which is focally disrupted and selectively removed through the phagocytic activity of macrophages/microglia. Recent studies emphasize heterogeneity in mechanisms of demyelination that parallel the heterogeneous appearances of lesions on neuroimaging and may also reflect the marked variation of clinical courses among different MS patients (2). In particular, potential contributions of antimyelin antigen antibody responses continue to be defined (3,4). Whether ECM alterations occurring in MS lesions contribute to retention of pathogenic antibodies in lesions or to the promotion of B cell differentiation and proliferation in the CNS is not known. However, ECM molecules that are deposited in MS lesions, e.g., fibronectin and vitronectin, do promote B cell migration and activation *in vitro* (5-7). Furthermore, antibodies to CNS white matter ECM components have been detected in MS patients (8). Therefore, additional investigation of interactions of B cells and antibodies with ECM components in MS is warranted.

Activated endothelial cells and matrix metalloproteinases

Alterations of endothelial cell intercellular adhesion and adhesion to ECM basement membrane molecules are among the earliest events in CNS immune reactions. Within endothelial cells, peripheral localization of proteins that form tight intercellular junctions is critical to their barrier functions. This localization may be modulated directly by the ECM (9) or indirectly as a consequence of ECM binding and presentation of soluble inflammatory mediators, e.g., tumor necrosis factor-α (Kuruganti PA, Hinojoza JR, Ehmann RA and Sobel RA, unpublished results). Altered expression of the integrin laminin receptors VLA-6 and VLA-1 in MS lesions may also result in endothelial cell retraction and detachment from vascular basement membrane laminin leading to increased permeability of the blood-brain barrier (10). Furthermore, up-regulation of molecules associated with endothelial cell injury, e.g., plasminogen activator inhibitor-1, urokinase plasminogen activator and matrix metalloproteinases (MMPs), is likely to facilitate leukocyte passage through blood vessel walls as the basement membrane ECM undergoes remodeling (11). Thus, endothelial cell-ECM-dependent interactions are critical events leading to the influx into the CNS of the soluble molecules and cells that are normally excluded, causing tissue damage.

MMPs also mediate a wide range of proteolytic activities that facilitate leukocyte migration and activation within the CNS parenchyma and directly degrade myelin components (12-14). Their presence and activities in cerebrospinal fluid of MS patients correlate with clinical disease activity (15-17) and MMP inhibition is a potential mode of MS treatment currently being investigated (18). MMP-mediated effects on both the blood-brain barrier and the interstitial ECM are, therefore, currently recognized as central to MS pathogenesis and of major clinical and potential therapeutic importance.

Promotion of remyelination

Oligodendrocytes, the cells responsible for myelination of the CNS, are lost in MS

lesions and there is only a limited oligodendrocyte precursor population capable of remyelinating the remaining axons (19,20). In normal development, CNS myelination is highly dependent on the ECM (21). Therefore, an abnormal white matter ECM in MS lesions probably precludes remyelination by both endogenous mature and immature oligodendrocyte populations. Therapies designed to repopulate MS lesions with xenografts of oligodendrocyte precursors or with stem cells (22,23) will, therefore, necessarily require knowledge of the precise composition and functions of an ECM that can promote remyelination. For example, MMP inhibition, which could be beneficial by decreasing the inflammatory response, might also impede MMP-dependent remyelination (21).

Neuronal/axonal pathology in lesions and normal-appearing white matter

Axonal injury has been recognized since the first descriptions of MS pathology (24). Relative sparing of axons is, however, the hallmark of acute lesions, whereas axonal loss is more characteristic of chronic MS plaques. Recent pathological studies have emphasized axonal injury in active, i.e., inflammatory, MS lesions (25,26) and diffuse axonal loss in normal-appearing white matter (27,28). The latter probably is a consequence of Wallerian degeneration following the axonal transection that occurs within frank lesions. Diffuse white matter axonal injury is also likely to result in the gross atrophy in the brains and spinal cords of MS patients that has been identified using newer neuroimaging methods (29-32). Taken together, these studies suggest that even in early stages of the disease there is a degenerative component to MS pathogenesis and that progresses independently of the dramatic fluctuations in neurologic function that characterize clinical relapses and remissions. Therefore, not only do focal lesions, particularly the chronic plaques which have large extracellular fluid volumes (1,33), have an altered ECM, but there may be widespread more subtle abnormalities accompanying axonal loss throughout the CNS white matter ECM in MS patients. These diffuse changes could contribute to persistently altered physiology and progressive neurologic dysfunction (34). Thus, pathologic analyses and neuroradiologic correlations underscore the importance of ECM molecules that prevent axonal regeneration, both within frank lesions and in normal-appearing white matter.

White matter proteoglycans

Numerous ECM molecules, including laminins, fibronectin, tenascins, collagens and proteoglycans are essential for CNS development (35). ECM molecule functions in the adult CNS following injury may, however, differ from those in development and need to be defined in pathological conditions. Proteoglycans, a complex group of macromolecules each of which is composed of a core polypeptide backbone and polysaccharide (glycosaminoglycan) chains that vary considerably both in amount and type, are major components of the adult CNS ECM. Specific proteoglycan functions depend on the interactions of both their core proteins and their side chains with numerous other ECM molecules (36). Proteoglycan glycosaminoglycan expression is affected by the inflammatory cytokines found in active MS lesions (37) and their differential expression may influence axonal growth and guidance and neurite outgrowth (38), i.e., processes that are critical to the failure of axonal repair.

Chondroitin sulfate and dermatan sulfate proteoglycans are the most abundant proteoglycans in the CNS. In general, chondroitin sulfate proteoglycans that are expressed in the injured CNS are considered to contribute to an environment that is inhibitory to neuron regeneration (39-42). In particular, the

brain-specific versican V2 splice variant has neurite and axon growth inhibitory activity (43,44).

We are using immunohistochemistry on CNS tissue sections of MS patients and controls to characterize alterations of the white matter proteoglycans versican and dermatan sulfate in MS. In control samples, both versican and dermatan sulfate proteoglycan immunostaining is stronger in CNS white matter than in gray matter (Figure 1A,B). In samples with active demyelinating MS le-

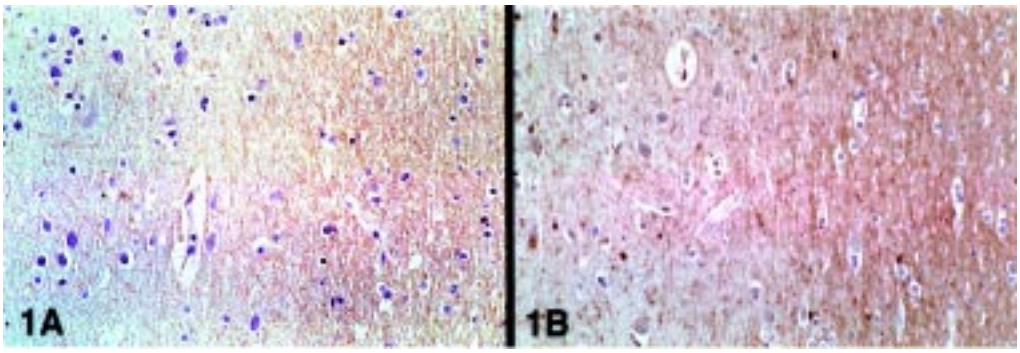

Figure 1. Immunostaining of versican (A) and dermatan sulfate proteoglycan (B) in the normal cerebral ECM. Staining is stronger in the white matter (right side of each field) than in the deep cerebral cortex (left side of each field). Both are paraffin sections of cerebrum from a control patient. Magnification = 258X for both.

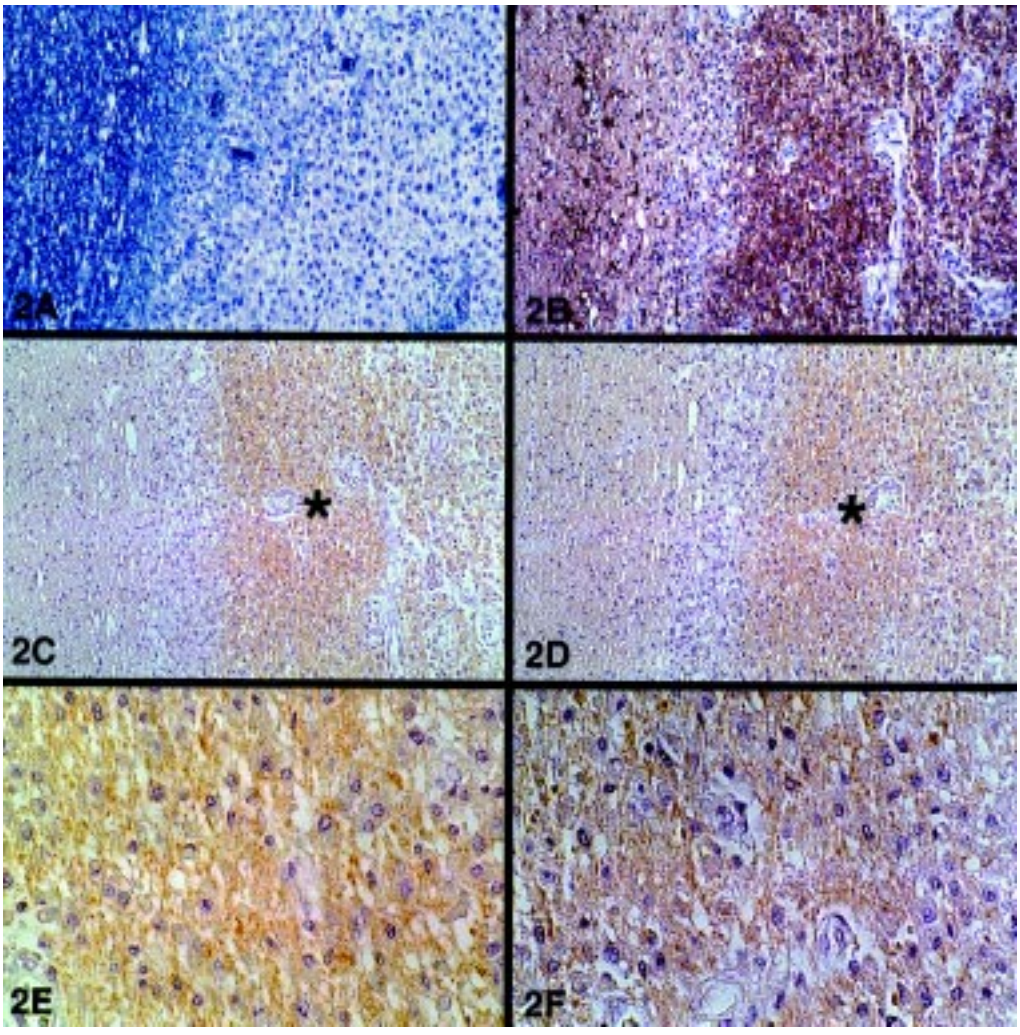

Figure 2. Versican and dermatan sulfate proteoglycan at the edges of an active MS lesion. Serial paraffin sections of brain white matter of an active MS lesion are stained with Klüver-Barrera stain for myelin (A) or immunostained with antibody to glial fibrillary acidic protein (B), versican (C, E), or dermatan sulfate proteoglycan (D, F). In *A-D*, intact white matter is on the left side of each field; a hypercellular vertical band in the middle of the field is the plaque edge, and the plaque center is on the right. In *A*, intact myelin is blue and the hypercellular area and plaque areas show loss of blue staining indicating demyelination. In *B*, there are individual dark staining reactive astrocytes in the intact white matter on the left and there is a diffuse increase in glial fibrillary acidic protein immunostaining in the plaque edge and center indicating astrocytosis. In *C* and *D*, there is pale staining of the intact white matter on the left and a zone of decreased staining corresponding to the hypercellular inflammatory plaque edge. In the middle, there is a band of increased ECM versican (C) and dermatan sulfate proteoglycan (D) staining (asterisks). This staining is less intense on the right, in the plaque center. *Panels E* and *F* show higher power of the indicated areas of increased ECM versican and dermatan sulfate proteoglycan staining. Magnification = 130X (A-D), 430X (E, F).

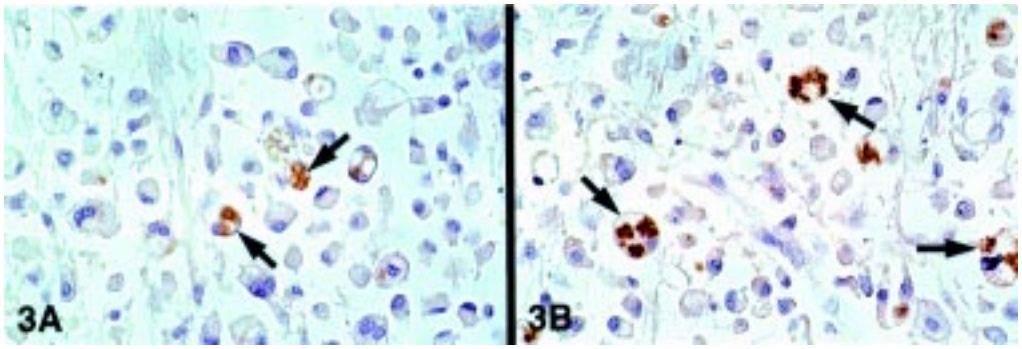

Figure 3. Versican and dermatan sulfate proteoglycan in the center of an active MS lesion. Each shows loss of extracellular versican (A) and dermatan sulfate proteoglycan (B) with staining for these molecules (arrows) in granules within the cytoplasm of foamy macrophages, indicating that they have been phagocytosed. Magnification = 514X for both.

sions (Figure 2A,B), there is a focal increase in the density of immunoreactivity of these molecules at the hypercellular edge of the plaque, i.e., the zone of advancing lesion growth (Figure 2C-F). This zone is also associated with an increase in glial fibrillary acidic protein immunoreactivity, suggesting that the increased deposition of the proteoglycans is linked to astrocyte activation. In the center of active plaques, there is loss of staining for these proteoglycans. Furthermore, dense staining within the cytoplasm of foamy macrophages indicates that the ECM molecules are phagocytosed along with myelin (Figure 3A,B).

These results suggest that an increase in these ECM proteoglycans occurs as MS lesions expand and that this increased deposition relates to the inflammatory cell infiltration, proinflammatory cytokine milieu and astrocytosis at the lesion edges. Moreover, since these molecules are known to impede axonal outgrowth, the CNS ECM may be altered towards a neurite/axon outgrowth-inhibiting environment at the earliest stages of lesion formation. A decrease of the ECM proteoglycans in active lesion centers as well as in chronic plaques (data not shown) demonstrates the dynamic nature of this ECM remodeling. The finding of proteoglycan phagocytosis suggests that the ECM alterations are part of the overall tissue injury, i.e., the molecules are phagocytosed because they are damaged (or recognized as foreign) and are, therefore, removed by macrophages.

Conclusion

In summary, complex alterations of the CNS ECM in MS patients continue to be identified. Additional distinct functions and evolving concepts on the roles of ECM alterations should provide a fertile ground for elucidating the pathogenesis, immunopathology and physiology of this important disease. Furthermore, these alterations have clear clinical relevance, both for monitoring disease progression and for developing new therapies.

Acknowledgments

I am grateful to Azam Ahmed and Mary Jane Eaton for assistance with this project and to Claudia Greco, M.D., of the University of California at Davis, for providing MS tissue samples.

References

1. Sobel RA (1998). The extracellular matrix in multiple sclerosis lesions. *Journal of Neuropathology and Experimental Neurology*, 57: 205-217.
2. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M & Lassmann H (2000). Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Annals of Neurology*, 47: 707-717.
3. Archelos JJ, Storch MK & Hartung H-P (2000). The role of B cells and autoantibodies in multiple sclerosis. *Annals of Neurology*, 47: 694-706.
4. Smith-Norowitz T, Sobel RA & Mokhtarian

FM (2000). B cells and antibodies in the pathogenesis of myelin injury in Semliki Forest virus encephalomyelitis. *Cellular Immunology*, 200: 27-35.
5. Salcedo R & Patarroyo M (1995). Constitutive αv ß3 integrin-mediated adhesion of human lymphoid B cells to vitronectin substrate. *Cellular Immunology*, 160: 165-172.
6. Shibayama H, Tagawa S, Hattori H, Inoue R, Katagiri S & Kitani T (1995). Laminin and fibronectin promote the chemotaxis of human malignant plasma cell lines. *Blood*, 86: 719-725.
7. Rafi A, Nagarkatti M & Nagarkatti PS (1997). Hyaluronate-CD44 interactions can induce murine B-cell activation. *Blood*, 89: 2901-2908.
8. Briani C, Santoro M & Latov N (2000). Antibodies to chondroitin sulfates A, B, and C: clinico-pathological correlates in neurological diseases. *Journal of Neuroimmunology*, 108: 216-220.
9. Savettieri G, Di Liegro I, Catania C, Licata L, Pitarresi GL, D'Agostino S, Schiera G, De Caro V, Giandella G, Giannola LI & Cestelli A (2000). Neurons and ECM regulate occludin localization in brain endothelial cells. *NeuroReport*, 11: 1081-1084.
10. Sobel RA, Hinojoza JR, Maeda A & Chen M (1998). Endothelial cell integrin laminin receptor expression in multiple sclerosis lesions. *American Journal of Pathology*, 153: 405-415.
11. Cuzner ML & Opdenakker G (1999). Plasminogen activators and matrix metalloproteases, mediators of extracellular proteolysis in inflammatory demyelination of the central nervous system. *Journal of Neuroimmunology*, 94: 1-14.
12. Cross AK & Woodroofe MN (1999). Chemokine modulation of matrix metalloproteinase and TIMP production in adult rat brain microglia and a human microglial cell line *in vitro*. *Glia*, 28: 183-189.
13. Yong VW, Krekoski CA, Forsyth PA, Bell R & Edwards DR (1998). Matrix metalloproteinases and diseases of the CNS. *Trends in Neurosciences*, 21: 75-80.
14. Lukes A, Mun-Bryce S, Lukes M & Rosenberg GA (1999). Extracellular matrix degradation by metalloproteinases and central nervous system diseases. *Molecular Neurobiology*, 19: 267-284.
15. Valenzuela MA, Cartier L, Collados L, Kettlun AM, Araya F, Concha C, Flores L, Wolf ME & Mosnaim AD (1998). Gelatinase activity of matrix metalloproteinases in the cerebrospinal fluid of various patient populations. *Research Communications in Molecular Pathology and Pharmacology*, 104: 42-52.
16. Waubant E, Goodkin DE, Gee L, Bacchetti P, Sloan R, Stewart T, Andersson PB, Stabler G & Miller K (1999). Serum MMP-9 and TIMP-1 levels are related to MRI activity in relapsing multiple sclerosis. *Neurology*, 53: 1397-1401.
17. Sellebjerg F, Madsen HO, Jensen CV, Jensen J & Garred P (2000). CCR5 δ32, matrix metalloproteinase-9 and disease activity in multiple sclerosis. *Journal of Neuroimmunology*, 102: 98-106.
18. Kieseier BC, Seifert T, Giovannoni G & Hartung HP (1999). Matrix metalloproteinases in inflammatory demyelination: targets for treatment. *Neurology*, 53: 20-25.
19. Wolswijk G (2000). Oligodendrocyte survival, loss and birth in lesions of chronic-stage multiple sclerosis. *Brain*, 123: 105-115.
20. Keirstead HS & Blakemore WF (1999). The role of oligodendrocytes and oligodendrocyte progenitors in CNS remyelination. *Advances in Experimental Medicine and Biology*, 468: 183-197.
21. Oh LY, Larsen PH, Krekoski CA, Edwards DR, Donovan F, Werb Z & Yong VW (1999). Matrix metalloproteinase-9/gelatinase B is required for process outgrowth by oligodendrocytes. *Journal of Neuroscience*, 19: 8464-8475.
22. Milner R (1997). Understanding the molecular basis of cell migration: implications for clinical therapy in multiple sclerosis. *Clinical Science*, 92: 113-122.
23. Del Bigio MR, Tchélingérian J-L & Jacque CM (1999). Expression of extracellular matrix degrading enzymes during migration of xenografted brain cells. *Neuropathology and Applied Neurobiology*, 25: 53-61.
24. Kornek B & Lassmann H (1999). Axonal pathology in multiple sclerosis. A historical note. *Brain Pathology*, 9: 651-656.
25. Ferguson B, Matyszak MK, Esiri MM & Perry VH (1997). Axonal damage in acute multiple sclerosis lesions. *Brain*, 120: 393-399.
26. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S & Bö L (1998). Axonal transection in the lesions of multiple sclerosis. *New England Journal of Medicine*, 338: 278-285.
27. Ganter P, Prince C & Esiri MM (1999). Spinal cord axonal loss in multiple sclerosis: a post-mortem study. *Neuropathology and Applied Neurobiology*, 25: 459-467.
28. Evangelou N, Esiri MM, Smith S, Palace J & Matthews PM (2000). Quantitative pathological evidence for axonal loss in normal appearing white matter in multiple sclerosis. *Annals of Neurology*, 47: 391-395.
29. Simon JH, Jacobs LD, Campion MK, Rudick RA, Cookfair DL, Herndon RM, Richert JR, Salazar AM, Fischer JS, Goodkin DE, Simonian N, Lajaunie M, Miller DE, Wende K, Martens-Davidson A, Kinkel RP, Munschauer III FE & Brownscheidle CM (1999). A longitudinal study of brain atrophy in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Neurology*, 53: 139-148.
30. Tortorella C, Viti B, Bozzali M, Sormani MP, Rizzo G, Gilardi MF, Comi G & Filippi M (1999). A magnetization transfer histogram study of normal-appearing brain tissue in MS. *Neurology*, 54: 186-193.
31. Leary SM, Davie CA, Parker GJ, Stevenson VL, Wang L, Barker GJ, Miller DH & Thompson AJ (1999). ^{1}H Magnetic resonance spectroscopy of normal appearing white matter in primary progressive multiple sclerosis. *Journal of Neurology*, 246: 1023-1026.
32. Lee MA, Blamire AM, Pendlebury S, Ho K-H, Mills KR, Styles P, Palace J & Matthews PM (2000). Axonal injury or loss in the internal capsule and motor impairment in multiple sclerosis. *Archives of Neurology*, 57: 65-70.
33. Gutowski NJ, Newcombe J & Cuzner ML (1999). Tenascin-R and C in multiple sclerosis lesions: relevance to extracellular matrix remodeling. *Neuropathology and Applied Neurobiology*, 25: 207-214.
34. Trapp BD, Ransohoff R & Rudick R (1999). Axonal pathology in multiple sclerosis: relationship to neurologic disability. *Current Opinion in Neurology*, 12: 295-302.
35. Pires-Neto MA, Braga-de-Souza S & Lent R (1999). Extracellular matrix molecules play diverse roles in the growth and guidance of central nervous system axons. *Brazilian Journal of Medical and Biological Research*, 32: 633-638.
36. Yamaguchi Y (2000). Chondroitin sulfate proteoglycans in the nervous system. In: Iozzo RV (Editor), *Proteoglycans - Structure, Biology, and Molecular Interactions*. Marcel Dekker, New York.
37. Jander S, Schroeter M, Fischer J & Stoll G (2000). Differential regulation of microglial keratan sulfate immunoreactivity by pro-inflammatory cytokines and colony-stimulating factors. *Glia*, 30: 401-410.
38. Bovolenta P & Fernaud-Espinosa I (2000). Nervous system proteoglycans as modulators of neurite outgrowth. *Progress in*

Neurobiology, 61: 113-132.

39. Zuo J, Neubauer D, Dyess K, Ferguson TA & Muir D (1998). Degradation of chondroitin sulfate proteoglycan enhances the neurite-promoting potential of spinal cord tissue. *Experimental Neurology*, 154: 654-662.
40. Stichel CC, Niermann H, D'Urso D, Lausberg F, Hermanns S & Muller HW (1999). Basal membrane-depleted scar in lesioned CNS: characteristics and relationships with regenerating axons. *Neuroscience*, 93: 321-333.
41. McKeon RJ, Jurynec MJ & Buck CR (1999). The chondroitin sulfate proteoglycans neurocan and phosphacan are expressed by reactive astrocytes in the chronic CNS glial scar. *Journal of Neuroscience*, 19: 10778-10788.
42. Asher RA, Morgenstern DA, Fidler PS, Adcock KH, Oohira A, Braistead JE, Levine JM, Margolis RU, Rogers JH & Fawcett JW (2000). Neurocan is upregulated in injured brain and in cytokine-treated astrocytes. *Journal of Neuroscience*, 20: 2427-2438.
43. Schmalfeldt M, Bandtlow CE, Dours-Zimmermann MT, Winterhalter KH & Zimmermann DR (2000). Brain derived versican V2 is a potent inhibitor of axonal growth. *Journal of Cell Science*, 113: 807-816.
44. Niederost BP, Zimmermann DR, Schwab ME & Bandtlow CE (1999). Bovine CNS myelin contains neurite growth-inhibitory activity associated with chondroitin sulfate proteoglycans. *Journal of Neuroscience*, 19: 8979-8989.