The extracellular matrix in multiple sclerosis: an update

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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.

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 anti-myelin 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
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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-
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.

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References

4. Smith-Norowitz T, Sobel RA & Mokhtarian


