

MECHANISMS OF NEURODEGENERATION IN MULTIPLE SCLEROSIS: PREMISE FOR NEUROPROTECTION?

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ABSTRACT

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), in which both focal demyelinating lesions and diffuse axonal loss are present. The relationship between the inflammatory/ demyelinating process and the neurodegenerative component of the disease are extremely complex and not yet fully understood, but recent data show that their coexistence is present very early during the evolution, even in the preclinical stages of the disease. There are also extremely important clinical correlations between the focal inflammatory/ demyelinating lesions and the potential reversible clinical relapses and between the permanent axonal loss and continuous neurodegenerative process (extended also beyond the focal lesions in the normal appearing white matter and gray matter) and the irreversible brain and spinal cord atrophy which are irreversible and is the cause of clinical invalidity, mainly in the progressive forms of disease. MS is a heterogenous disease and there are now described four pathophysiologic patterns which are also correlated with the clinical heterogeneity. Many factors, including genetic and environmental conditions, are responsible for this heterogeneity, influencing the extremely complex pathogenic pathways. Recent data put into evidence details concerning the mechanisms of the neurodegenerative pathways in MS, which could be the background for future neuroprotective therapeutic strategies which could have, in association with the immunomodulatory treatments (now available in clinical practice) the potential capacity to slow-down or even to stop the clinical deterioration of these patients. These potential therapeutic strategies are reviewed in the final discussion.

Key words: chronic inflammatory disease; disease heterogeneity; pathophysiological patterns; focal demyelination; diffuse axonal loss; normal appearing white matter (NAWM); brain and spinal cord atrophy; neuroprotection

Today, multiple sclerosis is considered a diffuse chronic inflammatory disease of the CNS, in which myelin sheaths are a main target of the focal tissue injury. The recent research, have shown in contrast to the „classic“ view of the pathophysiology of this disease, that the demyelination is present not only in the white matter but also in the gray matter, and that there is also an important and progressive axonal loss both in the demyelinating lesions and also beyond of these, in what has been called „normal appearing white matter“ (NAWM). The functional consequences of these lesions are extremely important because inflammation and demyelination are, at least in part reversible, and can be correlated with the clinical remissions, but the axonal loss is an irreversible process which correlates with the progressive and permanent neurological deficit and invalidation. The pathologic studies have also shown that in multiple sclerosis remyelination is also present and represents the background of the partially spontaneous repair, that it is quite extensive in the early stages of the disease in most patients, but in the late stages of the relapsing remitting forms and also in the progressive forms of disease its capacity is much decreased.

There are important clinico-pathological correlations between the episodes of inflammation and the clinical relapses which are more frequent during the initial stages of the relapsing- remitting forms on one hand, and between the progressive axonal loss and the brain and spinal cord atrophy and clinical worsening in the late stages and even earlier in the progressive forms of disease (1).

The treatment forms currently used today in multiple sclerosis (immunomodulatory and immunosuppressive drugs, corticoids) target only the inflammatory process, but not the degenerative component of the pathological process (at least not yet proven in the human disease, though for some drugs there is evidence of neuroprotection in the experimental models of experimental allergic encephalomyelitis - EAE). This recent progress in understanding the pathogenesis of multiple sclerosis could have important implications in the development of new therapies, which have to take into account the disease heterogeneity (as there are at least 4 main disease subtypes), the variable genetic background and susceptibility which may impact on treatment response and the changing relation of inflammation-

neurodegeneration during the progression of disease and in relation to the subtype of disease. So, the genetic and environmental factors may facilitate the movement of autoreactive T cells and demyelinating antibodies from the systemic circulation into the CNS through disrupting of blood-brain barrier and inside the CNS these factors may up-regulate the expression of endothelial adhesion molecules (ICAM-1, VCAM-1, E-selectin) further facilitating the entry of T-cells into the CNS. On the other hand the immunological/inflammatory cascade imply a complex process in which there are many „players“ as the inflammatory cells from systemic circulation, macrophages (from the systemic circulation and microglia), astrocytes, cytokines, chemokines, complement, Ig's, proteases affecting different nervous structures (myelin sheath, axons, oligodendrocytes).

Recently (2) have been identified 4 basic pathological patterns of multiple sclerosis: in patterns I and II the main process is that of autoimmune demyelination, mainly mediated by T-lymphocytes and activated macrophages, and microglia (pattern I) and with the prominent involvement of antibodies and complement (pattern II); in patterns III and IV the main process is the oligodendrocyte dystrophy, also associated with immune mediated-injury.

The axonal loss is triggered by more factors secondary to the initial lesions: cytotoxic T cells (through granzyme/ perforin and the activation of proapoptotic receptors and proteins) and macrophages/ microglia (as sources of reactive oxygen and nitrogen species, proteases and mediators of excitotoxicity); so, the axonal degeneration is the final consequence of the multiple injuries mediated by these factors: membrane disturbance, sodium and calcium intracellular influx, energy failure, activation of proteases and dissolution of the cytoskeleton (3). The detailed understanding of these process is of particular interest because it offers the rationale background for developing new therapeutic modalities destined to offer neuroprotection and hence to slow-down or stop the clinical deterioration.

The axonal loss offers different aspects in the established demyelinating lesions and in the normal apparent white matter and the progressive forms of disease.

In the established demyelinated lesions, axonal loss is present both in active demyelination and in inactive demyelinated plaques. In active demyelination locations, the earliest signs of axonal injury is the disturbance of axonal transport leading to focal accumulation of APP at the site of injury and focal axonal swelling; in inactive demyelinated plaques there is additional slow burning axonal injury and

loss, but these lesions could be absent if plaques are remyelinated.

In normal apparent white matter and the progressive forms of disease, there is a diffuse inflammatory process throughout the whole brain and spinal cord, associated with diffuse axonal injury and loss, followed by fiber demyelination and secondary myelin destruction (associated with extensive activated local microglia with increased expression of iNOS and oxidative stress).

The relation between primary demyelination and the axonal loss is extremely complex and it has been shown that when axons loose their myelin sheaths, there are more pathophysiological consequences (4):

1. Na⁺ channels primary located at the former nodes of Ranvier, redistribute along the whole internode and generates the conduction block;
2. Changes in the expression of Na⁺ channels subtypes disturb the functional properties of demyelinated axons: Na⁺ voltage-dependent channels (Nav1.6 subtype) are up-regulated and expressed along the whole internode of demyelinated axons, where they produce persistent sodium current; the persistent sodium current can drive reverse sodium/calcium exchange and leads to accumulation of intraaxonal calcium, triggering injurious secondary cascades and axonal lesion;
3. Other molecules located at the myelin/axon interface show abnormalities in demyelinated MS plaques, and these abnormalities are extended beyond the borders of plaques;
4. Disturbed axonal transport in acutely injured axons leads also to accumulation of N-type voltage-gated Ca⁺⁺ channels;
5. The presence of abnormalities also in surviving axons with severe changes in their composition and function determined by deranged cytoskeletal proteins (5).

The final pathway of axon destruction is the result of mitochondrial dysfunction, ion influx into the axon and activation of proteases (6).

As a consequence of disruption of the axonal continuity, outside the plaques appears the degeneration of distal portion of the nerve fiber (wallerian degeneration) and in addition, retrograde neuronal degeneration, which associated with the intracortical demyelinating lesions is the main cause of progressive brain and spinal atrophy.

Other important aspects of cellular pathology in multiple sclerosis refer to abnormalities of premyelinating oligodendrocytes associated with axons. In chronic lesions of MS appears the differentiation of oligodendrocytes to a premyelination cell type and

the tendency of association of this type of cell with axons, expressing myelin oligodendrocyte glycoprotein (MOG), but finally myelination fails. We do not have an explanation of the inhibition of myelination in MS chronic lesions, but there are some data implying possible disregulated growth factors, the altered molecular composition of axons, the possible existence of an inhibitory signal; there are evidence of a number of growth-inhibiting substances in the gliotic scar which perpetuate the development arrest of precluding axonal outgrowth and myelin repair; among these some agonists for Nogo-receptors like Nogo and MOG have been shown to arrest axonal growth, so it seems rationale that Nogo-receptor blockade, as a possible future therapeutic strategy, might promote axonal regeneration (7). All these studies finally suggest that the dystrophic axons limit the remyelination of chronic MS lesions (7).

Taking into account all these data concerning the mechanisms of axonal injury, at least theoretically they are potential targets for neuroprotection which could target:

- the excitotoxic mechanisms (glutamate activation, calcium and sodium cellular influx);
- the inflammatory mechanisms (mediated by nitric oxide, CD8⁺ cytotoxicity, TNF- α , PGE₂, the proinflammatory cytokines, antibodies, oedema);
- the increased vulnerability to damage of demyelinated axons;
- the dying-back mechanisms;
- the cellular energy depletion (mitochondrial dysfunction, free radicals generation);
- the genetic programme of the degeneration;
- the apoptotic mechanisms;
- the depletion of growth factors.

So, the neuroprotective strategies in MS could be reached by at least 3 therapeutic pathways:

- anti-inflammatory treatment;
- anti-demyelinating treatment and promotion of early remyelination;
- trophic support for the axons.

Maybe the most critical and complex aspect in MS is the role of the inflammatory cells which are one of the triggers of the pathophysiologic pathways of the disease, but which in the same time produce growth factors, remove myelin-associated inhibitory molecules and may adapt a protective suppressor phenotype (8). So, we have to understand that inflammation is essentially implied both in the neurodegenerative and the neuroprotective component of the pathophysiological cascade, and in this complex process the differentiation of Th0 cells to Th1 phenotype (with a proinflammatory activity) or Th2

phenotype (with anti-inflammatory and neuroprotective consequences) is a critical step. Many studies have shown that in patients with MS the shift to Th1 phenotype is dominant and is correlated with the disease activity.

Also other nervous cell types, like astrocytes, implied in the pathophysiology of MS have both pro-inflammatory activity and are also a source of the trophic support by producing neurotrophic factors. Among these, in MS the most often target of the experimental studies is BDNF, which has been shown to be present in MS and EAE lesions both in immune cells and in astrocytes while neurons and nearby astrocytes express the BDNF receptor (9, 10).

So drugs, as glatiramer acetate and statins which experimentally demonstrated the capacity to promote the shift of Th0 cells to Th1 phenotype are among the candidate molecules with possible neuroprotective effects in MS, but this hypothesis has to be demonstrated by controlled clinical trials.

Other molecules are also studied as potential neuroprotective treatments in MS as: sodium-channel blocking agents (flecainide, lamotrigine, phenytoin), AMPA-receptors antagonists (talampantel and E2007), minocycline (which antagonizes the anti-apoptotic intracellular signaling pathways and decreases the glutamate excitotoxicity), erythropoietin (interfering with more intracellular signaling pathways involved in neuroprotection) combined with corticoids or with a selective inhibitor of phosphatidylinositol 3-kinase, the fumaric acid esters and fingolimod (extensively studied in this moment in large international clinical trials, both for their clinical efficacy and for their safety profile).

Very interesting observations might be the starting point of other therapeutic pathways in MS: there natural autoreactive antibodies of IgM type which may enhance the endogenous myelin repair, associated with proliferation or preservation of mature oligodendrocytes (11); these IgMs probably bind to antigens on the oligodendrocytes surface membrane exerting a direct stimulation of myelin-producing cells. So, possible human monoclonal antibodies of IgM type which might promote remyelination might be also possible candidates for neuroprotection in patients with MS (12).

Also the transplantation of oligodendrocyte progenitors and of bone marrow derived cells may have a therapeutic potential in MS and are currently the subject of many experimental studies, in order to understand how to manipulate these cells to obtain therapeutic benefits in human diseases, including MS, and in the same time to have a biologically safety profile on short and long term (13).

In conclusion:

- Demyelination is necessary but not sufficient for development of permanent deficits in primary demyelinating disorders of human and animals;
 - Demyelination predisposes axons to subsequent secondary injury or loss;
 - Axonal injury and loss determine secondary myelin destruction;
 - Injury to the axon may result as either T cell toxicity or/ and failure of neurotrophic support from death by myelinating oligodendrocytes;
 - Success in remyelination therapy may be achieved either by enhancing endogenous repair or by grafting exogenous remyelinating cells;
 - Several neurotrophic factors have been shown to enhance endogenous remyelination;
 - Many immature cells have been shown to induce efficient exogenous remyelination in animal models;
- Statins, cyclins and immunophilin ligands are orally available immunomodulatory agents that may protect neurones;
 - Other promising possibilities could be modulation of excitotoxicity, modulation of nitric oxide synthesis and modulation of cationic channels;
 - Despite the increasing number of putative therapeutic targets:
 - no treatment to achieve remyelination or neuroprotection has yielded positive clinical results in humans;
 - forging a link between basic biology and treatment of patients will require us to overcome several challenges: assessment of efficacy of repair, improving tolerance to neurotrophic factors, delivery of neurotrophic factors, better defining the indications for and limitations of transplantation (14).

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