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Review

Regeneration in the Central Nervous System: *The Inhibitory Factors*

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تجديد الخلايا العصبية المركزية و العوامل المحبطة لها

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الملخص العربي

إن الخلايا العصبية في الجهاز العصبي المركزي و الطرفي بالرغم من تشابهها التشريحي إلا أنها تسلك طرقا مختلفة بعد تلفها. إن تجديد طرف الخلايا العصبية في الجهاز العصبي الطرفي (Axons) ليس فقط في الصفة التشريحية ولكن أيضا الوظيفية. و لكن من الواضح أن عملية التجديد للخلايا في الجهاز العصبي المركزي للثدييات بعد إتلافها يتم إجهاضها بسرعة. هناك بحث حديث ركز على أسباب إحباط عملية التجديد في الجهاز العصبي المركزي بعد تلفه من جوانبه المتعددة مثل التيبس العصبي، خلل البناء التركيبي العصبي و تأثير العوامل الثانوية لتلف الخلايا. و قد تم فصل بعض المركبات المسببة لإحباط هذا التجديد ودراسة تأثرها إذا تعرضت للعوامل المختلفة. إن آلية التجديد للخلايا العصبية المركزية أصبحت الآن منطقة جاذبة للأبحاث و قد تضمنت هذه المرجعية العوامل المختلفة المسئولة عن إحباط تجديد الخلايا العصبية المركزية باختصار. فبالرغم من أن عملية إحباط تجديد خلايا الجهاز العصبي المركزي يحيطها الكثير من الألغاز و الغموض فانه يبدو أن هذا الغموض بدأ ينقشع تدريجيا. و يبدو أن هناك عوامل متعددة و متداخلة لها دور فعال في آلية هذا الإحباط المعقد.

الكلمات الدالة: تجديد – الجهاز العصبي المركزي – العوامل المحبطة – إحباط – التيبس العصبي – الإحباط المرتبط بالميلين

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ABSTRACT

The neurons in the central and peripheral nervous systems, in spite of being morphologically similar, exhibit different behavior after injury. Regeneration of the axons in the peripheral nervous system is not only elaborate but is also functionally effective; while it is peculiar that in the adult mammalian central nervous system, following an injury or trauma, the regenerative process is quickly aborted. Recent research has focused on this inhibitory process and has studied its various aspects such as glial scar, disruption of the geometrical architecture, the impact of secondary injury process. Certain molecules incriminated for growth cone collapse have been isolated and their effects under varying conditions studied. The mechanism involved in the control of regenerative capacity of the central nervous system neurons has become an area of intense research. In this review, the various factors responsible for inhibiting the regenerative efforts in the central nervous system are briefly presented. Although the process of inhibition remains largely shrouded in mystery, the mist seems to be gradually receding. It seems likely that multiple, rather than a single, interacting factors and mechanisms are involved in this highly complex phenomenon.

Key words: Regeneration, central nervous system, CNS, inhibitory factors, inhibition, glial scar, myelin-associated inhibitors

INTRODUCTION

Whereas there is hardly any obvious morphological difference between the neurons of the central nervous system (CNS) and the peripheral nervous system (PNS), it is well known that lesions in the CNS are irreversible due to an almost complete lack of regenerative growth of the axons, while in the PNS the growth is not only substantial but also functionally effective. Nevertheless, it was almost a century ago that Raymond y Cajal provided the first definitive experimental evidence that the central nervous system does, in fact, have a limited capacity of self-renewal and regeneration[1]. His studies stimulated further work towards the noble yet so far elusive goal of restoration of CNS regenerative capacity. In the years to follow and more recently in the last two decades a huge body of interesting observations, although largely inconclusive and with problems of interpretations has been evolved. The capital question is: if the neurons in the CNS and PNS have the same morphological and functional attributes and characteristics then why is the CNS reparative process so abortive?(Fig.1).

A hostile environment and decreased regenerative capacity may contribute towards the failure of axonal regeneration in the adult central nervous system. Taken that the concept of regeneration in the CNS is not completely foreign, some enthusiastic studies to explore the process were undertaken. In this regard Aguayo and his

colleagues[2] in their pioneer study, bridged lesions of the spinal cord in rats with a piece of sciatic nerve; after a short time the explants were invaded by growing neuritis from both sides eventually bridging the injury site. But function was not restored because the neuritis stopped growing after re-entering the spinal cord. Their experiments showed that motor neurons in the spinal cord established connections in the periphery but not in the CNS indicating that the central nervous system neurons could perhaps regenerate in a favorable environment. There could be two possibilities for the poor regenerative behavior of the CNS neurons; either there are active inhibitors to the neurite growth operating in the environment or certain growth promoting factors are lacking in the milieu. A brief overview of the inhibitory factors is given here.

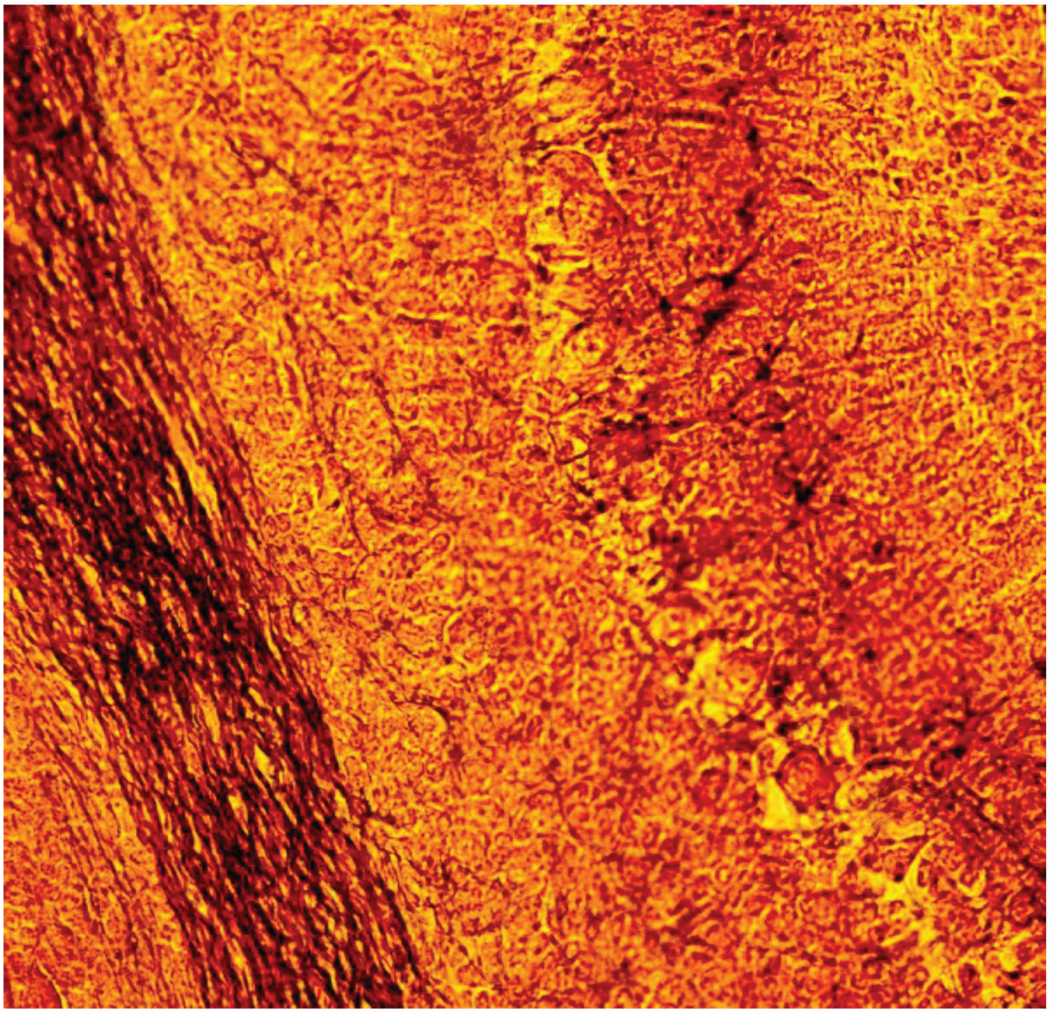

Figure 1: If the neurons in the CNS and PNS have the same morphological and functional attributes and characteristics then why is the CNS reparative process so abortive?

The impact of secondary injury:

After the first acute injury the nervous tissue is subjected to a secondary injury process that may occurs during the following hours to days. The cellular and biochemical events that take place during this period are significantly cytotoxic and include such phenomena as influx of cations, free radicals, inflammatory mediators and glutamatergic elements[3]. As a result necrosis and apoptotic cell death ensue leading to extensive morphological and functional alterations in the injured CNS tissue. Ongoing death of neurons as a result of this secondary injury process is one of the fundamental factors limiting the regenerative capacity in the CNS. The surviving neurons after the secondary injury are exposed to an unfavorable balance of abundant inhibitory factors which may be basically classified to be related to one of the three groups: the neuron itself, the extra-cellular milieu and the diffusible molecules.

Disruption of the architectural geometry:

Whether the CNS white matter supported neuronal attachment and neurite outgrowth has been extensively studied by Pettigrew and his associates[4,5]. In their *in vitro* studies they cultured sympathetic neurons on cryostat sections of adult rat forebrain or spinal cord. Neuronal attachment occurred to the white matter (as also to the gray matter) and the neurite growth was generally limited to the long axis of the tract. The white matter seemed to support long neurite growth but only along its parallel axis and not in an orthogonal direction. These studies and others[6] suggest that disrupted geometry may contribute to the non-permissive properties of the injured tissue.

Is it possible that the rate of degeneration and/or reconstruction of the peritraumatic region might have a role to play? Wallerian degeneration normally occurs much faster in the peripheral nerves than in the white matter [7,8,9]. The clearance of myelin debris from the area of trauma may take as long as 52-60 days in the central fibers.

Although neurons appear to possess the intrinsic capacity to regenerate their axons this capacity may not be retained indefinitely following injury. Whereas in the peripheral nerves the appropriate geometry is reconstructed at a fast pace, in white matter such reconstruction apparently fails or does not succeed in sufficient time to permit regeneration.

A limitation of the in vitro tissue section culture is that it accounts for only the two of the three dimensions. The behavior of the growth cones on section, therefore, cannot be assumed to be the same as that normally occurring in more complex three-dimensional structure of the tissue in vivo.

Glial scar:

A complex cellular interaction at the site of CNS injury represented by the activation of astrocytes, microglial cells and the invasion of macrophages, fibroblasts and meningeal cells results into what is commonly referred to as the "glial scar" [10,11,12]. The glial scar is generally accepted to represent an impediment to axonal regeneration but the relative significance of its molecular and cellular constituents in inhibiting axonal growth is not entirely clear and the extent to which this inhibition is due to physical environment created by the tightly interwoven astrocytic processes is also uncertain[13]. Davies et al[14] eloquently demonstrated the inhibitory nature of the scar: they used a microtransplantation technique that avoided glial scarring and myelin damage. In their experiment dorsal root ganglion (DRG) neurons injected into the CNS grew axons for long distances when myelin remained intact and glial scarring was absent but growth halted on contact with an established scar!

In addition to forming a three dimensional physical barrier to neurite outgrowth, the glial scar is inhibitory to growth because its cellular components secrete a number of inhibitory substances.

Reactive astrocytes and surrounding inflammatory cells along with oligodendrocytes progenitors express inhibitory chondroitin sulphate glycoprotein (CSPGs) at the site of injury. Application of bacterial chondroitinase to the site of injury disrupts the CSPG component of the glial scar and thus promotes substantial axonal growth and functional recovery in a rat model of in vivo spinal cord injury[15] ___implying thereby that CSPG molecules do indeed play a role in blocking axonal regeneration after injury.

Growth cone inhibition and collapsing:

The growth of an injured axon occurs at its distal tip where the growth cone plays the pivotal role in axonal regeneration by negotiating with the environment and integrating signals from the guiding molecules. The growth cone is primarily composed of filamentous actin (F-actin) and microtubules extending along the entire length of an axon. The peripheral part of the growth cone comprises of two dynamic structures: filopodia made from polarized bundles of F-actin filaments and lamellopodia made from a meshwork of F-actin[16,17]. These actin-rich structures provide the propulsive force necessary for the forward extension of the growth cone [18,19]. Whereas growth cone extension is a steady process in the PNS an early collapse is an equally intriguing problem in the central nervous tissue.

Kapfhammer and Raper[20] in Germany have recorded the dramatic change and behavior of growth cone in the CNS after injury. Using time-lapse video-analysis they

observed that a few minutes after contact between the filopodia of a growing retinal axon and a sympathetic axon the retinal growth cone thickened and shortened its filopodia and the neurites retracted. Plasma membranes from chick embryonic brain have been found to contain components that cause collapse of the growth cone of dorsal root ganglion neurons in culture. Furthermore, membranes prepared from the chick posterior optic tectum have been shown to collapse growth cones of axons from temporal retina explants[21].

More recent studies have provided with further insight into the problem. The members of the Rho family of small guanosine triphosphatase (GTPase) and in particular Rho A, Rac1 and Cdc42 appear to play a critical role in the control and regulation of actin in the growth cone and have emerged as potentially important molecular targets for inducing axonal regeneration[22]. While Rac and Cdc42 promote the formation of lamellopodia and filopodia, the Rho activity causes neurite retraction and growth cone collapse[23,24]. Activated (i.e., GTPase bound) Rho modulates the activities of numerous proteins among which Rho-kinase/ROKa/ROCK-2 (ROCK) is the best characterized. ROCK mediates Rho signaling to the actin cytoskeleton[25] leading to a chain of reactions which culminate in growth cone collapse[26]. Inhibiting the Rho-ROCK activity by using specific ROCK-inhibitors has been shown to promote axonal regeneration of retinal ganglion cells by Wahl and his associates[27]. Rho inhibition by C3 exoenzyme has also been shown to overcome the inhibitory effect[28]. In vivo application of C3 exoenzyme was found to significantly increase the growth in crushed optic neurons in adult rats[29], and to promote axonal regeneration of the corticospinal tract after a complete spinal cord transaction injury[30].

Myelin associated inhibitors:

Myelin in the central nervous system has been studied for its properties as a neuronal substratum and as a source of inhibitory components. Although myelin had been proposed to inhibit regeneration before, it was the pioneering work of Martin Schwab and co-workers[31,32], which firmly established that oligodendrocytes and their myelin are major inhibitors within the CNS. Corroborating this finding, John Steves and colleagues demonstrated that the failure of axonal regeneration within chick spinal cord coincided with the onset of spinal myelination; in fact the experimental delay of myelination resulted in extending the permissive period for axonal regrowth[33]. More recent studies have lead to the identification of several myelin-associated inhibitors and their signaling molecules providing opportunities to assess the contribution of these inhibitory molecules in restricting axonal regeneration[34].

MAG: The first such molecule to be identified and described was a myelin- associated glycoprotein (MAG)[35,36]. As a member of the immunoglobulin super-family and a sialic acid-binding glycoprotein MAG is a siglec family protein (Siglec 4). In the central nervous system, MAG is found in the periaxonal myelin membrane, and in the

peripheral nervous system it is also found in the outermost membrane of the myelin sheath[37,38]. MAG has been shown to inhibit neurite outgrowth from adult CNS neurons in vitro[35,36]. The role of MAG as an inhibitor in the PNS is clear from the increased axonal growth of peripheral nerve fibers manifested in certain mice, which are deficient in MAG[39]. Within the CNS, however, the importance of MAG as an impediment to axonal regeneration has been difficult to establish. This may be due to the presence and concomitant influence of other inhibitors. How does MAG work is not completely understood although several candidate receptors for MAG have been identified[40,41,42,43].

Nogo: Schwab and associates biochemically separated inhibitory fractions from the CNS myelin and developed an antibody that could block their inhibitory property in vitro[44,45]. Subsequent in vivo application of the antibody resulted in substantial axonal sprouting and some long-distance corticospinal axonal regeneration within the adult mammalian CNS resulting in improved performance, although it could not be ascertained that functional recovery was due to regeneration of injured axons or due to sprouting and compensation from the uninjured axons[46]. The protein inhibiting factors being called as Nogo; three types were later defined as A, B and C[47,48]. Nogo-A is highly expressed by oligodendrocytes but not by the Schwann cells [49]. There remains some uncertainty regarding the actual component of Nogo protein responsible for axonal inhibition. Whereas Schwab and Walsh ascribe this effect to the N-terminal domain of Nogo-A [50,51], the Strittmatter group assign it to a loop of 66 amino acids (and hence the name Nogo-66) present in all the three isoforms of Nogo[49]. The relationship of Nogo and inhibition of axonal regeneration has been clearly shown more recently where it was observed that in Nogo-deficient mice following dorsal root hemisection of the spinal cord the corticospinal tract fibers growing towards and into the lesion were extensive[52].

OMgp: In contrast to its name Omgp is expressed not only by oligodendrocytes but also at high levels in various neurons. It is a minor component of myelin with a relevant abundance much lower than the MAG, and is found largely in the paranodal loops, next to the node of Ranvier. Omgp is localized on the outer leaflet of the plasmamembrane. Its in vivo function is not known. Previously termed arretin, Omgp, has recently been shown to be a potent inhibitor of axonal extension and capable of inducing growth cone collapse[53,54].

Detailed domain analysis of Nogo has revealed two distinct inhibitory domains: N-terminal cytoplasmic and the extracellular domain Nogo-66 [49,55,56]. Given the extracellular nature of the Nogo-66 and its potential as a receptor-binding site Fournier et al.[57] began searching for a Nogo-A receptor at this location. This effort resulted in the identification of the Nogo receptor (NgR), a protein that is attached to the extracellular surface of the neuronal membrane. It is an interesting, although

unexpected, finding that in addition to Nogo-A binding to NgR, both MAG and Omgp have recently been identified as NgR ligands[42,57,58]. Therefore NgR seems to act as a major convergence point on the surface of the growth cone where it detects the multiple inhibitory cues of the CNS myelin. It is likely that NgR modulates the initial signaling that promotes cytoskeletal alteration and subsequent growth cone collapse [59]. This is an exciting discovery and offers a focal point for the development of new strategies to overcome axonal growth inhibition. In this direction a recent study demonstrates that competitive inhibition of Nogo-A to NgR by an antagonistic peptide NEP1-40 [59] or soluble NgR binding of Nogo-A[60] blocks myelin induced axonal growth inhibition.

NgR has no transmembrane or intracytoplasmic domain and, therefore, must produce inhibition through binding with a co-receptor capable of transducing the extracellular signal and inhibiting intracellular signaling. It is revealed in a recent study that p75 neurotrophin does not interact directly with MAG but is required for its neurite inhibitory activity[61]. Wand et al.[62] have found that the p75 functioned at the NgR coreceptor for Nogo-A, MAG and Omgp. p75 seems to interact with the C-terminal of NgR via its extracellular domain whereas the intracellular domain of p75 is required to mediate myelin-associated activities. p75 modulates the inhibitory activities of all known NgR ligands in a neutrotrophin-independent manner[62].

The intrinsic capability of the CNS neurons to repair after injury:

The neurons in the PNS exhibit regular axonal regeneration after axotomy or crush injury indicating plasticity of the adult mammalian nervous system. Peripheral axonal injury switches a fully differentiated adult neuron into a "growth mode" in which specialized neuronal functions are shut down and a specific gene expression is initiated to initiate axonal growth. Efforts to elucidate the identity and function of these regeneration-associated genes (RAGs) have been under way for years in the PNS[63].

The importance of RAG expression and transcriptional regulation in modulation of the CNS regenerative response has been demonstrated by Neumann and Woolf[64]. Dorsal root ganglion neurons are known to mount a robust regenerative response after injury to their peripheral processes, but transection of the central processes of the DRGs in the dorsal funiculus of the spinal cord does not result in regenerative growth. However, Neumann and Woolf[64] noted that if a peripheral axotomy is performed before dorsal funiculus injury (conditioning lesion), transected central axons could grow for long distances within the dorsal column of the spinal cord. It appears that the peripheral lesion provided the DRG cells with an intrinsic growth capacity to overcome the non-permissive CNS environment. It is the response at the neuronal nucleus through activation of RAGs that is pivotal to the regenerative response manifested at the growth cone. The regenerative process is thought to involve the expression of genes that encode a wide spectrum of proteins including transcription factors, cytoskeletal proteins, growth cone proteins and cell adhesion molecules[65].

The best known example of a RAG product is GAP43. Upregulation of GAP43 gene expression after injury results in increased GAP43 at the growth cone[66]. Furthermore, transgenic over expression of GAP43 and functionally related CAP23, in combination, results in the activation of an intrinsic growth capacity in DRG neurons akin to that observed by Neumann and Woolf [64]. The GAP43 and CAP23 are known to elicit an effect on actin dynamics at the growth cone by sequestering specific required phospholipids along the axonal shaft, which in turn stabilizes axon cortical actin and inhibits axonal branching[67]. These studies by Bomze et al. draw a direct correlation between RAG expression and growth cone activity. It is at the growth cone that not only the machinery required for actin polymerization and subsequent neurite elongation is located but it is also the site where the axon encounters inhibitory cue from the myelin and the extracellular milieu.

But what signaling mechanisms dictate expression of RAGs and lead to unmasking of the CNS neuron's intrinsic program? Neural data lend some insight into this question: developmentally cAMP and cyclic guanosine monophosphate act as switches that dictate changes from positive to negative growth responses and vice versa. High intracellular levels of these nucleotides seem to promote growth cone extension, whereas low levels cause its collapse. Two recent articles address the function of cAMP and in CNS axon regeneration and reveal that by increasing levels of cAMP it is possible to mimic the effect of a conditioning injury on DRG central process outgrowth into the dorsal funiculus of the spinal cord[68,69]. Qui et al. also showed that the classic conditioning lesion of Neumann and Woolf induces an elevation in the cAMP levels in the DRG neurons[69]. Specific mechanisms by which cAMP elicits this response, however, remains unknown.

CONCLUSION

Significant progress has been made during the past two decades in the field of neural regeneration. Some aspects of the factors that inhibit regeneration and the mechanisms by which they do so have been elucidated,

Successful axonal outgrowth depends on the integrity of the white matter and any traumatic injury disrupting its geometry and organization may alter the permissiveness of the tract for neurite growth. This along with slow rate of Wallerian degeneration and restoration of the white matter geometry are important factors to be considered in planning future strategic studies.

The glial scar not only forms a complex three-dimensional physical barrier to neurite outgrowth, it is also inhibitory to growth because its cellular components secrete a number of inhibitory molecules including extracellular matrix molecules. A better understanding of the molecular aspect of the glial scarring and its association with inflammation and myelin are, therefore, required.

Exciting recent advances have revealed a convergence of intrinsic and extrinsic signaling mechanism mediated at the growth cone. Now that the NgR complex has been identified, attention should turn towards eliciting the intracellular signaling molecules downstream from the NgR-p75 complex that transduce the binding of

Nogo-A, MAG and Omgp and ultimately result in growth cone collapse. The small membrane-bound GTPase-Rho seems to be the most likely and promising candidate. Although the specific mechanism of action of the cAMP is not understood at the moment a number of possibilities remain: in addition to directly preventing the signal transduction of external inhibitory cues by inhibition of Rho GTPase, cAMP could also initiate a separate transcription-dependent program that promotes axonal growth through an inhibitory environment. Further investigation of the gene transcription and specific signal transduction pathways responsible for activation of the growth response are required to elucidate the specific mechanism with new possibilities.

It would be naïve to suggest at this juncture that following a single straight road would lead to a clinically significant success. More likely, multiple pathways are involved in this highly complex process and continued intensive research efforts, especially on the molecular front, may sometime in future unfold the mystery of CNS neural inhibition that seems to foil all efforts of axonal regeneration.

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