Article
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Schwann cell expression of an oligodendrocyte-like remyelinating pattern after ethidium bromide injection in the rat spinal cord

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ABSTRACT

Schwann cells are recognized by their capacity of producing single internodes of myelin around axons of the peripheral nervous system. In the ethidium bromide (EB) model of primary demyelination in the brainstem, it is observed the entry of Schwann cells into the central nervous system in order to contribute to the myelin repair performed by the oligodendrocytes that survived to the EB gliotoxic action, being able to even remyelinate more than one axon at the same time, in a pattern of repair similar to the oligodendroglial one. The present study was developed in the spinal cord to observe if Schwann cells maintained this competence of attending simultaneously different internodes. It was noted that, on the contrary of the brainstem, Schwann cells were the most important myelinogenic cells in the demyelinated site and, although rare, also presented the capacity of producing more than one internode of myelin in distinct axons.

Key words: demyelination, ethidium bromide, remyelination, Schwann cells, spinal cord.

Expressão pelas células de Schwann de um padrão de remielinização semelhante ao oligodendroglial após injeção de brometo de etídio na medula espinhal de ratos

RESUMO

As células de Schwann são reconhecidas por sua capacidade de produzir internodos de mielina únicos ao redor de axônios do sistema nervoso periférico. No modelo de desmielinização primária do brometo de etídio (BE) no tronco encefálico, tem sido observada a entrada destas células no sistema nervoso central. Isso pode contribuir para o reparo mielínico desempenhado pelos oligodendrócitos que sobreviveram à ação glitóxica do BE, chegando a remielinizar mais de um axônio ao mesmo tempo, em um padrão de reparo semelhante ao oligodendroglial. O presente estudo foi realizado na medula espinhal para observar se as células de Schwann mantinham esta competência de atender simultaneamente diferentes internodos. Foi observado que, ao contrário do tronco encefálico, as células de Schwann foram as células mielinogênicas mais importantes no sítio de desmielinização induzida pelo BE e, embora raro, também apresentaram a capacidade de produzir mais de um internodo de mielina em axônios distintos.

Palavras-chave: brometo de etídio, células de Schwann, desmielinização, medula espinhal, remielinização.

Schwann cell remyelination in the central nervous system (CNS) is a well described event in demyelinating diseases such as multiple sclerosis (MS)[1-4] and in some experimental models of primary demyelination like the ethidium bromide

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(EB) gliotoxic model[5-14], where there is early oligodendroglial and astrocyte disappearance and subsequent myelin loss and disruption of both glia limitans and blood-brain barrier. As astroglial processes disappear in most areas of the EB-induced lesions, Schwann cell entry in the CNS is facilitated, although the exact source and the precise mode of entry of these cells remain obscure[5-7].

Ultrastructural examination of the central area of EB-induced lesions in the rat brainstem often reveals the presence of Schwann cells related to one or more naked axons or at initial remyelinating stages[10,11]. Although rare, it is described that the same Schwann cell may be found producing myelin in more than one axon of small caliber. Axons associated to Schwann cells usually show more advanced remyelination, with thicker myelin sheaths than those related to surviving oligodendrocytes at the same period[10,11].

Differently to the brainstem, there are no reports of Schwann cells remyelinating more than one axon in the spinal cord. In this context, the aim of this study was to investigate Schwann cell behavior after a gliotoxic injection in this site in order to see if this oligodendrocyte-like remyelinating pattern is exclusively seen in the brainstem or can also be found in the spinal cord.

METHOD

Twenty male Wistar rats were used in this experimental study approved by the Scientific and Ethics Comission of the University Paulista (UNIP) under protocol no. 002/09. All rats were anaesthetized with ketamine and xylazine (5:1; 0.1 ml / 100g) and submitted to a laminectomy on the first lumbar vertebra to allow 1 µl of 0.1% EB solution (group I) or 0.9% saline solution (group II) to be injected into the dorsal columns by means of a Hamilton syringe.

The rats were anesthetized and submitted to intracardiac perfusion with 4% glutaraldehyde in 0.1 M Sorensen phosphate buffer (pH 7.4). Three animals from group I and 2 from group II were perfused at each of the following periods – 7, 15, 21 and 31 days after injection. The roofs of all vertebrae in the thoraco-lumbar region were removed by using bone nippers. The spinal cord was removed and the region of the spinal cord containing the lesion was cut into a series of 1 mm thick transverse slices which were collected and post-fixed in 1% osmium tetroxide, dehydrated with graded acetones and embedded in Araldite 502 resin, following transitional stages in acetones. Thick sections were cut from each block and stained with 0.25% alkaline toluidine blue. Selected areas were trimmed and thin sections were stained with 2% acetate and lead citrate and examined using a Philips EM-210 transmission electron microscope.

RESULTS

The EB-induced lesions in animals from group I varied in size and the central areas presented naked axons, conspicuous macrophagic infiltration, myelin-derived membranes in the extracellular space and few lymphocytes. Demyelinated axons were later remyelinated by invasive Schwann cells, chiefly in subpial and perivascular areas, or by oligodendrocytes in areas close to normal tissue and presenting reactive astrocytes. In all lesions macrophages were recognized by their round nuclei and abundant cytoplasm containing lipid droplets and myelin debris.

Each Schwann cell showed oval to elongate shape, its

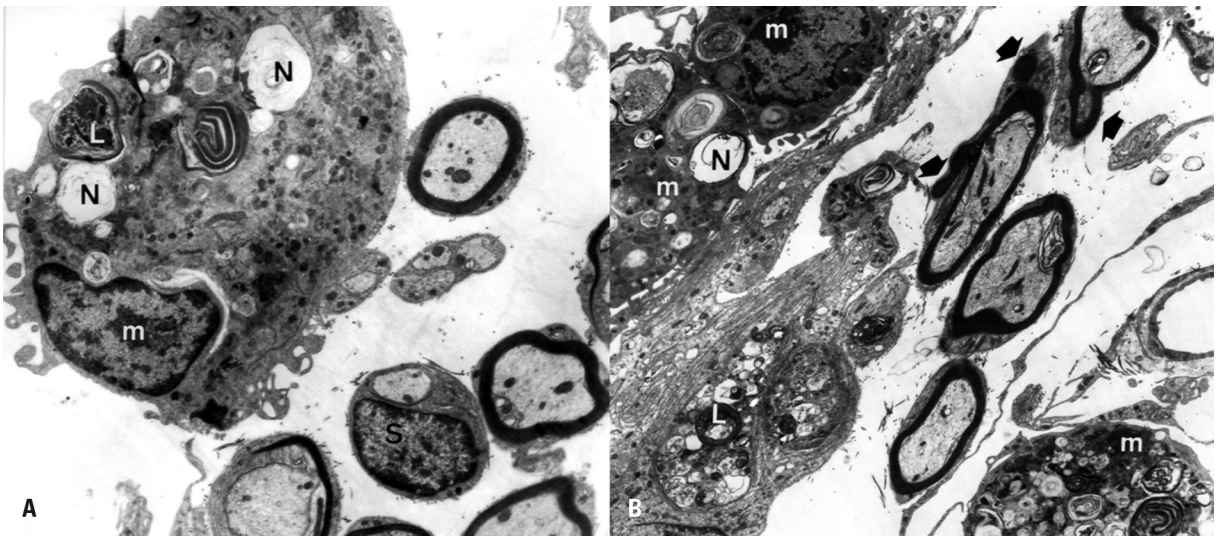

Fig 1. [A and B] Group I - 31 days after EB injection. Schwann cells (S) contact individually axons exhibiting myelin sheaths of variable thickness, even forming redundant loops (arrows). Note phagocytic cells (m) containing myelin in different stages of degradation, including lamellae (L) and fat neutral droplets (N) in areas of enlarged extracellular space. Electron micrograph, [A] 6.645×; [B] 4.305×.

nucleus was often indented and the nucleolus was large and usually located in apposition to the inner nuclear membrane. The Golgi system was prominent and tended to concentrate close to the nuclear indentation. The cytoplasm presented extensive rough endoplasmic reticulum, usually arranged parallel to the cell membrane, abundant polysomes and 6-9 nm filaments uniformly distributed within the cell. When the cell had surrounded an axon it was coated by basal lamina. The extracellular space round Schwann cells contained variable number of large diameter collagen fibers. After encirclement of axons, when a basal lamina had been formed, small collagen fibers were seen in contact with the basal lamina. In those areas where remyelination was performed by Schwann cells, the glia limitans was disrupted, astrocyte processes disappeared and the extracellular space was expanded allowing an easy access and mobility to their intrusion. Schwann cells were seen related to one or more naked axons from the 7th to the 31st day post-injection and at the 15th day they were at initial stages of remyelination, already forming thick myelin sheaths around individual internodes, in a much faster way than oligodendrocytes did, even forming redundant loops around some axons at 21 days. (Figs 1A and B). Many Schwann cells persisted around groups of naked axons like they do around PNS non-myelinated axons. Oligodendrocytes also began to remyelinate at peripheral sites 15 days after the EB injection (Fig 2).

In rare situations, it was noted that the same Schwann cell was simultaneously producing myelin to 2 or more axons of small diameter, adopting an oligodendrocyte-like pattern of repair from the 21st day post-injection (Fig 3).

Rats that received saline injection (group II) presented discrete and circumscribed lesions noted at the 7th day after injection and consisting of Wallerian degeneration along the needle track. No lesion was observed by 15, 21 and 31 days post-injection Ultrastructural examination of the lesion revealed a light and focal expansion of the extracellular space, containing some loose lamellae, few myelin-derived membranes and clearing of cellular debris by phagocytic cells. Some fibers showed periaxonal edema and signs of degeneration, but there was no evidence of either primary demyelination or loss of neuroglia. No Schwann cell invasion into the spinal cord was noted in this group.

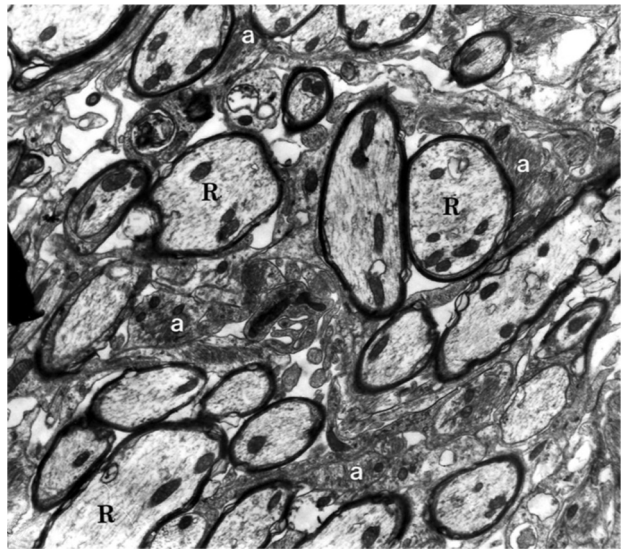

Fig 2. Group I - 31 days after EB injection. Oligodendrocyte remyelinated axons (R) associated with astrocytic processes (a). Electron micrograph, 11.200×.

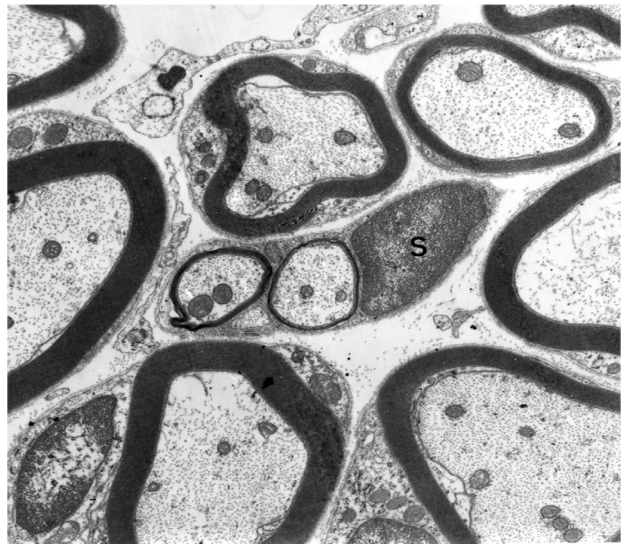

Fig 3. Group I - 21 days after EB injection. Schwann cell remyelinated axons. A basal lamina and some collagen fibers can be seen on the surface of these Schwann cells. Note 2 small caliber axons being remyelinated by the same Schwann cell (S). Electron micrograph, 10.253×.

DISCUSSION

Although the major remyelinating cells in the brainstem were the oligodendrocytes[9-15], Schwann cells appeared to be the most important in the spinal cord remyelination as stated by previous studies[5-8].

Apart the presence of ectopic Schwann cells in the CNS in a variety of spontaneous[1-4] and experimental[5-14] pathologic situations, they were also reported in subpial areas of apparently normal rabbit, guinea pig or human spinal cord[16].

Nevertheless it is generally accepted that disruption of the glia limitans is a prerequisite for Schwann cells to intrude into the CNS. It has been proposed that invading Schwann cells come from peripheral nerve source close to central lesions - the most commonly considered sources being the dorsal roots or the pial nerves and the autonomic fibers accompanying blood vessels[4].

Schwann cell remyelination in the spinal cord can be prevented if astrocytes are introduced into the sub-

arachnoid space to reconstruct the glia limitans, or when mixtures of oligodendrocyte precursors and astrocytes are transplanted to reconstruct a normal CNS environment[17,18]. In these transplantation studies, myelin repair by Schwann cells is only observed near to the injection point, either in association with blood vessels or adjacent to basal lamina-covered astrocytes, both of which are situations where an extracellular matrix (ECM) is present[19]. Schwann cells that make contact with axons away from either blood vessels or astrocytes can be observed associating with axons but failing to produce myelin sheaths, mimicking their inability to myelinate axons in vitro in the absence of a steady ECM[19]. This leads to a curious paradox - the requirement of astrocyte disappearance to permit that transplanted Schwann cells gain access to demyelinated axons versus the hability of Schwann cells to myelinate only if they find a stable ECM (that astrocytes are able to provide).

Central nervous system has a distinctive ECM, with paucity of otherwise frequent molecules, like fibronectin and collagens; it is mainly composed of proteoglycans of the lectican / hyalectan-family, such as aggrecan, versican (V0, V1, V2), neurocan, brevican, phosphacan, and their binding partners, hyaluronan, link proteins (HAPLNs - hyaluronan and proteoglycan binding link proteins) and tenascins (Tn)[20]. Once established, the composition of the mature type of ECM is rather stable with little or no turnover of their components. This radically changes when lesions to the adult CNS occur, thus reactivating the juvenile-type of matrix previously found during early nervous system development[20]. For detailed information about ECM in the CNS, see recent review by Zimmermann, Dours-Zimmermann[20].

In addition it is known that Schwann cell migration is integrin-dependent and is inhibited by astrocyte-produced aggrecan[21], fact that may explain Schwann cell invasion in the astrocytic-free environment observed in EB-induced lesions. However, injection of this gliotoxin deeply destabilizes the delicate microenvironment found in the CNS, leading to areas of unstable matrix that somehow present a different balance of the trophic factors required for cell migration, tissue repair and, particularly, myelin reconstruction.

In our study, Schwann cells were able to enter and remyelinate in areas apparently destituted of close astrocytes, perhaps because the locations where they were found constructing myelin (around blood vessels and below piamater) provided this requirement for a stable matrix, thus exempting the need for proximity to astrocytic processes.

Schwann cells also proliferate in the CNS - they appear to divide on contact with demyelinated axons and to stop dividing when they form myelin[22,23]. Several factors such as neuroregulin-1, insulin-like growth factors (IGFs), fibroblast growth factor (FGF) 1 and 2, platelet-derived growth factor (PDGF), transforming growth factor β (TGF β), act as mitogens for Schwann cells. Some inhibit myelination like neuroregulin-1, neurotrophin-3 (NT3) and TGF β; others like brain-derived neurotrophic factor (BDNF) and glial derived neurotrophic factor (GDNF) stimulate myelin deposition[24].

Schwann cell myelination represents an exceptional example of a transforming cell-cell interaction, in which contact with certain axons, the large diameter ones, triggers a radical change in the phenotype of immature Schwann cells, leading to the generation of one of the most highly specialized cell types in the body, the myelinating Schwann cell. Although the Schwann cell myelinating process has been widely studied, to date, no myelination-inducing signal has yet been isolated from axons, nor have myelin-signal receptors been identified in Schwann cells[24].

In the PNS a single Schwann cell is intimately related to the single internode it forms, whereas in the CNS a mature oligodendrocyte can produce multiple internodes of myelin (from 30 to 50)[25,26]. These internodes in the CNS are laid down on different axons either in the same or in different tracts and the myelin sheaths are connected to the oligodendrocyte cell body by long and tortuous processes, so that this cell body may be at some distance from the internodes it forms.

As observed in the brainstem after EB injection into the basal cisterna[10,11], the finding of some Schwann cells compromised to 2 or more axons of small diameter in the spinal cord may be the result of an intense inflammatory response induced by the gliotoxin with subsequent appearance of stimulating factors that may represent the necessary stimuli to drive Schwann cell migration from the PNS into the CNS and allow myelin formation in more than one embraced axon that should be myelinated. Nerve growth factor (NGF)-related neurotrophins (NGF, BDNF), GDNF-family ligands (GDNF, NTN - neurturin), neuropoetic cytokines (CNTF - cilliary neurotrophic factor, LIF - leukemia inhibitory factor), as well as IGFs, PDGF, FGF, among many others, are assumed to be probable candidates for this stimulating role[27].

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