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BIOLOGY OF THE REPAIR OF CENTRAL NERVOUS SYSTEM DEMYELINATED LESIONS

AN APPRAISAL

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ABSTRACT - The integrity of myelin sheaths is maintained by oligodendrocytes and Schwann cells respectively in the central nervous system (CNS) and in the peripheral nervous system. The process of demyelination consisting of the withdrawal of myelin sheaths from their axons is a characteristic feature of multiple sclerosis, the most common human demyelinating disease. Many experimental models have been designed to study the biology of demyelination and remyelination (repair of the lost myelin) in the CNS, due to the difficulties in studying human material. In the ethidium bromide (an intercalating gliotoxic drug) model of demyelination, CNS remyelination may be carried out by surviving oligodendrocytes and/or by cells differentiated from the primitive cell lines or either by Schwann cells that invade the CNS. However, some factors such as the age of the experimental animals, intensity and time of exposure to the intercalating chemical and the topography of the lesions have marked influence on the repair of the tissue.

KEY WORDS: toxic demyelination, remyelination, central nervous system, peripheral nervous system.

Biologia da reparação de lesões desmielinizantes do sistema nervoso central: uma avaliação

RESUMO - A integridade da bainha de mielina é fornecida pelos oligodendrócitos e pelas células de Schwann, no sistema nervoso central (SNC) e no sistema nervoso periférico, respectivamente. O fenômeno de desmielinização refere-se à remoção das bainhas de mielina de axônios e este fato é característico na esclerose múltipla, a doença desmielinizante do SNC mais comum no homem. Muitos modelos experimentais têm sido utilizados para o estudo da biologia da desmielinização e remielinização no SNC, face à dificuldade de estudo de material humano. No modelo experimental da droga intercalante, gliotóxica, brometo de etídio, a remielinização do SNC pode ser efetuada por oligodendrócitos sobreviventes à lesão e/ou oriundos de diferenciação de linhagens celulares mais primitivas e por células de Schwann que invadem o SNC. No entanto, fatores como a idade dos animais, a intensidade, e o tempo de exposição ao agente intercalante e a topografia da lesão influenciam significativamente a reparação da lesão.

PALAVRAS-CHAVE: desmielinização tóxica, remielinização, sistema nervoso central, sistema nervoso periférico.

The central nervous system (CNS) develops from a thickened area of the embryo ectoderm, the neural plate. The neural tube formation and the neural crest development will respectively give rise to the CNS and

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most of the peripheral nervous system (PNS). Thus histologically the nervous tissue of the CNS is made up of neurons and glial cells from a single layer of proliferating neural epithelium or by cells of the primitive neural tube (for details see Moore[20]; Pereira & Graça[24]) and a non-collagenic extracellular matrix[3].

Raff et al[26] and Raff[27] defined the neuroglia according to the expression of cellular antigens along their fetal development, using immunocytochemical methods. Astrocytes may be classified into two distinct classes: Type I (former protoplasmic), RAN-2$^+$ and Type II (former fibrous) A2B5$^+$. Type II astrocytes assist interfascicular oligodendrocytes in the formation and maintenance of the myelin sheath and in saltatory conduction[27]. Interrelationships between Type II astrocytes and oligodendrocytes (GC$^+$) come from a common embryonic origin, the O-2A cell[27].

In the CNS oligodendrocytes put many layers of their cytoplasmic membrane around one or more axons, forming a spiralled structure, the myelin sheath under the form of short tubes named internodes[1]. In the PNS myelin sheath formation is made up by Schwann cells. Each Schwann cell forms one 1 mm long internode in a single axon composed of up to 300 concentric lamellae[1]. Oligodendrocytes form similar segments although they extend cytoplasmic processes that may myelinate many axons simultaneously, in some cases up to two hundred[10]. This fact and the proliferation potentialities of Schwann cells explain the larger repair capacity of demyelinated lesions in the PNS.

DEMYELINATION/REMYELINATION

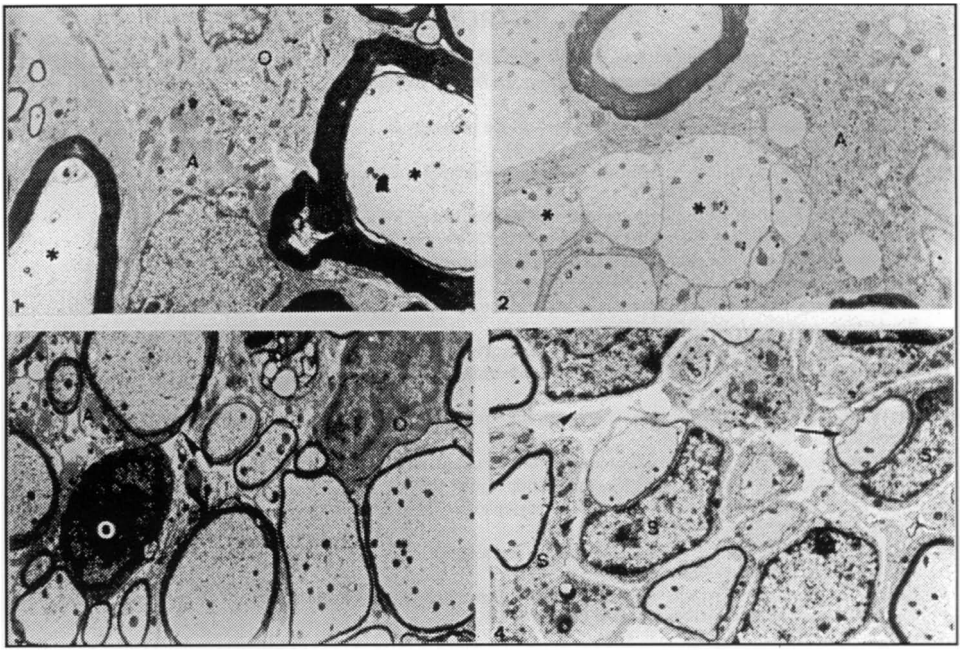
The phenomenon of demyelination consists of the removal of myelin sheaths from normal CNS and PNS axons usually with perivascular infiltration of small lymphocytes, red blood cells and large mononuclear cells[29]. Such myelin loss is frequently referred as primary demyelination[5] and must be differentiated from degeneration of myelin sheaths following axonal degeneration, known as Wallerian degeneration.

Many experimental models have been used to study the biology of primary demyelination and remyelination that take part in spontaneous CNS diseases. Those models seek to mimic demyelinating pathologies and to search for physiological features of those diseases including the behavior of glial cells involved with repair processes after the action of harmful agents. Among these demyelinating CNS diseases, multiple scleroses (MS) and distemper, in humans and canines respectively, are the most striking, being the most marked histological feature of such disorders the presence of persistent demyelinated areas which consistently fail to remyelinate and are responsible for the symptoms of the disease.

Inoculation of lytic viruses that infect oligodendrocytes, such as Theiler's virus[18]; induction of immunologic reactions against cells that express viral antigens, such as those of Semliki Forest virus[15]; induction of *in vivo* immunological reactions by intradermal inoculation of emulsified brain[17]; *in vitro* addition of myelinotoxic EAE (experimental allergic encephalomyelitis) serum[16]; systemic administration of toxic substances such as cuprizone[19] and hypocholesterolemic drugs[32]; local injection of either myelinotoxic or gliotoxic agents such as lysolecithin[6], 6-aminonicotinamide[4] and ethidium bromide (EB)[5,9,12,13,23,31,34], are among the experimental models which have been developed in order to clarify the cellular processes of demyelination and remyelination.

As in individuals suffering of MS there is a persistent failure in the repair of lost myelin sheaths, the viewpoint that remyelination did not occur in the CNS was sustained for many years. However in 1961, Bunge et al.[7] firstly reported CNS remyelination after demyelination following cerebral fluid barbotage in the cat. Albeit the remyelinating process is viable, it depends upon factors as the intensity, the exposition time to the demyelinating agent[10] as well as the CNS areas involved[23]. In the EB model of demyelination when the lesion is inflicted to the spinal cord of Wistar rats, remyelination may be accomplished either by surviving oligodendrocytes over the edges of the lesions or Schwann cells that invade the CNS along subpial and perivascular areas (Fig 1 to 4)[9,13]. When EB is injected into the brain stem of Wistar rats, remyelinating-oligodendrocytes- repair myelin sheaths chiefly around the periphery of the lesions and Schwann cells remyelinate axons also in places other than subpial and perivascular areas[23,25].

Although remyelination is well recognized both in experimental models and spontaneous diseases the origin of oligodendrocytes and Schwann cells remains a controversial issue. Three hypothesis are proposed for the presence of remyelinating oligodendrocytes: 1) they would be surviving cells from the edges of the lesions in MS cases[30]; 2) oligodendrocytes would be able of generating remyelinating daughter-cells[19], although the absence of morphological demonstration of oligodendrocyte mitosis in adult individuals, is an intriguing feature; 3) oligodendrocytes might come from the differentiation of the adult O-2A lineage cells, once autoradiographic



Figs 1 to 4: EB model of demyelination of the CNS. 1. Normal white matter of the spinal cord in an adult Wistar rat: astrocyte (A), oligodendrocyte (O); myelinated axons (*) 4300X; 2. demyelinated axons (*) separated by processes from an astrocyte (A) 4300X; 3. Oligodendrocyte (O) remyelination of demyelinated axons. There are irregularly packed myelin lamellae (arrowhead) and the presence of astrocytic processes (A) closely related to the new sheaths. 4300 X. 4. Schwann cell (S) remyelination of demyelinated axons. Myelin sheaths show loosely packed myelin lamellae (arrow). Some interstitial collagen fibres (arrowhead) abut Schwann cells basal laminae. 4300 X. IB. UNICAMP.

experiments show poor oligodendrocyte proliferation in adult mice[21]. Oligodendroglial replacement in adult animals is being studied by immunocytochemical identification of the adult O-2A lineage cells and to find out which stimuli trigger the proliferation and differentiation of cells of such lineage constitute the goal of numerous investigations[33]. However the most relevant feature is that oligodendrocytes may be a replacement source since profuse astrocytic gliosis, observed in most lesions of the CNS, does not hinder remyelination[22].

On the other hand, the presence of myelinating Schwann cells in the CNS has been depicted in normal[28] as well as in several demyelinating processes related to human diseases such as MS[14] and many of the experimental models mentioned above. The way and stimulus for the migration of Schwann cells into demyelinating lesions in the spinal cord has been comprehensively discussed by Graça[9]. The origin of Schwann cells in those lesions appears to be related to blood vessels, pial and dorsal nerve root[9,11].

Although remyelination is a fact in most experimental models of demyelination, it is necessary to understand the mechanisms that interfere with that function in MS lesions. One limiting factor could be the gliosis[8] made up by Type I astrocytes although oligodendrocyte remyelination in gliotic areas in the brain of Wistar rats[23] and humans[2] has been consistently reported. Thus, astrocytosis *per se* can not be blamed for the lack of remyelination [23,25]. The main problem seems to be the lack of appropriate stimuli for the differentiation of cells from the O- 2A lineage. Astrocytic gliosis and the integrity of the blood brain barrier, on the other hand, could interfere with the CNS invasion of the Schwann cells[23].

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