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Presence of α-globin mRNA and migration of bone marrow cells after sciatic nerve injury suggests their participation in the degeneration/regeneration process

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Abstract

We have previously reported that in the distal stump of ligated sciatic nerves, there is a change in the distribution of myelin basic protein (MBP) and P_0 protein immunoreactivities. These results agreed with the studies of myelin isolated from the distal stump of animals submitted to ligation of the sciatic nerve, showing a gradual increase in a 14 kDa band with an electrophoretic mobility similar to that of an MBP isoform, among other changes. This band, which was resolved into two bands of 14 and 15 kDa using a 16% gel, was found to contain a mixture of MBP fragments and peptides with great homology with α- and β-globins. In agreement with these results, we have demonstrated that the mRNA of α-globin is present in the proximal and distal stumps of the ligated nerve. It is also detected at very low levels in Schwann cells isolated from normal nerves. These results could be due to the presence of α- and/or β-globin arising from immature cells of the erythroid series. Also, they could be present in macrophages, which spontaneously migrate to the injured nerve to promote the degradation of myelin proteins. Cells isolated from normal adult rat bone marrow which were injected intraortically were found to migrate to the injured area. These cells could contribute to the remyelination of the damaged area participating in the removal of myelin debris, through their transdifferentiation into Schwann cells or through their fusion with preexisting Schwann cells in the distal stump of the injured sciatic nerve.

Keywords: Myelin proteins; Peripheral myelin; Wallerian degeneration; α-Globin; Macrophages; Bone marrow mononuclear cells

Introduction

Wallerian degeneration is a well characterized process in which one of the hallmarks is myelin breakdown. Although in the last years, enormous progress has been made in the understanding of the cellular events and molecular changes occurring during degeneration and regeneration of peripheral nerves, the majority of the molecular players involved in nerve regeneration await identification. Understanding the molecular mechanisms of the regeneration of the peripheral nervous system (PNS) is essential to allow experimental designs facilitating the complete recovery after injury.

In a recent paper (Setton-Avruj et al., 2002) we have shown changes in the expression of MBPs and P_0 in sciatic nerves of young and adult rats following nerve ligation with immunohistochemistry and SDS-PAGE of isolated myelin. In adult rats, there was an increase in MBP immunostaining from 3 to 7 days postligation, followed by a marked dec-

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rease at 14 days. P_0 immunolabeling increased from 3 to 14 days postligation. Young animals showed an acceleration in the process of protein redistribution and digestion within ligated nerves, which followed a pattern similar to that of adult animals. With reference to the myelin composition, the most prominent result was the unusual increase in both groups of animals of a 14 kDa protein band which could correspond to one of the MBP isoforms. Analyzing this 14 kDa band in another SDS-PAGE system, we detected two different bands of 14 and 15 kDa and, in a preliminary analysis, we also found that these bands contained a mixture of fragments of low molecular weight isoforms of MBP with protein products that could probably derive from α- and β-globin.

De Leon et al. (1991) have shown that under certain circumstances, the mRNA of α-globin is expressed in peripheral nerves, particularly after injury. In coincidence with our results, they demonstrated that the expression of the α-globin sequence was repressed on the distal side of the injured nerve when compared to the contralateral side, 3 days after crushing. This gene is also known to be expressed by macrophages and by several primitive cells derived from the bone marrow (Liu et al., 1999; Herzog et al., 2003). Active SCs and recruitment of macrophages seem to play an important role in Wallerian degeneration as well as in nerve regeneration after PNS injury (Bunge, 1993). Macrophages could benefit remyelination by exerting two different roles: phagocytic clearance of myelin debris (Smith, 1999; Shen et al., 2000) and release of trophic factors for the repair of injury (Miyauchi et al., 1997; Dailey et al., 1998; Siebert et al., 2000). Other primitive cells derived from bone marrow could also have a role in peripheral nerve injury repair or, as it has been recently described, they could participate through their differentiation into neural cells (Dezawa et al., 2001; Cuevas et al., 2002; Mimura et al., 2004; Zhang et al., 2004).

In this paper we demonstrate that the 14 kDa band that we observed in our previous studies can be resolved when isolated myelin is studied in a 16% tricine gel, into two different bands of 14 and 15 kDa. Sequence analysis of these bands showed that they contain a mixture of MBP fragments and of α- and β-globin peptides. Using an RNAase protection assay, Northern blot analysis and real time-PCR, we clearly demonstrate the presence of the mRNA of α-globin in the sciatic nerve of the rat. Adult SCs studied with the same methodology contain negligible levels of the α-globin mRNA. Cells which express markers of macrophages and undifferentiated bone marrow cells, which also express the mRNA of α-globin were detected in the injured nerve, and in the myelin isolated from the proximal and distal stumps. Isolated bone marrow mononuclear cells (BMMCs) expressing α-globin, intravascularly injected into the experimental animals, were found to migrate to the injured area of the nerve, most likely in order to help repair the damaged tissue and suggesting that they could have an important role in the degeneration–regeneration process that takes place after peripheral nerve injury.

Materials and methods

Materials

Polylysine, trypsin, collagenase, cytosine arabinoside, sucrose, leupeptin, pepstatin A, Coomasie blue, bovine serum albumin, penicillin, streptomycin, proteinase K, 3,3′-diaminobenzidine using glucose oxidase and nickel salts were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Dulbecco modified Eagle′s medium (DMEM) high glucose with L-glutamine, and α modified Eagle's medium (α MEM) were from GIBCO (Gran Island, NY, USA). Fetal calf serum was purchased from Natocor (Córdoba, Argentina). The ECL Plus kit was from Amersham-Pharmacia Biotech (UK). Anti-ED1 antibody was purchased from Ultraclone (Isle of Wight, UK). Anti-CD11b and anti-CD45 antibodies were purchased from Chemicon International Inc. (Temecula, CA, USA) and anti-CD34 antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Secondary antibodies for immunocytochemistry and Western blot studies were purchased from Jackson (West Grove, PA, USA). Biotinylated secondary antibodies, FITC-conjugated secondary antibodies and the ABC Elite kit for immunohistochemistry were purchased from Vector Lab. (Burlingame, California, USA). The OCT compound was from Tissue Tek, Miles Laboratories (Elkhart, IN, USA). The RNAse kit was from Qiagen (Venlo, The Netherlands) and RNase A and RNase T1 were purchased from Promega Corp (Wisconsin, USA). Trizol reagent was purchased from Invitrogen (Carlsbad, CA, USA).

Animals

Adult Wistar rats (70 day old, 250–280 g body weight) of either sex were anesthetized with chloral hydrate (1.5 g/kg i.p.), and their right sciatic nerves were exposed and ligated at the midthigh level. The animals were allowed to survive for 3 and 5 days. At the end of each survival period, the animals were re-anesthetized and perfused via the ascending aorta with 50 ml of 10% formalin containing 0.2% picric acid in 0.1 M phosphate buffer (37°C) followed by 150 ml of the same fixative at 4°C. Both sciatic nerves were carefully dissected out, and the proximal and distal regions to the ligation were identified in the right nerve (ipsilateral).

Isolation of peripheral nerve myelin

Purified myelin was obtained from the proximal and distal regions of ligated nerves as well as from the contralateral sciatic nerves of adult rats by homogenizing the tissue in 2 ml of 0.32 M sucrose containing 1.1 M leupeptin and 1.02 M pepstatin A, in glass-Teflon homogenizer at 3000–5000 rpm. Subcellular fractionation was carried out by a modification of the procedure of Iyer et al. (1996). The homogenate was layered on 4 ml of 0.85 M sucrose in 16 × 102 mm tubes and centrifuged for 45 min at 85,000 × *g* in a SW 28 Ti rotor. The band at the interface was removed and resuspended in 4 parts of cold water.