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Research Report

Increased phosphorylation of caveolin-1 in the sciatic nerves of Lewis rats with experimental autoimmune neuritis

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ABSTRACT

The levels of phosphorylated caveolin-1 (p-caveolin-1) were analyzed in the sciatic nerves of Lewis rats with experimental autoimmune neuritis (EAN). Western blot analysis showed that the phosphorylation of caveolin-1 increased significantly in the sciatic nerves of EAN-affected rats at the paralytic stage of EAN on day 14 post-immunization (PI) ($P<0.05$) and declined slightly thereafter during the recovery stage. Immunohistochemistry showed intense p-caveolin-1 immunostaining in some inflammatory macrophages, as well as in T-cells in individual nerve fascicles at the peak stage of EAN, while p-caveolin-1 was weakly expressed in some of the vascular endothelial cells and Schwann cells of normal sciatic nerves. The inflammatory cells with intense p-caveolin-1 expression in the EAN-affected individual nerve fascicles were not positive for terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL), while the TUNEL-positive apoptotic cells in the perineurium, where infiltration initially occurred, were weakly positive for p-caveolin-1. Based on these findings, we postulate that caveolin-1 is phosphorylated in inflammatory cells soon after they infiltrate the sciatic nerve, as well as in the perineurium, and that p-caveolin-1 activates intracellular signaling in inflammatory cells, leading to cell death, which ultimately eliminates the infiltrating inflammatory cells from the sciatic nerves of animals with EAN.

1. Introduction

Experimental autoimmune neuritis (EAN), which is a T-cell-mediated autoimmune disease of the peripheral nervous system (PNS), is used as a model of human demyelinating diseases, such as Guillain–Barré Syndrome (Hartung and Toyka, 1990; Zhu et al., 1998). The clinical course of EAN is characterized by weight loss, ascending progressive paralysis, and spontaneous recovery. It has been proposed that inflammatory mediators produced in the affected spinal nerves roots and sciatic nerves are involved in the pathogenesis of EAN (Zhu et al., 1998). EAN lesions in susceptible animals are

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characterized by T cells and macrophages infiltrating into the sciatic nerves during the peak stages of the disease (Moon et al., 2005, 2006). In addition, the apoptotic elimination of inflammatory cells is a common feature of autoimmune diseases, such as EAN (Moon et al., 2005). Although many factors are involved in the process of apoptosis in EAN lesions (Conti et al., 1998; Moon et al., 2005), little is known about the activation of lipid raft proteins, such as caveolins.

Caveolin is a transmembrane adaptor molecule that recognizes glycosylphosphatidyl inositol-linked proteins and interacts with downstream cytoplasmic signaling molecules, which include the Src-family tyrosine kinases and heterotrimeric G proteins (Li et al., 1996; Okamoto et al., 1998). Under certain activation conditions, caveolin is phosphorylated, and the phosphorylation of caveolin at Tyr-14, Ser-88, and other residues in v-Src-transformed cells leads to the flattening, aggregation, and fusion of caveolae and caveolae-derived vesicles (Li et al., 1996). In addition, the phosphorylated form of caveolin-1 (p-caveolin-1) is thought to be a downstream element of p38 mitogen-activated protein (MAP) kinase and c-Src in NIH 3T3 cells (Volonte et al., 2001), which are also important signals in inflammatory diseases.

In autoimmune disease models, we have reported that the expression of caveolins is significantly elevated in the spinal cord in experimental autoimmune encephalomyelitis (EAE) (Shin et al., 2005) and in the sciatic nerve in EAN (Ahn et al., 2006). Subsequently, we have found that the level of caveolin-1 phosphorylation increases in the spinal cords of EAE animals, particularly in the activated microglia and macrophages present in the EAE lesions (Kim et al., 2006). In EAE, caveolin-1 is not phosphorylated in all caveolin-1-positive cells (Shin et al., 2005). These findings suggest that caveolin-1 is phosphorylated in a limited cell population (mainly macrophages and activated microglia) in rat EAE. Although a variety of cells in the EAN lesions express caveolin-1 (Ahn et al., 2006), little is known about the status of caveolin-1 phosphorylation in the sciatic nerves of rats with EAN. Thus, we examined the phosphorylation of caveolin-1 in the PNS in autoimmune-targeted sciatic nerves.

2. Results

2.1. *Clinical progression of experimental autoimmune neuritis and histopathological findings*

Rats with EAN immunized with the SP26 peptide developed floppy tails (grade 1, G1) on days 10–12 post-immunization (PI)

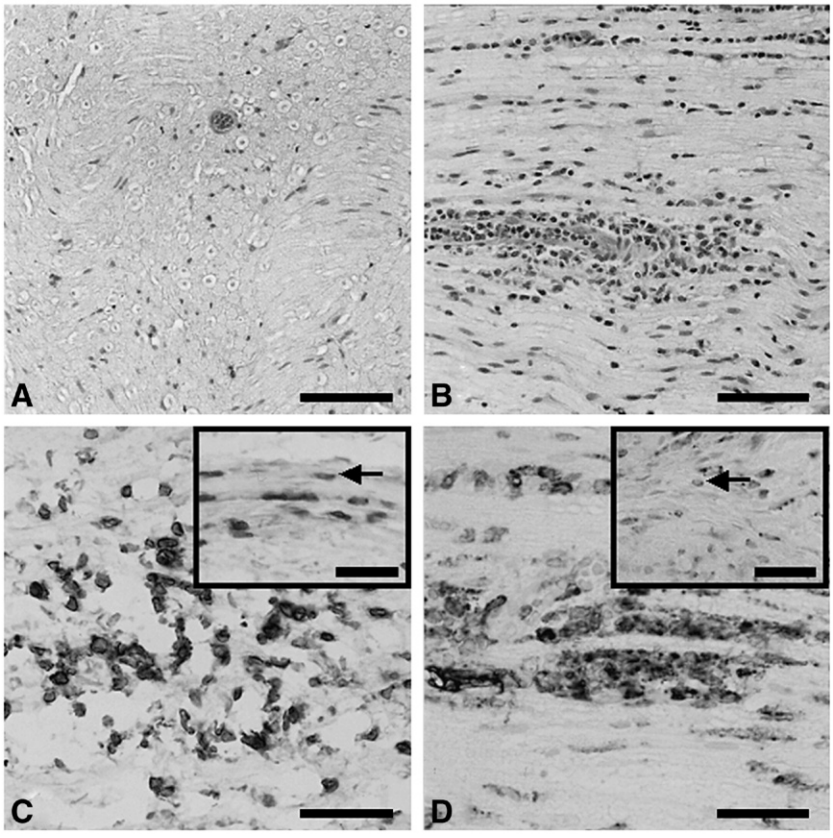

Fig. 1 – Histopathological examination of normal and EAN-affected sciatic nerves of rats. No inflammatory cells were present in the sciatic nerves of the normal control rats (A). On day 14 PI, many inflammatory cells were found in the sciatic nerves (B). In the EAN-affected sciatic nerve (D14PI), the majority of the inflammatory cells were R73 (TCR αβ)-positive T cells (C) and ED1-positive macrophages (D) in the individual nerve fascicles and perineurium (insets, arrow). (A and B) Hematoxylin–eosin staining. (C and D) Immunostained with either R73 (C) or ED1 (D) and counterstained with hematoxylin. Scale bars: in A and B, 80 μm; in C and D, 40 μm; in inset, 30 μm.