Introduction
Schwann cells play an important role in nerve regeneration as a source of neurotrophic factors and macrophages stimulate secretion of neurotrophic factors from Schwann cells. In addition, macrophages themselves have the function of producing neurotrophic factors as well as phagocytosing necrotic axons and myelin sheaths at the injured distal nerve segment. Thus, it is of note that macrophages play critical roles in both degenerative and regenerative processes following peripheral nerve injuries. In the process of macrophage recruitment, macrophage migration inhibitory factor (MIF) was the first lymphokine reported to prevent the random migration of macrophages out of capillary tubes. In recent years, it has been found that MIF is present in skin, scutum tissues, bone, kidney and vascular endothelial cells and its role in tissue healing has been noted. However, presence of MIF in peripheral nervous system has not been identified yet and its kinematics after nerve injury is unclear. In this study, we reveal for the first time the expression and localization of MIF in myelinated peripheral nerves using reverse transcription-polymerase chain reaction (RT-PCR), Western blotting and immunohistochemistry. Furthermore, we examined effects of nerve transection on MIF expression by Northern blotting and immunohistochemical analysis, and discuss an possible role of this protein during the process of nerve degeneration-regeneration.

Materials and Method
All experiments were carried out on male 10-week-old Wistar-King-Aptekman rats weighing around 300 g. First, rats were anesthetized with sodium pentobarbital (50 mg/kg, i. p.) and perfused transcardially with 100 ml of saline at 1 h after nerve transection. The sciatic nerve was resected, stripped of excess connective tissue, and immediately frozen in liquid nitrogen. Total RNA and protein were extracted from the nerve tissue. We performed RT-PCR analysis to confirm expression of MIF mRNA and Western blot analysis to confirm expression of MIF protein in peripheral nerves without lesions. To identify the histological localization of MIF in peripheral nerve tissues, immunohistochemical analysis was carried out. Animals were anesthetized and perfused transcardially with 50 ml of saline followed by 100 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.0). Ten-micrometer-thick frozen sections were incubated with the rabbit anti-rat MIF polyclonal antibody and they were stained with the avidin-biotin-peroxidase complex procedure. As positive controls, we used anti-S100 and anti-neurofilament antibodies for Schwann cells and neuronal fibers. As nerve injury models, left sciatic nerves were transected just distal to the obturator tendon. At 24 h after transection, immunohistochemical staining of transected nerves, positive staining of MIF was observed by RT-PCR analysis of MIF in normal rat sciatic nerves. According to immunohistochemical analysis, the immunostaining pattern of MIF (Fig. 1A) was similar to that of S-100 in normal sciatic nerves (Fig. 1B). Underlying myelin sheaths and axons lacked specific MIF immunoreactivity, whereas axons were positively stained by the anti-neurofilament antibody (Fig. 1C). These results suggested that MIF protein was localized in Schwann cells of normal sciatic nerves. Northern blot analysis showed the level of MIF mRNA began to increase 12 h after nerve transection in both proximal and distal segments. Then the level remained high from 24 h to 7 days after injury. After this it decreased gradually between 14 and 21 days after transection (Fig. 2A and 2B). Moreover, immunohistochemical analysis of transected sciatic nerve segments at day 4 after the operation was carried out. At the proximal segments of transected nerves, positive staining of MIF was observed in axons as well as non-neuronal cells (Fig. 3A). On the other hand, Schwann cells and endoneurial fibroblasts that migrated around neuronal fibers showed immunoreactivity to MIF at distal segments (Fig. 3B).

Discussion
In this study, we found that Schwann cells of peripheral nerves were sources of MIF protein. It should be noted that the tissue distribution of MIF was not uniform in the case of nerve injury. Immunohistochemical localization of MIF at day 4 after nerve transection showed different features from normal nerves. At the distal segments, MIF was observed in Schwann cells that migrated to phagocyte myelin debris. On the other hand, MIF was identified in axons at the proximal segments. From these facts, it appears that MIF is carried by retrograde axonal transport after peripheral nerve injury in a manner similar to interleukin-1. The increase of MIF mRNA corresponds to the period when macrophages are recruited to injured nerves. In this period, it is speculated that MIF may regulate recruitment of macrophages, which stimulates the release of neurotrophic factors, e.g. nerve growth factor, from Schwann cells. We revealed the expression of MIF in rat peripheral nerves, in which MIF might play an important role in regeneration of peripheral nerves. The precise pathophysiological function of MIF in nerve regeneration remains unclear; however, the present results could contribute to further understanding of MIF in relation to peripheral nerve degeneration and regeneration.