ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IN PERIPHERAL NERVE REGENERATION: ANTI-MIF ANTIBODY INDUCES DELAY OF NERVE REGENERATION AND THE APOPTOSIS OF SCHWANN CELLS

INTRODUCTION:
Macrophages play an important role in the peripheral nerve degeneration-regeneration process by producing cytokines and stimulating Schwann cells to proliferate and to play the role of phagocytes. In the process of macrophage recruitment from the peripheral vasculature, macrophage migration inhibitory factor (MIF) is considered to play a pivotal role to concentrate macrophages at inflammatory loci. In our previous reports, we demonstrated the presence of MIF in the peripheral nervous system, where Schwann cells were the potential sources of MIF protein in peripheral nerves and MIF mRNA expression was up-regulated after axotomy. However, the precise pathophysiological functions of MIF in nerve regeneration remained to be elucidated. In the present study, we locally administered anti-MIF antibodies into regenerating rat sciatic nerves using the silicone chamber model, and the effect of anti-MIF antibody administration on nerve regeneration was assessed using the regeneration length of nerve measured by a pinch reflex test. We also evaluated the number of regenerating axons, immunohistochemical analyses revealed that p53 and, to a lesser extent, Fas were more up-regulated in the anti-MIF antibody-treated nerves than in the controls.

DISCUSSION:
As shown in this study, administration of the anti-MIF antibody led to a delay of peripheral nerve regeneration. In a previous study, we showed that MIF mRNA expression was up-regulated after nerve transection, but decreased within three weeks after nerve injury. In this context, it is conceivable that MIF may exert its stimulant effect on nerve regeneration within four weeks after axotomy. Taken together, these findings indicate that MIF may play a pivotal role in the early stages of the peripheral nerve degeneration-regeneration process. Furthermore, we demonstrated that MIF convincingly affected the survival of Schwann cells. Marked apoptosis of Schwann cells was detected in the anti-MIF antibody-treated axotomized nerves. These data strongly suggest that MIF plays a key role not only in peripheral nerve regeneration, but also in viability of Schwann cells by the suppression of apoptosis-related proteins such as p53. The current study may shed light on the novel role of MIF in the nervous system, and could auger therapeutic application of this cytokine to nerve damage.

MATERIALS AND METHODS:
All experiments were carried out on male 10-week-old Wistar King rats weighing around 350g. The rats were anesthetized with sodium pentobarbital (50 mg/kg, i. p.), and then sciatic nerves were exposed bilaterally and transected midthigh level under aseptic conditions. Each nerve end was sutured 2 mm into a silicone tube (internal diameter, 2.0 mm; external diameter, 3.0 mm; and length, 14 mm) leaving a gap of 10 mm between the divided ends. The chamber at the left leg was filled with 40 µl non-immune rabbit IgG (50 µg/ml) as a control, and the chamber at the right was filled with 40 µl anti-rat MIF antibodies (50 µg/ml). The skin wound was closed with 4-0 nylon sutures. Every three days, the animals were anesthetized and non-immune rabbit IgG or anti-rat MIF antibody was administered into the silicone chambers up until the time at which animals were sacrificed. At weeks 2, 4, and 6 after surgery, a pinch reflex test was performed. In brief, rats were anesthetized, and the sciatic nerves were re-exposed. The leading axons were localized by pinching the nerve in a proximal direction with a pair of forceps until a withdrawal reflex was elicited. The length between this point and the marked crush site was measured with a caliper and regarded as the length of regeneration of the sensory axons. To examine the number of regenerating axons, immunohistochemical analysis of the neurofilament, which is a specific protein for axons, was performed. Slides were photographed at 400x magnification, and the number of visible fluorescence-labeled axons was counted. In addition, we carried out TUNEL assay and immunohistochemical analysis of the damaged nerve segments with regard to apoptosis-related proteins such as p53 to evaluate the effects of anti-MIF antibodies on apoptosis during the regeneration process.

RESULTS:
The regeneration length of nerve measured by a pinch reflex test in the anti-MIF antibody-treated group was significantly shorter than that in the control nerve at week 4 after surgery (Fig. 1B). TUNEL assay showed that a large number of apoptotic cells, mostly Schwann cells, were observed in the intratubal and distal nerve segments at weeks 4 and 6 after surgery by the anti-MIF antibody treatment (Fig. 2). Consistent with these results, immunohistochemical analyses revealed that p53 and, to a lesser extent, Fas were more up-regulated in the anti-MIF antibody-treated nerves than in the controls (Fig. 3).

Fig. 1. Time-course study on regenerating axons. (A) The number of axons in the silicone chamber. (B) The number of axons in the distal nerve segment.

Fig. 2. Effect of anti-MIF antibody with regard to apoptosis at week 6 after nerve damage (TUNEL assay). Intratubal nerve segments in the control (a) and the anti-MIF antibody-treated group (b). Distal nerve segments in the control (c) and the anti-MIF antibody-treated group (d).

Fig. 3. Immunohistochemistry for p53, which is one of apoptosis-related proteins, in the intratubal nerve segment at week 6 after damage. Intratubal nerve segments in the control (a) and the anti-MIF antibody-treated group (b). Distal nerve segments in the control (c) and the anti-MIF antibody-treated group (d).