Posttraumatic Survival of Sensory Neurons during Allotransplantation of Rat Embryonic Tissues into the Nerve

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Allotransplantation of the liver, hindlimb, and bone marrow tissues from 14-day-old embryos into the sciatic nerve had a modulatory effect on survival of various populations of axotomized neurons in L5 spinal ganglion in an adult rat.

Key Words: *sensory neurons; allotransplantation; posttraumatic apoptosis*

Much recent attention is paid to the use of cell technologies for therapy of neurodegenerative diseases. The effect of transplantation of various cells and tissues on peripheral nerve regeneration was previously studied [1,2]. After transplantation into the peripheral nerve, mesenchymal stem cells (MSC) acquire the phenotype of Schwann cells. However, the efficacy of peripheral nerve regeneration after MSC transplantation is unsatisfactory [1]. Electrophysiological study showed that bone marrow cells from 14-15-day-old rat embryos reinnervate the soleus muscle by the 12th week after transplantation [3].

The degree of posttraumatic regeneration of the nervous system is determined by the number of survived neurons. Previous studies of the effect of cell transplantation on peripheral nerve regeneration were focused on the state of these cells, but not on neuron survival in spinal ganglia.

Here we studied survival of spinal ganglion neurons after transplantation of progenitor cells of different origin.

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MATERIALS AND METHODS

Experiments were performed on male outbred rats under urethane anesthesia (600 mg/kg intraperitoneally, Sigma). A silicone tube (length 7 mm, inner diameter 2.2 mm) was sewed to the proximal nerve segment in the middle part of the femur. In animals of the treatment group (n=20), this tube contained a transplant of the thoracolumbar spine (n=7), hindlimb (n=6), and liver (n=7) from 14-day-old rat embryos. This transplant was adjacent to the proximal segment. The transplant was absent in control animals (n=7). The wound was sutured layer by layer. The L5 spinal ganglion was isolated after laminectomy on day 30 of the postoperative period. The sample was fixed with 10% neutral formalin and embedded in paraffin by the standard method. Each fifth section of the spinal ganglion (thickness 7 μ) was stained with methylene blue. The number of small (<30 μ^2), intermediate (30-50 μ^2), and large neurons with visible nucleoli (>50 μ^2) was estimated using an ocular grid [4,5].

The results were analyzed by Student's t test.

RESULTS

The examination was performed on day 30 after nerve transection. The total number of survived

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axotomized neurons in the L5 spinal ganglion significantly increased under conditions of spinal cord transplantation (by 62.2%), but remained unchanged after transplantation of the liver and limb (Fig. 1). The count of large (proprioceptive) and intermediate (mechanoreceptor) neurons increased by 61.6 and 139.2%, respectively, after transplantation of embryonic spinal cord (Fig. 2). However, the number of small (nociceptive) neurons remained unchanged. Limb transplantation was followed by an increase in the number of intermediate neurons (by 55.5%) and decrease in the count of small cells (by 58%). Opposite changes were found after liver transplantation. The number of small neurons increased by 29.1%, while the count of large and intermediate cells did not differ from the control. Therefore, transplantation of various tissues has a modulatory effect on survival of various populations of axotomized neurons in adult animals. The spinal cord mainly contributes to survival of large and intermediate neurons, while the liver affects the population of small cells. This fact is probably related to high expression of NGF by mesenchymal progenitor cells [6], which serves as a neurotrophic factor for small neurons. During ontogenesis of neurons, glial cells secrete the factors that stimulate neuronal growth and survival. Our findings indicate that these factors can inhibit posttraumatic apoptosis in adult animals. An ambiguous effect was observed after transplantation of embryonic hindlimb tissue into the nerve. It was probably associated with the presence of several types of cells in this tissue. Previous experiments with coculturing of rat embryonic neurons and potential target cells showed that dermal and muscle cells stimulate the growth of processes in sensory and motor neurons. Cells of the epidermis and muscle connective tissue inhibit the growth of fibers in both types of neurons [7]. These features can determine an increase in the number of large and intermediate neurons and decrease in the count of small cells.

We conclude that sensory neurons whose processes constitute the peripheral nerve can survive after nerve injury and do not undergo apoptosis under the influence of factors from cells of the developing spinal cord. Therefore, transplantation of several types of cells is followed by high-efficacy regeneration.

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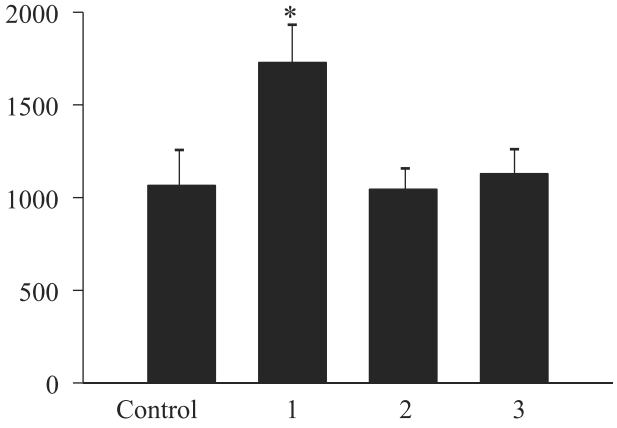

Fig. 1. Total number of neurons in the L5 spinal ganglion of a rat on day 30 after sciatic nerve injury. Here and in Fig. 2: spinal cord (*1*); hindlimb (*2*); liver (*3*). **p*<0.05 compared to the control.

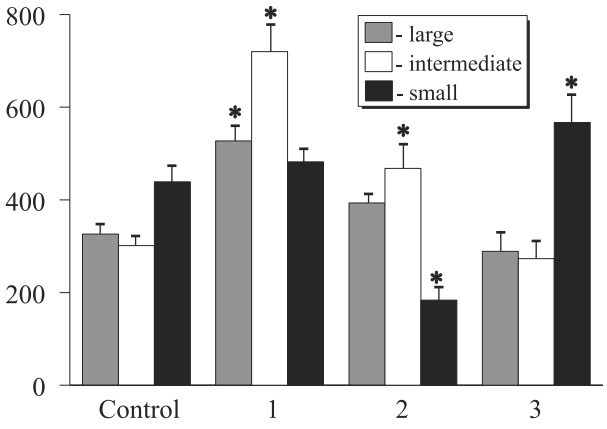

Fig. 2. Number of small, intermediate, and large neurons in the L5 sensory spinal ganglion on day 30 after sciatic nerve injury.

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