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Development of Rat Embryonic Spinal Ganglion Cells in Damaged Nerve

E. S. Petrova, E. N. Isaeva, and D. E. Korzhevskii

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The development of dissociated cells from rat embryonic spinal ganglion after transplantation to damaged nerve of adult animals was studied using immunohistochemical differentiation markers of neural and glial cells. The cell suspension obtained after dissociation of rat embryonic spinal ganglia (embryonic day 15) was injected into the proximal segment of crushed sciatic nerve. The nerve was damaged by ligation for 40 sec. Progenitor cells were labeled with 5-bromo-2'-deoxyuridine (BrdU) before transplantation. BrdU-immunopositive cells were detected in the nerve trunks of recipients on days 1, 21, and 28 after transplantation. Dissociated cells of rat embryonic spinal ganglion (embryonic day 15) survived for at least 4 weeks after transplantation to the nerve and differentiate into NeuN-immunopositive neurons with morphological properties of sensory neurons and satellite cells containing S100 protein.

Key Words: *nerve; spinal ganglion; bromodeoxyuridine; S100 protein; NeuN antigen*

Cell technologies for the stimulation of regenerative processes in the nervous system after damages are now intensively developed. Various cell types (neural stem/progenitor cells, cells of olfactory structures, multipotent stromal bone marrow and adipose tissue cells, *etc*.) are tested in experimental damage to spinal cord, brain [2,7], and peripheral nerve [4,11,13]. The cells are transplanted directly into the nerve trunks or into special bioengineering structures bridging the segments of the damaged nerves. Some investigators use fragments of embryonic anlages of the brain for transplantation [14]. However, these transplants undergo destructive changes at delayed terms [3], which can negatively affect nerve regeneration. It was hypothesized that these processes can be prevented by using dissociated cells from embryonic brain and transplantation of progenitor cell suspension into the nerve trunk. Survival progenitor cells isolated from embryonic anlages of rat spinal cords and neocortex and their differentiation into the appropriate cell lineages were previously demonstrated [5,8,10]. Transplantation of embryonic cells from the peripheral nervous system (PNS) is less studied. It was shown that neuronal elements survive and differentiate after transplantation of embryonic ganglia cells to the dorsal spinal ganglion of adult rats [1]. Attempts were undertaken to transplant these cells into the nerve [12]. However, differentiation of progenitor cells obtained from embryo cells from PNS and their effects on the regeneration of damaged nerve are poorly studied.

Here we studied the development of dissociated embryonic cells from the spinal ganglion of rats after transplantation into damaged nerve of adult animals using immunohistochemical differentiation markers of neural and glial cells.

Laboratory of Functional Morphology of Central and Peripheral Nervous System, Department of General and Special Morphology, Research Institute of Experimental Medicine, North-West Division of the Russian Academy of Medical Sciences, St. Petersburg, Russia. ***Address for correspondence:*** iemmorphol@yandex.ru. E. S. Petrova

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MATERIALS AND METHODS

The study was performed on Wistar rats (*n*=15). The animals were housed and the experiments were conducted in accordance to the Regulations of the Work using Experimental Animals. Rat embryos (embryonic day 15) served as the donors. For labeling of the majority of spinal ganglion progenitor cells, 5-bromo-2'-deoxyuridine (BrdU; Sigma) in a dose of 50 mg/kg was injected 3 times (every 6 h) to pregnant female rats 1 day before embryo isolation. Fragments of the spinal cord ganglion with dorsal roots were isolated from 15-day embryos (E15) and placed in nutrient medium 199 (BioloT) containing 0.2% chymopsin (Samson-Med) for 10 min at 37°C. Then, the fragments were pipetted, the suspension was twice washed with medium without chymopsin and centrifuged (15 min at 200*g*). The pellet was resuspended in 1 ml fresh medium and cell viability was evaluated by staining with 0.2% trypan blue and counting in a Goryaev's chamber. Smears were prepared from an aliquot of the suspension and fixed in 96% ethanol for 40 min. Cell suspensions with more than 85% of viable cells were used for transplantation. The cells in a dose of $(30\text{-}70)\times10^3$ in 5 µl medium were transplanted into the proximal end of preliminary damaged sciatic nerve of recipients. The nerve was damaged by ligation for 40 sec. The animals were kept under standard vivarium conditions and sacrificed by ether overdose on days 1 and 21-28 after the surgery. The material was fixed in a zinc–ethanol–formaldehyde mixture, dehydrated, and embedded into paraffin. Detection of BrdU-immunopositive cells in E15 ganglion, suspension obtained by dissociation, and nerve trunks of the recipients was performed using murine monoclonal antibodies (Bu20a, Dako).

Differentiation of transplanted progenitor cells was analyzed by immunohistochemical detection of nuclear antigen NeuN (neuronal differentiation marker) and S100 protein (marker of satellite and Schwann cells, lemmocytes). We used monoclonal murine antibodies (1:400, clone A60, Chemicon) for NeuN detection, and polyclonal rabbit antibodies (1:600, Z0311, Dako) for S100 detection. Reagents from EnVision+System Labeled Polymer-HRP Anti-Mouse kit (K4001, Dako) were used as second reagents for NeuN detection. Reagents from Super Sensitive Polymer-HRP Kit HRP/Dab (Bio Genex) were used as second reagents for S100 detection. Some smears were additionally stained with toluidine blue (Nissl method) or astra blue.

RESULTS

Embryo cells of the spinal ganglion from E15 rats served as an initial material for transplantation. During this period, the spinal ganglia consist of neuronal and glial progenitor cells. After preliminary administration of BrdU, most of these cells were labeled (Fig. 1, *a*). The dorsal roots containing lemmocytes with BrdU were isolated too. After dissociation of embry-

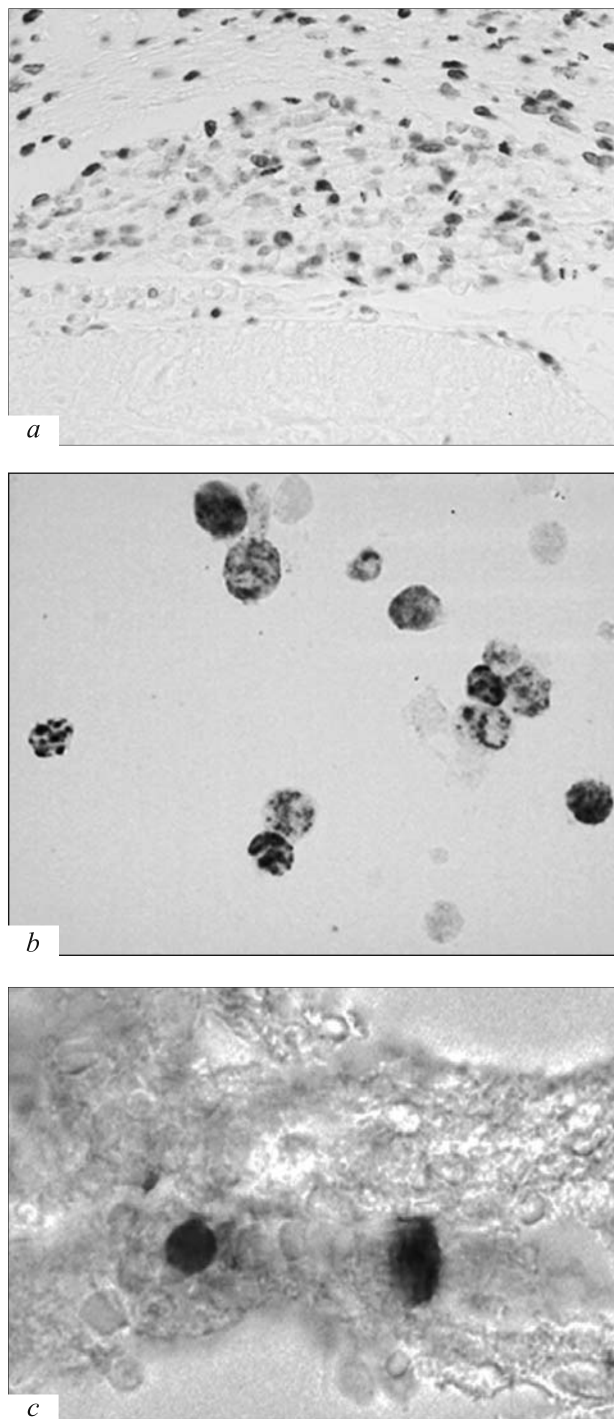

Fig. 1. BrdU-positive cells of the spinal ganglion from E15 rats *in situ* (*a*), in a sample prepared immediately after dissociation (*b*) and 1 day after transplantation into the nerve of mature animals (*c*): immunohistochemical reaction for detection of BrdU. *a*: ×400; *b*, *c*: ×1000.

onic spinal ganglion cells, analysis of cell suspension smears was performed. Immunohistochemical staining showed that the majority of samples were labeled with BrdU (Fig. 1, *b*).Histochemical assay performed 1 day after surgery revealed some BrdU-positive cells in the endoneurium of nerve trunks (Fig. 1, *c*).

BrdU-immunopositive cells were detected in the nerve trunks on days 21-28 after transplantation. The distribution of BrdU in the nuclei varied in different cells. Some nuclei were homogenously and intensively stained, while others contained only solitary aggregates of BrdU-immunopositive chromatin (Fig. 2, *a*). The label can probably be diluted in some nuclei due to proliferation of labeled cells. However, even in these cases the nuclei definitely contained BrdU (Figs. 2 and 3, *a*). Staining with toluidine blue (Nissl staining) showed that stained cells varied in size. Some of these cells were similar to the sensory cells of the spinal ganglion. These cells were large, round-shaped (diameter up to 20-25 μ), with great amount of cytoplasm. Other cells were small. Immunohistochemical reaction for detection of a marker of mature nerve cells (NeuN protein) showed that some transplanted progenitor cells labeled with BrdU differentiated into neurons (Fig. 2, *c*). Analysis of serial sections showed that some small BrdU-immunopositive cells expressed S100 protein typical of satellite cells (Fig. 3, *b*). It can be hypothesized that glial progenitor cells also differentiated to lemmocytes and disappeared between endogenous Schwann cell of a recipient. Our experiment did not confirm this assumption. We could not trace the fate of lemmocytes transplanted into the nerve.

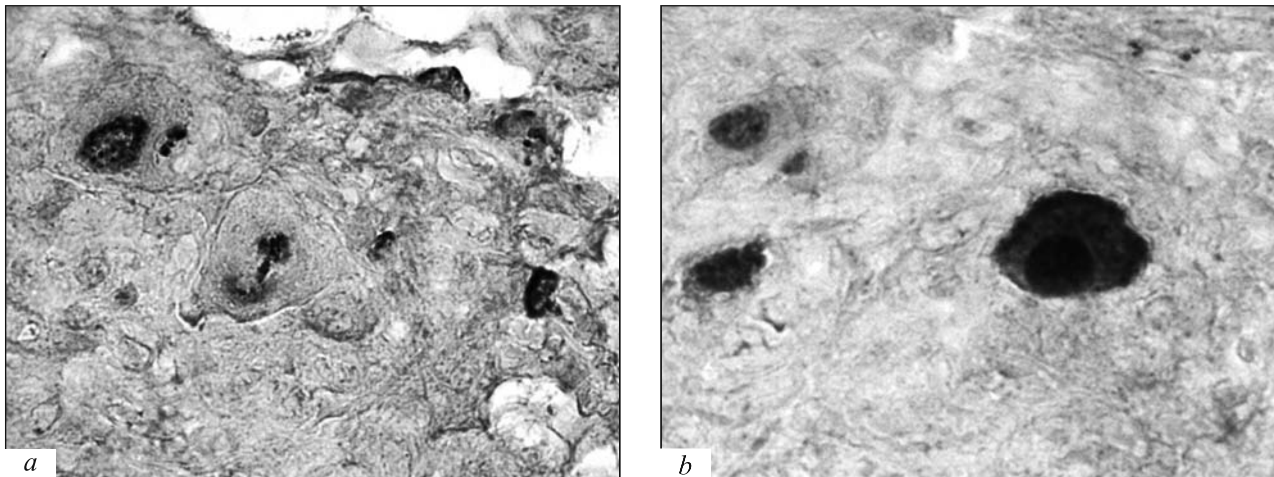

Fig. 2. Sensory neurons in the recipient nerve on day 28 after transplantation: immunohistochemical reaction for BrdU (*a*) and nerve cell antigen NeuN (*b*). ×400.

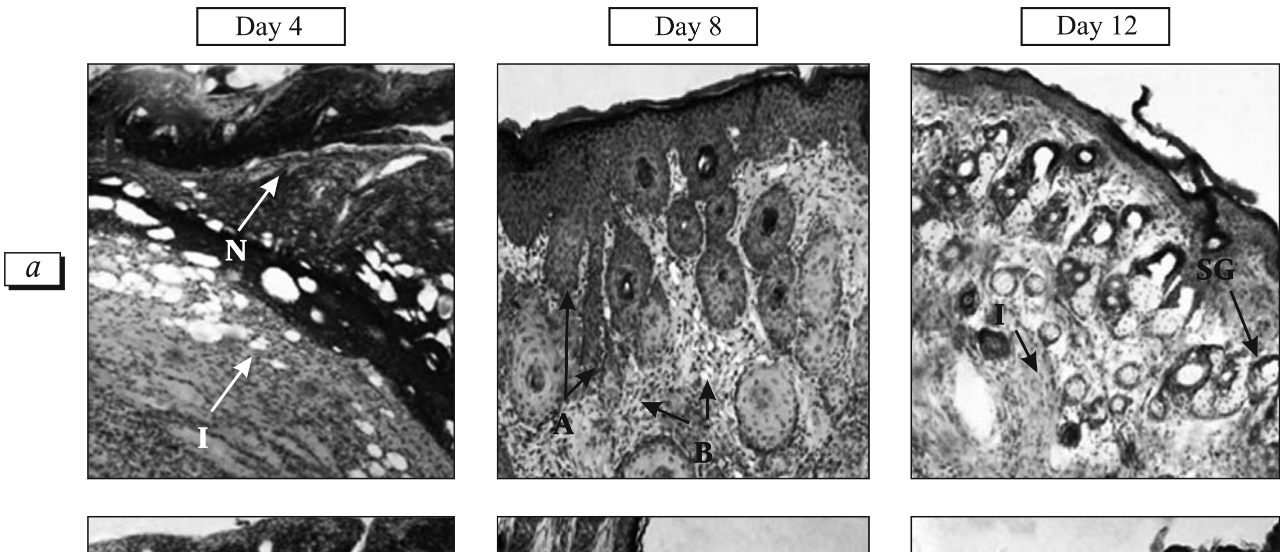

Fig. 3. Satellite cells (arrow) in the nerve trunk on day 28 after transplantation. Immunohistochemical reaction for BrdU (*a*) and S100 protein (*b*): serial sections (×1000).

The number of transplanted neurons in the nerve was low. These features were shown previously during transplantation of dissociated cells and other embryonic cells into the damaged nerve [5]. The experiments on the model of neural transplantation into the brain of mature animals indicated that the transplanted suspensions of dissociated cells have some specific features in comparison with the transplants of fragments of embryo cells [6,9,15]. A part of transplanted dissociated cells died at early stages due to apoptosis [15]. Less than 1% of transplanted cells survive for 10 weeks after transplantation of suspension into the nerve. Neurotrophic factors are administrated with the cells into the nerve for increasing of cell survival [10].

Thus, we found that dissociated cells of the spinal ganglion from E15 rat survive for 28 days after transplantation to the damaged nerve. The transplanted cells differentiate into NeuN-immunopositive neurons with morphological properties of sensory neurons and satellite cells containing S100 protein. These data showed that transplanted cells are glial satellite cells.

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