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Immunohistochemical Localization of Laminin-1 in the Acellular Nerve Grafts is Associated with Migrating Schwann Cells which Display Corresponding Integrin Receptors

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Abstract. The presence of laminin-1, collagen-IV, $\alpha 6$ and $\beta 1$ integrin chains was detected by indirect immunohistochemistry using biotin/streptavidin/HRP or gold-conjugated secondary antibody at the light and electron microscope level, respectively Cryo-treated segment of the peripheral stump without living Schwann cells (S-100$^-$) did not display immunoreactivity for laminin-1 and integrin's chains, while the migrating Schwann cells in the marginal regions were immunostained for the antigens Isolated acellular nerve segments protected from migration of Schwann cells (S-100$^-$) exhibited laminin-1$^-$, $\beta 1^-$, and $\alpha 6^-$ integrin chains immunoreactivities Position of the basal lamina was verified by collagen-IV$^+$ immunoreactivity Results indicate that presence of the laminin in the peripheral nerve is related with living Schwann cells

Key words: Acellular nerve graft — Laminin-1 — Integrins — Schwann cell migration

The basal lamina tubes of axon-Schwann cell units play an important role in the nerve regeneration preparing conduits for growing axons (Idé *et al* 1983) Laminin, the

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major glycoprotein of the basal lamina is referred as a potent stimulator of axon outgrowth (Manthorpe *et al* 1983) and Schwann cell migration (Bailey *et al* 1993) Schwann cells can synthesize laminin (Cornbrooks *et al* 1983), but it is not clear whether the molecules survive in the basal lamina after elimination of the cells during preparation of the acellular nerve grafts Many authors using the acellular nerve grafts for experimental nerve reparation (Idé *et* al 1990, Tohyama *et al* 1990) believe that the basal laminae remain intact after cryo-treatment including laminin molecules

To investigate immunohistochemically detected laminin molecules in the basal lamina of acellular nerve grafts we prepared two types of acellular nerve segments i) isolated and protected from Schwann cell migration and ii) merged with the distal nerve stump which enables Schwann cell migration

In the first group of adult rats ($n = 12$) we prepared the isolated sciatic nerve segment protected from both reinnervation and Schwann cell migration, but with preserved vascularization Isolated nerve segments were cryo-treated by forceps cooled with liquid nitrogen The cryo-treatment was one ($n = 4$), three ($n = 4$), and five times ($n = 4$) to prepare the standard acellular nerve segment (Idé *et al* 1983) The sciatic nerve of the second group of rats ($n = 4$) was exposed, transected at the midthigh level and the distal stump was protected from reinnervation The nerve portion, a 10 mm long, marked out with two epineurial sutures (10-0 Ethicon) in the distal stump was frozen 5 times by forceps pre-cooled with liquid nitrogen

Immunoreaction for laminin-1, collagen-IV, $\alpha 6$ and $\beta 1$ integrin chains was detected by indirect immunohistochemistry using biotin/streptavidin/HRP or gold-conjugated secondary antibody at the light and electron microscope level, respectively Light microscopic immunohistochemical staining was intensified by means of indirect catalyzed signal amplification

The isolated nerve segment cryo-treated 3 or 5-times did not exhibit immunoreactivity for S-100 protein 7 and 14 days after the injury, indicating absence of Schwann cells in the nerve segments The single cryo-treated segment contained residual cells stained for S-100 protein, therefore, the single cryo-treatment was insufficient to kill all Schwann cells A faint immunoreaction for laminin-1 and integrin chains investigated was observed as parallel lines in the sections of the segment cryo-treated 1 or 3 times In latter way of the cryo-injury, intensity of the reaction was lower than following single treatment Distinct decrease of immunostaining intensity was related with time of survival after cryo-injury (more after 14 days in comparison to 7 days) Sections through segment cryo-treated 5-times did not display immunostaining for laminin and integrin chains besides evident course of blood vessels Moreover, positive immunostaining for collagen-IV verified position of the basal lamina tubes

Longitudinal sections through the cryo-treated portion demarcated in the distal nerve stump exhibited the lines of S-100 immunoreactivity at both marginal regions, while the central part was S-100 protein-free This indicated that Schwann cells migrated into cell-free part of the segment from the marginal regions merging with untreated parts of the distal nerve stump Absence of immunohistochemical staining for laminin in the acellular nerve segment was identified by means of very high sensitive method (Indirect Catalyzed Signal Amplification) In addition, the light microscopic results were confirmed by immunogold method at the electron microscopic level including association of laminin-like immunoreactivity with migrating Schwann cells

Our findings demonstrate that standard preparation of the acellular nerve segment by freeze-thawing treatment may result in a fundamental diminution of laminin molecules in the basal lamina tubes Distinct reduction of laminin immunoreactivity in the basal

lamina following powerful freeze-injury can be explained by structural (conformation) changes in the laminin molecules related with impossibility to be recognized by used antibody Second possibility corresponds with a short turnover of laminin molecules in the basal lamina and thus, they disappear very quickly without presence of Schwann cells

The reappearance of laminin molecules in the acellular nerve segment coincides with the Schwann cell migration from undamaged marginal regions The migration of Schwann cells without an axon association confirms earlier observations (Anderson *et al* 1991) The laminin molecules synthesized by migrating Schwann cells probably enrich the basal lamina scaffolds in the acellular nerve portion Similar dependence of extracellular molecules upon presence of living cells was observed in the case of specialized Schwann cells They secrete molecules of the collagen-tailed forms of non-specific cholinesterase into the extracellular matrix of the sensory corpuscles The collagen-tailed molecular forms of the enzyme in the extracellular matrix are strictly confined to the presence of viable specialized Schwann cells They continuously support the ECM of the sensory corpuscles by nChE molecules (Dubový and Sviženská 1990)

Our results demonstrate that molecular content of the basal lamina is changed after powerful cryo-treatment Insertion of such prepared acellular nerve grafts of a 1 cm length in the rat sciatic or median nerves revealed very good growth and maturation of the regenerating axons comparable with published results This suggests that the basal lamina tubes devoid of viable Schwann cells and laminin molecules after freezing injury make only mechanical scaffolds for the growing axons or migrating Schwann cells

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