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Presence of Schwann cells in neurodegenerative lesions of the central nervous system

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Ultrastructural analysis of neurodegenerative CNS lesions produced by an excitotoxic substance revealed that the majority of cells ensheathing axons were not oligodendrocytes. By their morphology and the presence of both a basal lamina and collagen fibers they were identified as Schwann cells. The presence of Schwann cells, whose growth-promoting role in the peripheral nervous system has been largely documented, may account for the development of regenerating growth cones which have been observed in the excitotoxically lesioned central nervous system. Further support for this hypothesis came from the analysis of fetal neural transplants implanted into the lesioned area. Schwann cells ensheathing axons were indeed numerous in the neuron-depleted area surrounding the transplants, where neurite outgrowth of graft origin occurred.

In the peripheral nervous system (PNS), neural regeneration occurs extensively after an axonal lesion [10]. In contrast, in the damaged adult central nervous system (CNS), regenerative capacity is thought to be very limited. This difference may be due to the presence of the growth-promoting Schwann cells in the PNS [8, 9], while in the CNS, oligodendrocytes appear to play an inhibitory role [17]. One particular case of regenerative ability demonstrated by CNS fibers is the formation of regenerating growth cones by afferent fibers deprived of target neurons following an excitotoxic lesion [11, 12]. These growth cones are subsequently able to form new synaptic contacts with fetal neurons transplanted into the neuron-depleted area [13, 14]. The present study was therefore undertaken to analyze the cellular content of excitotoxic CNS lesions, in order to determine which cellular environment may support, or promote, this particular form of CNS regeneration.

Neuronal depletion of the right ventrobasal thalamic complex (VB) was obtained in 8 rats via a stereotaxic injection of kainic acid (KA) as described previously [12]. Briefly, the rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and received

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a slow pressure injection of 5 nmol of KA (in 0.15 μl of water, over 15 min), via a 1 μl Hamilton syringe. Injections were aimed into the internal capsule and not directly into VB to avoid direct trauma of afferent fibers with the injection needle. The neuronal loss was then produced by diffusion of KA. Rats were allowed to survive for 10–90 days after lesion. They were then perfused transcardially with saline followed by a phosphate-buffered solution (pH 7.4) containing 2% paraformaldehyde, 2% glutaraldehyde and 4% sucrose. Vibratome sections were prepared for electron microscopy. They were osmicated for one hour (OsO_4 2% and S. Collidine buffer 0.2 M at pH 7.4, 1:1 solution), dehydrated and embedded flat in a mixture of Epon and araldite. Blocks of tissue were trimmed out and thin-sectioned using an ultramicrotome. Thin sections were mounted on grids and stained with lead citrate.

In areas depleted of neurons, many cellular elements ensheathing axons displayed morphological features atypical for CNS at times after lesion longer than 20 days.

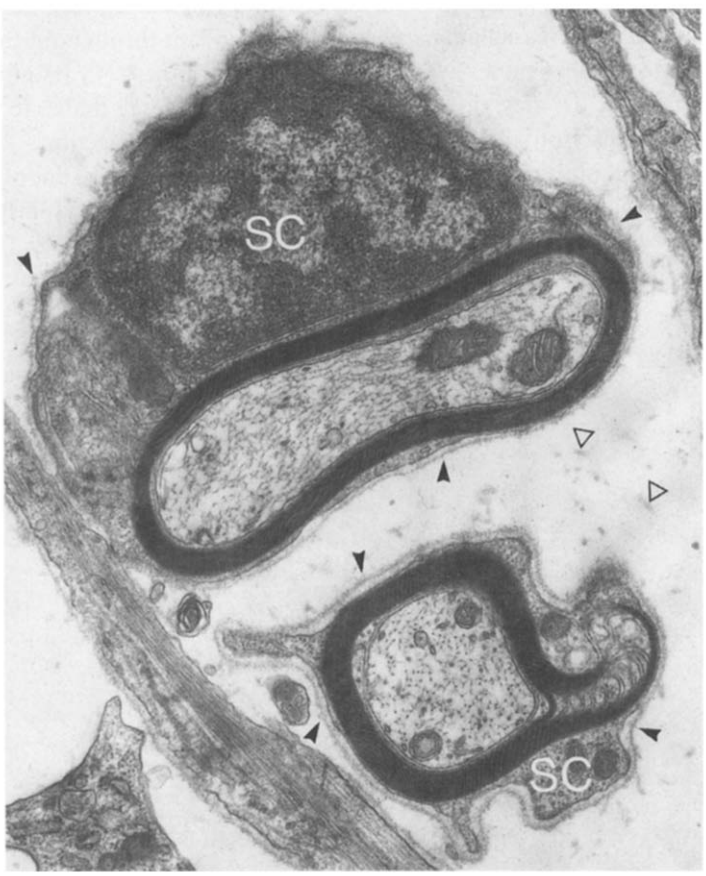

Fig. 1. Two Schwann cells (SC) with typical morphological characteristics: a one-to-one relationship between a myelinated axon and SC, presence of cytoplasm between the myelin sheath and the plasma membrane, basal lamina (dark arrows) and collagen fibers (open arrows) in the adjacent extracellular space.

A major number of both large myelinated and thin unmyelinated axons were ensheathed by these atypical cells, and there was no apparent preference for axonal type. When myelinated, axons were ensheathed by a cell exhibiting a large nucleus and a dark cytoplasm, in a one to one relationship (Fig. 1). The complex made by a cell and its myelinated axon was always surrounded by a basal lamina and collagen fibers were visible in the adjacent extracellular space. When unmyelinated, several axonal profiles were ensheathed by one of these atypical cells. Basal lamina and collagen fibers were also present in these cases. In the same area, other axons displayed normal CNS characteristics, ensheathed by profiles from oligodendrocytes, without basal lamina or collagen fibers.

All the characteristics of these atypical cells ensheathing CNS axons correspond to the classical description of the cells ensheathing axons in the PNS, the Schwann cells [15]. A small number of such cells are known to exist within the intact CNS. They are found in the perivascular spaces where they ensheath unmyelinated sympathetic fibers of the blood vessels [4]. In the neuron-depleted regions, Schwann cells were not confined to perivascular spaces but were abundant throughout the neuropil.

To investigate whether these Schwann cells, present in the CNS lesion may facilitate neural growth, as they do in PNS lesions [8, 9], fetal thalamic tissue was implanted into the excitotoxic lesion one month after kainate injection.

In 8 rats, transplantation of fetal thalamic cells was carried out 1 month after KA injection, according to a technique extensively described elsewhere [13]. Briefly, dorsal thalamic primordia were dissected out of rat fetuses (gestational age 15–16 days), treated with trypsine, then mechanically dissociated in a 0.6% D-glucose saline so-

Fig. 2. Presumably growing dendrite in the marginal zone of a transplant, 4 months after grafting. The main shaft of the dendrite (D) is bordered by glial processes and a basal lamina (arrows). Schwann cells are present in the neuropil (SC). A synaptic contact onto the dendrite is indicated (asterisk).

lution to a concentration of 60 000–80 000 cells/μl. A total of 200 000 cells were stereotaxically implanted into the lesioned thalamus using a 5 μl Hamilton syringe. Rats were allowed to survive for 7–120 days after grafting. At the end of the survival time, rats were sacrificed and thalamic tissue prepared for electron microscopy as described previously.

In the electron microscopic analysis, no Schwann cell was observed in the neuropil of the 7- to 120-day-old neural transplants and myelinating cells were oligodendrocytes. In contrast, Schwann cells were present in the area adjacent to the transplants, ensheathing myelinated (Fig. 2) and unmyelinated axons of unknown origin. In this marginal zone, which at the light microscopic level appears as a neuron-free glial band [13], dendrites of graft origin could be recognized. These dendrites were intermingled with Schwann cells, and some of them were surrounded by glial processes bordered by a basal lamina (Fig. 2). Axons of graft origin are also most probably present in this marginal zone, but their identification was not possible in our preparations.

The present results demonstrate that Schwann cells can be present in neuron-depleted CNS areas and myelinated axons. The origin of these Schwann cells is presently unknown. A possible hypothesis could be that they have proliferated and migrated out of perivascular spaces where they are known to ensheath fibers of the autonomic nervous system. One can, however, exclude the possibility that Schwann cells ensheathed only potential sympathetic and parasympathetic fibers growing into the lesion since these fibers are unmyelinated. The mechanism by which Schwann cells are induced to appear in the CNS lesion is unknown. An attractive hypothesis stems from previous work in the PNS showing that macrophages which have phagocytosed myelin produce factors that are mitogenic for Schwann cells [2]. Macrophages invade the area of lesion during the first hours after a kainate injection [7], but we have observed in the tissue taken for the present study that they phagocytose most myelin debris only weeks later, when Wallerian degeneration of thalamo-cortical axons is completed. It is therefore possible to hypothesize that a large number of Schwann cells might then appear in the lesion as a result of myelin elimination. An alternative hypothesis is that Schwann cells are stimulated to migrate into the CNS following loss of oligodendrocytes [3]. However, although one study has described some acute signs of limited demyelination following kainate injection [18], no actual loss of oligodendrocytes has been reported.

The ability of Schwann cells, experimentally introduced into the CNS, to promote neural growth and regeneration is extensively documented [5, 16]. The presence of Schwann cells in the KA-lesioned area suggests that they may be able to play a similar role in neurodegenerative areas in the CNS. Our results using fetal neural transplants failed to demonstate a role for Schwann cells in the maturation of grafted neurons. It is interesting to note, however, that dendritic outgrowth of graft origin occurs into the neuron-depleted area surrounding the transplant and that this phenomenon takes place in the presence of Schwann cells. These cells may, therefore, assist in this growth process by supplying, in particular, neurotrophic factors and a basal lamina.

Neurodegenerative disorders in humans can be experimentally mimicked by the

use of excitotoxic substances [6]. An excitotoxic mechanism has also been recently demonstrated in epileptic, ischemic and hypoglycemic brain damage [1]. The observation of Schwann cells in the excitotoxically lesioned CNS, possibly participating in processes of growth, may be a mechanism by which CNS regeneration may take place after neurodegeneration. These results raise new possibilities in the attempts to enhance regenerative phenomena in the CNS following neurodegeneration.

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