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Neural transplants in spinal cord injury

J-C Horvat

Professor, Director of the Research Group 'Transplantation and Repair of the Spinal Cord and of its Motor Connections', Laboratoire de Neurobiologie, Université René Descartes, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France.

Spontaneous recovery from severe traumatic or neurodegenerative lesions of the central nervous system (CNS), which includes the brain and the spinal cord, does not occur in adult mammals because the lost neurons are not replaced; the surviving nerve cells do not regenerate the missing part of their cut axon. The likely reason for this is inhibitory effects from the mature glial cells: reactive astrocytes[1] and differentiated oligodendrocytes.[2,3] Conversely, lengthy axonal regrowth is known to take place in the peripheral nerves of adult mammals, as well as in the CNS of fishes and amphibians.

With regard to traumatic injury to the spinal cord, damage will result in an immediate loss of intrinsic spinal neurons as well as in axotomy of both intrinsic and extrinsic (projection) neurons. In turn, axotomized nerve cells may either die after a variable delay, or survive axotomy. Yet, the surviving axotomized neurons will not re-establish their original functional connections. For instance, severance of the corticospinal tracts and/or important loss in the motoneuronal pool will result in a functional disconnection between the 'upper motor neurons' and the skeletal muscles, thus causing chronic paralysis.

However, transplantation strategies that aim at repairing the injured mammalian CNS have been developed. Thus, to some extent: (1) lost neurons can be replaced by foetal nerve cells,[4] grafted either as solid pieces of CNS tissue[5,6] or as CNS cell suspensions;[7,8] (2) axonal (re)growth and extension, from both host and transplanted neurons, can be triggered and guided by long segments of peripheral nerve autografts (PNGs).[9,10,11]

Several types of transplant are commonly used in animal experimentation aimed at spinal cord repair or reconstruction. They differ mainly by their nature and origin: foetal extraspinal (heterotopic)[12–14] or spinal (homotopic)[12,14,15] CNS tissue, autologous peripheral nerve;[11,14,16–20] but also by their mode of action: protective and/or trophic effect on the host neural tissue,[12,15,16] inhibitory influence on glial reaction,[12,21], guiding effect on axonal regrowth,[11,14,17–20] and role of substitution.[8,14,15,18]

However, peripheral nerve autografts, used to join the 2 stumps of a completely transected spinal cord, are unable to promote any functional restoration as: (1) corticospinal axons do not regenerate into the grafted nerves;[20] (2) axons originating from spinal or brain stem neurons may grow throughout the grafted nerves but their possible re-entry into the caudal stump of the host spinal cord is restricted to a few millimeters,[20] suggesting that injured adult CNS tissue is a rather non permissive terrain for axonal growth and extension. Along the same lines, in less severe spinal injuries, surviving motoneurons will grow axons into PNGs[11] as well as into reimplanted,[22] but not into intact,[23] spinal roots.

Taking all of these data into account, our research group in Paris V University is studying, in the adult rat, the possibilities of an anatomical and functional reconstruction of the injured spinal cord by means of the above mentioned transplantation techniques, used alone or in combination with molecules supposed to be active in reducing glial scarring,[24] and/or in permitting axonal regrowth and extension.[25,26] At present, emphasis is laid on the reconnection of the injured spinal cord with its peripheral motor effectors.

Several experimental models are being studied which correspond to different types of spinal severance (focal and mild lesion, without substantial neuronal loss; mechan-

ical or chemical depletive lesions, involving important neuronal loss) and, consequently, to different ways of repairing the injured spinal cord (with or without foetal neural tissue).

In a first model,[11] one end of an autologous PNG (a 30 mm segment of the common peroneal nerve), was introduced into the cervical spinal cord, thus determining a small and localised lesion which did not cause any apparent functional deficit. The other end of the PNG was inserted into an aneural area of a nearby skeletal muscle of the dorsal musculature, which was carefully denervated prior to grafting. After 2 to 21 months following surgery, it was noticed that the reconnected muscle contracted under an adequate electric stimulation of the nerve bridge.

Application of different axonal tracers (horse radish peroxidase = HRP, fluorescent dyes) to the nerve bridge led to an extensive neuronal labelling in the whole spinal grey matter, between C3 and C7. Yet, when the tracers were injected directly into the muscle, the neuronal labelling was mainly restricted, in the same segments, to typical motoneurons of the ventral horn, different from those that normally innervate the experimental muscle (as they are located unilaterally in C1 and C2).

In the reconnected muscle, morphological studies revealed that motor endplates had been reformed not only at the sites of original innervation but also, and mainly, in ectopic locations all around the grafted nerve. These neuromuscular junctions were quite functional and necessarily formed by regenerated axons in the PNG, as electrical stimulation of this graft triggered the contraction of the muscle to which it was attached. In addition, the reformed endplates were cholinergic insofar as the endplate potentials, evoked by the stimulation of the PNG, could be suppressed by the action of curare, added to in vitro preparations.

Thus was reformed a functional motor system of substitution which, however, appears anatomically far removed from the original model as its motoneuronal pool, the course of its motor axons and the sites of terminal innervation are different. In this sense, this experimental model constitutes an additional example of the remarkable plasticity of the adult mammalian central and peripheral nervous systems.

The studies concerning a second experimental model[14] have been developed more recently. Their main objective is an attempt at repairing larger spinal lesions, implicating important neuronal loss. The last stage of this experimentation will consist in joining, by means of a PNG, a foetal neural transplant of substitution, designed to fill a cavity made by unilateral aspiration of the host grey matter and dorsal funiculi in C5, and the muscle used in our first set of experiments. The studies carried out so far concern a preliminary stage where the distal end of the PNG, unconnected to the muscle, is made blind by crushing and stitching it to extraspinal tissues. Should the aspiration procedure be gentle enough, the resulting motor deficit is apparently restricted to paralysis of the right upper limb. The cavity is filled with solid pieces of neural tissue (cortex, spinal cord or dorsal root ganglia), removed from E13 to E18 inbred embryos.

From one to 6 months following the double transplantation, healthy appearance of the 3 types of transplants and integration with the host tissues were consistently observed. Surviving neurons in the grafted tissues developed processes, some of which became myelinated. Yet, the ability of the grafted neurons to extend axons into the PNG differed strikingly from one type of graft to another, being apparently non existent for cortical grafts, moderate for spinal cord grafts and quite extensive for dorsal root ganglia transplants. Interestingly, these differences reflected, at least in part, what was observed for the corresponding, fully differentiated neurons in adult animals when their cut axons were also placed in contact with the non neuronal components of peripheral nerves. In addition, some host spinal neurons, mainly located around the transplants, appeared HRP-labelled, indicating that they had also grown axons into the PNG.

Other models are being developed with external collaborations. For instance, kaïnic acid-induced cavitation lesions of the lumbar spinal cord have been filled with dissoci-

ated foetal spinal cord tissue.[18] A great number of grafted neurons did survive and some were shown to grow axons into blind-ended PNG.

Studies of the possibilities of all these neurons of substitution to form whatever connections with denervated skeletal muscles, as well as of their eventual reafferentation by regenerating central nerve fibres of the host, are in progress.

In conclusion, embryonic neurons and autologous peripheral nerve segments constitute selected materials for studying CNS plasticity and repair in adult mammals. Transplanted to the brain or the spinal cord, the former are possible substitutes designed to replace lost or deficient host neurons while the latter have useful stimulating and guiding effects upon axonal regrowth from surviving axotomized neurons.

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