22.S3

Transplantation Strategies to Reconstruct the Injured Spinal Cord and its Peripheral Motor Connections in the Adult Rat

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We are currently working, in the adult rat, on two main experimental models of spinal injury (with special reference to peripheral motor connectivity) and its subsequent repair with the help of PNS and/or CNS transplants.

A first model involved a limited spinal lesion (determined by the insertion of one end of a 30 mm segment of a peripheral nerve autograft (PNG) into the right side of the cervical enlargement of the spinal cord) causing no apparent functional deficit. The other extremity of the PNG was inserted into a normally aneural area of a nearby skeletal muscle of the dorsal musculature which was carefully denervated prior to grafting.

Two to 21 months following surgery, it was noticed, in the anaesthetized animal, that the reconnected muscle contracted under an adequate stimulation of the nerve bridge. Application of horseradish peroxidase (HRP) to the PNG led to an extensive neuronal labelling in the whole spinal grey matter, between C3 and C7. However, when the tracer was injected directly into the muscle, the neuronal labelling appeared to be mainly restricted, in the same segments, to typical motoneurons of the ventral horn, different from those (located unilaterally in C1 and C2) that normally innervate the experimental muscle.

In the reconnected muscle, morphological and electrophysiological studies revealed that motor endplates had been reformed not only at the sites of original innervation but also, and principally, in ectopic locations, all around the intramuscular tip of the PNG. These neuromuscular junctions were quite functional and necessarily formed by regenerating axons in the PNG, as electrical stimulation of the grafted nerve triggered the contraction of the muscle to which it was attached. In addition, the reformed endplates were proved to be 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* PNG/muscle preparations, just removed from the animal.

Thus was reformed a functional and stable (up to 21 months post grafting) substitute motor system, which, however, appears anatomically far from the original model as its motoneuronal pool, the course of its motor axons and the sites of terminal innervation are all different.

A second experimental model was developed to investigate the possibilities of reconstructing the spinal cord and its peripheral motor connections after larger injuries with significant neuronal loss.

Unilateral (right) aspiration of the grey matter and of the dorsal funiculi, including the cortico-spinal tract, was made at the level of the cervical enlargement. Should the aspiration procedure be gentle and limited enough, the consequent motor deficit was apparently restricted to the paralysis of the right forelimb. The resulting cavity was filled with either solid homotypic (E14 spinal cord = SC) or heterotypic (E14-18 neocortex = CT) foetal CNS tissue; or, alternatively, with foetal or adult PNS neural tissue (dorsal root ganglia = DRG). In addition, one end of a PNG was inserted in the centre of the transplanted tissues, while its other, extraspinal end was either made blind or inserted into the skeletal muscle used in our first experimental model.

After a post-grafting period ranging from 1 to 6 months, it was found that the three types of transplants had survived and become integrated with the host spinal cord, although their overall organization remained atypical. Surviving graft neurons had developed processes, some of

which had become myelinated. A number of them expressed expected neuropeptides, neurotransmitters or related enzymes.

These similarities in the overall development of the different transplants did, however, contrast with the striking differences in the extent of neuronal labelling following HRP application to the extraspinal part of the PNG. Thus, the average number (per animal) of retrogradely labelled neuronal somata was (mean ± SEM) 0, 16.8 ± 4.7 and 1070 ± 420, inside the foetal CT, SC and DRG transplants, respectively. Labelled cells were also observed in the host spinal cord, close to the transplanted tissues. The average numbers of 64.4 ± 28.9, 117 ± 30 and 134 ± 103, around CT, SC and DRG transplants, respectively, were consistent with those of host spinal neurons which could be labelled from a PNG in the absence of a neural transplant (as in the first experimental model). Interestingly, very similar results were obtained when adult DRG transplants were used instead of foetal ones. Thus, it could be concluded that axogenesis into PNGs was apparently non-existent from foetal CT grafts, moderate from foetal SC grafts, but quite extensive from both foetal and adult DRG transplants. In bridging experiments, some axons in the PNGs established functional contacts with skeletal muscles. However, their origin was ambiguous since both host and transplanted neurons grew axons up to the muscle.

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