Myelin formation following transplantation of normal fetal glia into myelin-deficient rat spinal cord

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Summary
Structurally normal myelin sheaths develop in the spinal cord of juvenile myelin-deficient rats (mdr) 11 days after transplantation of normal fetal spinal cord fragments or cultured cells that do not yet express galactocerebroside. Cultures result in more extensive myelin formation, and in both cases the myelin that forms is located primarily at or near the site of transplantation.

Myelin formation also occurs after transplantation of postnatal donor tissue, but the extent diminishes with donor age, and none was seen after transplantation of adult donor tissue over the two-week period studied. Injection of killed tissue, tissue derived from mouse donors or an extract of myelin also did not lead to myelin formation. The results imply that myelin formed in the host following transplantation was generated by oligodendrocytes newly differentiated from donor precursor cells rather than by donor oligodendrocytes that were already mature at the time of transplantation or by host oligodendrocytes that took up components of the injected material.

We conclude that exogenous fetal glial cell precursors are able to survive, differentiate and form myelin in the environment of the juvenile mdr spinal cord.

Introduction
Myelin formation by transplanted Schwann cells around peripheral axons was demonstrated by Aguayo et al. (1978). The possibility of myelin formation by exogenous oligodendrocytes around myelin-deficient CNS axons was later raised by Wolff et al. (1981), who showed that explants of neonatal cerebellum from a myelin-deficient mouse mutant become partially myelinated when co-cultured in direct contact with normal optic nerve. Subsequently, these studies were repeated using other mutants, and it was also shown radioautographically that labelled normal oligodendrocytes from the optic nerve migrate into the mutant cerebellum and can be identified there in the vicinity of normal-looking myelin segments (Billings-Gagliardi et al., 1983).

In vivo studies have also demonstrated myelin formation following transplantation of normal brain fragments or cultured normal oligodendrocytes into the CNS of myelin-deficient mice and rats (Gumpel et al., 1983; Lachapelle, et al., 1984; Gansmuller et al., 1986; Kohsaka et al., 1986; Baulac et al., 1987; Duncan et al., 1988b). These studies utilized neonatal or older donors and hosts, which, in most cases, were littermates. In addition, Friedman et al. (1986) reported immunohistochemical evidence for patches of myelin basic protein in shiverer mouse brain following transplantation of fetal cerebral cortex, and we have recently shown that transplantation of fetal spinal cord fragments into the spinal cord of juvenile myelin-deficient rats (mdr) results in the formation of ultrastructurally normal myelin in the hosts (Rosenbluth et al., 1988, 1989a).

In this study we report further studies of fetal transplants into juvenile mdr host spinal cord. The results of injecting CNS fragments are compared with those obtained using cell cultures, and we present the outcome of experiments designed to determine whether it is the donor cells that form the myelin or, alternatively, whether some other component of the transplant stimulates host oligodendrocytes to form myelin. In addition, we report the results of experiments in which the age of the host animal was held constant and the age of the donor varied, in order to assess the effect of donor maturity on success of transplantation, and to determine whether is mature oligodendrocytes or precursor cells in the transplant that are responsible for the myelin formation.

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Materials and methods

Donor tissue fragments

Wistar rats, 15–18 days pregnant, were anaesthetized with chloral hydrate and the fetuses removed by Caesarian section. The spinal cord and brain stem were dissected from each, the meninges were removed, and the pooled tissue was minced and triturated through a 27-gauge syringe needle. Postnatal and juvenile rats up to 16 days, adult rats and 15–18 day fetal mice were also used as donors. In some cases, 0.05% fast blue was added to the resulting tissue fragment suspensions (Del Cerro et al., 1988). Donor tissue suspensions were subdivided into ~0.01–0.02 ml aliquots in insulin syringes equipped with 27 gauge needles, and injected into the dorsal portion of the thoracic spinal cord of host animals 8–13 days old.

Gliarial cell cultures

The procedures used were essentially those of Rosen et al. (1989). Briefly, fetuses were removed aseptically from 15–18 day pregnant Wistar rats. In several cases, specimens were taken from neonatal rats, and in one case from an adult rat. Sixteen-day mouse fetuses were also used as a source of culture material. In each case, the spinal cord and medulla were used a dorsal approach. The cord/medulla was transferred to cold L-15 medium and stored at 4°C. The meninges were then stripped away with forceps, and the cords were placed into 2.5 ml of Ca<sup>2+</sup>Mg<sup>2+</sup>–free Hanks balanced salt solution containing 0.05% 3x crystallized trypsin (Sigma) and 400 µg ml<sup>–1</sup> DNAase I (Sigma). The cords were hemisected longitudinally, cut into ~0.5 mm<sup>3</sup> fragments and incubated in the enzyme solution for 1 h at 37°C, spun down, triturated, filtered and centrifuged in Percoll/sucrose. The band of cells just above the red cell band near the bottom of the tube was collected and washed in cold L-15 medium. In some instances, fast blue was added, to a final concentration of 0.025%.

Some of the cultures were labelled using monoclonal antibodies to either galactocerebroside (culture supernatant, courtesy of B. Ranchst) or A2B5, diluted 1:100 (courtesy of E. Napolitano). The primary antibodies were visualized with rhodamine conjugated to goat anti-mouse serum (Cappel) at a 1:200 dilution, or fluorescein conjugated to goat anti-rabbit serum (Cappel), also at a 1:200 dilution. Cultures were then fixed and permeabilized in 5% acetic acid/EOTH at ~20°C. Some of these were then labelled with polyclonal antibodies to either neurofilaments, diluted 1:100, or GFAP, diluted 1:100 (both courtesy of R. Liem). Primary antibodies were visualized as described above. Cultures were then examined and photographed with a fluorescence microscope.

Killed donor tissue

Three procedures were used: (i) cultured cells from day 15–18 rat fetuses were heated to 60°C for 45 min, (ii) cultured cells from day 15–18 rat fetuses were fixed in 4% formaldehyde, spun down at 1000 rpm and reconstituted in HBSS, (iii) tissue fragments from day 15–18 rat fetuses were frozen by immersion of the tube containing them into liquid nitrogen. When boiling of the LN2 had ceased and the suspension was completely frozen, the tube was thawed at room temperature and simultaneously sonicated. The freezing and thawing/sonication steps were repeated four times, and the suspension was then either subdivided into aliquots as above, or frozen and stored for later use. In one case, an aliquot of the frozen and thawed suspension was cultured according to the method described above. No living cells were seen in the culture dish at 24 or 48 h.

Extract

A 300 g rat was anaesthetized with chloral hydrate. The brain stem and spinal cord were removed and weighed (1 g), and then extracted according to the procedure of Lees and Sakura (1978); 19 ml of a 2:1 mixture of chloroform/methanol were added to the tissue in a Waring blender. The tissue was homogenized for 2 min, filtered, and the filtrate set out in a Petri dish to evaporate. After evaporation of the solvents the result was a cream-coloured, waxy residue containing high concentrations of both proteolipid protein and myelin basic protein. This was mixed with 0.3 ml of Hanks balanced salt solution, placed into the barrel of a syringe and forced through a 27-gauge needle four times. The material was then subdivided into 0.01–0.02 ml aliquots in insulin syringes and injected into the dorsal region of the thoracic spinal cord of the recipients (see below). Since the final mixture was very viscous, considerable pressure was needed to force the mixture out through the syringe needle. In order to prevent movement of the needle tip and laceration of the spinal cord during this procedure, the needle was secured in position with forceps.

Transplantation procedure

Pups were anaesthetized with chloral hydrate and a lower thoracic laminectomy was carried out. The dura was incised over the dorsal columns and ~0.01–0.02 ml of donor material, suspended in balanced salt solution, was injected below the surface of the spinal cord with an insulin syringe and 27-gauge needle. Recipient animals were allowed to recover and were reintroduced into the mother's cage the following day. Details of the methods used are presented elsewhere (Hasegawa & Rosenbluth, 1990). Recipient animals were followed for 8–16 days. For fixation, pups were reanaesthetized with chloral hydrate and perfused through the heart with either 4% formaldehyde (freshly prepared) or 3% glutaraldehyde plus 2% formaldehyde, both in 0.1 M cacodylate buffer (pH 7.4). Animals were dissected on the following day. The site of injection was identified by the location of a 10-0 black nylon suture placed at the time of surgery. The spinal cord was removed and transversely cut into 1–2 mm slices, which were successively lettered and placed individually into vials.