Tissue Culture Models of Myelination After Oligodendrocyte Transplantation

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SUMMARY

Studies of myelination after transplantation of mature oligodendrocytes to cerebellar cultures in which oligodendrocyte maturation and myelination had been irreversibly inhibited by exposure to cytosine arabinoside were reviewed. Transplanted oligodendrocytes were derived from three sources, including cerebellar explants treated with kainic acid, dissociated oligodendrocyte cultures, and optic nerve fragments. Oligodendrocytes from all sources migrated into the host explants and myelinated appropriate axons. The time of appearance of myelin and the percentage of host cultures myelinated differed for the three sources of oligodendrocytes, however. Myelin was visible earliest and in the highest percentage of host explants transplanted with cultured dissociated oligodendrocytes, which were presumably the most free to migrate into the host tissue, and latest and in the lowest percentage of host cultures transplanted with optic nerve, from which oligodendrocytes were presumably least free to migrate. Some myelin-like membranes unassociated with axons appeared in cerebellar cultures transplanted with cultured dissociated oligodendrocytes, and not in cerebellar explants transplanted with oligodendrocytes from other sources. The formation of such myelin-like membranes was interpreted as a manifestation of oligodendrocyte hyperreactivity induced by culture in isolation.

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INTRODUCTION

Cerebellar explants derived from neonatal Swiss-Webster mice are unmyelinated when initially placed on collagen coated coverslips in Maximow chambers for maintenance as organotypic cultures /6, 16/. Such cultures begin to myelinate after 7 days *in vitro* (DIV), as observed in the living state, with the most active period of myelination being between 9–12 DIV. Generally, 80% or more of cerebellar cultures myelinate when maintained in normal nutrient medium which includes 40% fetal calf serum and a glucose concentration of 600 mgm% (6 mgm/ml medium). The myelin is most abundant in the area between cortex and intracerebellar nucleus, where Purkinje cell axons are concentrated, an area equivalent to the white matter zone *in vivo*.

We developed a tissue culture model in which oligodendrocyte maturation and myelination were irreversibly inhibited by exposing neonatal mouse cerebellar explants to cytosine arabinoside (Ara C) incorporated in the nutrient medium (5–10 μg Ara C/ml medium) for the first 5 DIV, followed by maintenance in normal medium /5, 21/. Purkinje cells survived in increased numbers in such cultures and sprouted recurrent axon collaterals. Thus there was an enhanced population of myelin-receptive axons in this culture system, without resident oligodendrocytes capable of myelination. This preparation was an ideal host system to test the myelinating capability of oligodendrocytes transplanted from other sources. What follows is a review of some studies of myelination

of Ara C treated cultures after transplantation with oligodendrocytes from various origins /4, 11, 17, 19, 20, 22, 24/.

OLIGODENDROCYTE TRANSPLANTATION

Oligodendrocytes Derived From Other Explants

Upon exposure of neonatal mouse cerebellar explants to 10^{-4} M kainic acid for the first 5 DIV, all intracerebellar nucleus and cortical neurons were destroyed except for the cerebellar granule cells, which were selectively resistant to kainate toxicity /18/. Both astrocytes and oligodendrocytes in such preparations were intact, but the cultures were not myelinated because the only available axons, the granule cell axons, do not ordinarily myelinate in cerebellar cultures /16/. That oligodendrocytes in kainate exposed cerebellar cultures were capable of myelinating was evident from the occasional presence of myelinated granule cell somata, a phenomenon that is also seen in normal cultures, but not in Ara C treated cultures /16, 18, 21/.

When we removed kainic acid treated mouse cerebellar cultures from their collagen substrates at 9 DIV and superimposed them upon 9 or 16 DIV Ara C treated mouse explants, myelination of axons was evident in 78% of host cultures 3–5 days after transplantation /17, 20/. Equivalent results were obtained by transplanting Ara C treated mouse cerebellar cultures with kainate exposed cerebellar explants derived from neonatal Sprague-Dawley rats /19/. Although not as abundant as in normal cultures, the myelin was easily observed by light microscopy of explants in the living state, as shown in Fig. 1, which illustrates mouse axons myelinated by oligodendrocytes from a kainate exposed

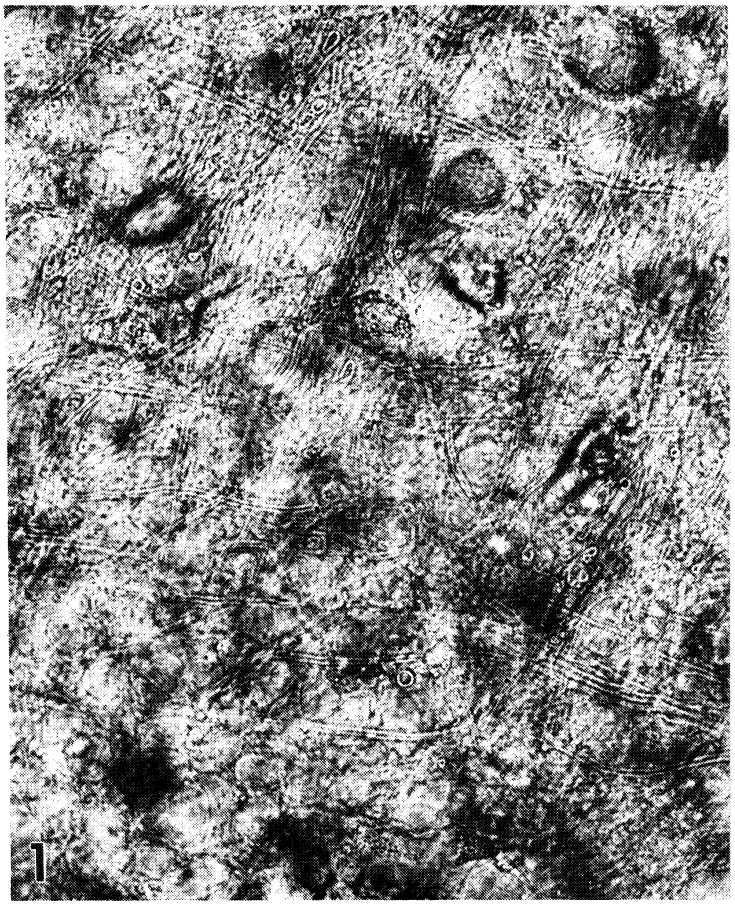

Fig. 1. Lengths of living myelinated axons in a 15 DIV Ara C treated mouse cerebellar culture that had been transplanted at 9 DIV with a 9 DIV rat cerebellar explant exposed to kainic acid. Prior to transplantation, the Ara C treated mouse cerebellar culture was devoid of mature axons while the kainate exposed rat cerebellar axons contained mature oligodendrocytes, but no myelin-receptive axons. Myelination of the host axons was achieved after transplantation. Photographed in the living state with brightfield optics, x555.

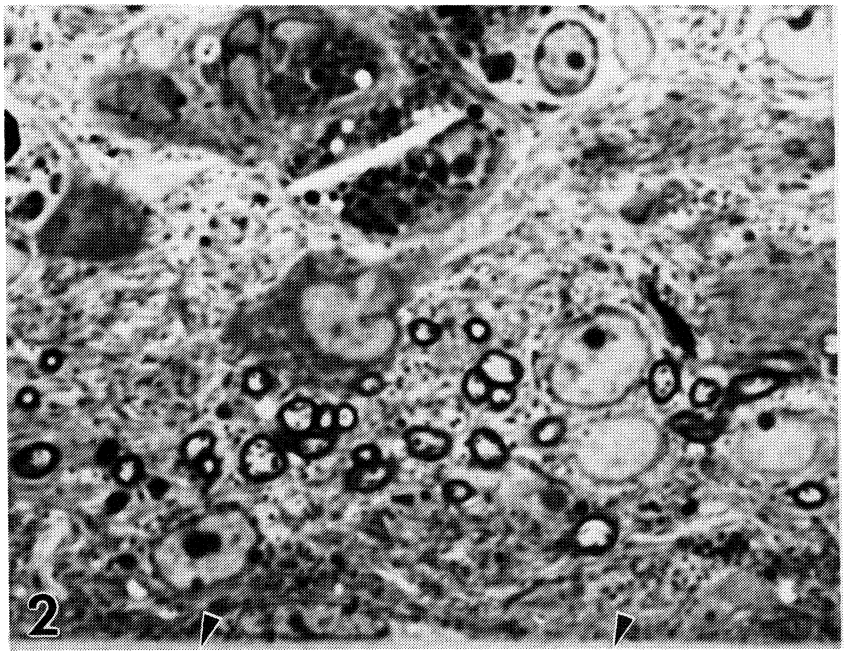

Fig. 2. Myelinated fibers in cross section in a 19 DIV Ara C treated mouse cerebellar explant overlain at 9 DIV with a 9 DIV mouse cerebellar culture exposed to kainic acid. The myelin is well within the host explant, near the collagen substrate (arrowheads). Plastic embedded thick section stained with toluidine blue, x1900.

rat cerebellar explant. The myelinated fibers appeared in greatest concentration in the area between cortex and intracerebellar nucleus. Myelinated axons were evident well below the culture surface in thick sections of plastic embedded tissue stained with toluidine blue (Fig. 2). Ultrastructurally, many mature dense oligodendrocytes were present within the host explants /4/. Compact myelin with normal periodicity surrounded axons, occasional dendrites of large cortical neurons, and a more than usual number of granule cell somata. Aberrant myelin was not present, nor were oligodendrocytes in mitosis evident.

If the kainic acid exposed explants were placed adjacent to rather than superimposed upon the Ara C treated cultures, myelination did not occur /17/. Similarly, if paired cerebellar explants were exposed to Ara C, and only one member of the pair subsequently received a superimposed kainic acid treated cerebellar transplant, only the transplanted member of the pair myelinated.

Dissociated Oligodendrocytes

Nishimura *et al.* /11/ provided the first demonstration of the myelinating capability of cultured purified dissociated oligodendrocytes prepared by the differential substrate adhesion method of McCarthy and de Vellis /10/. Cultures of rat oligodendrocytes prepared by this method were applied as cell pellets to Ara C treated mouse cerebellar explants. A few myelinated axons were observed by light microscopy in only 10% of the transplanted cultures. All of the cultures examined ultrastructurally had myelinated axons, but such axons were rarely found within the host cerebellar explants. Rather, they were present among the aggregates of transplanted oligodendroglia overlying the host cerebellar cultures, that is, the myelinated axons were axons that had grown out from the cerebellar explants into the oligodendrocyte pellets. There appeared to be little or no penetration of the host explants by the transplanted oligodendrocytes. This represented a pattern quite different from that seen with transplantation of host Ara C treated explants with cerebellar cultures exposed to kainic acid, as described above.

In a subsequent study, we applied cell suspensions of cultured (for 16–34 DIV) rat oligodendrocytes prepared by the differential substrate adhesion method to host mouse Ara C treated cerebellar explants /19/. Myelinated axons were observed by light microscopy 2–5 days after transplantation in 92% of the host explants. Most of the myelin appeared in the *in vitro*

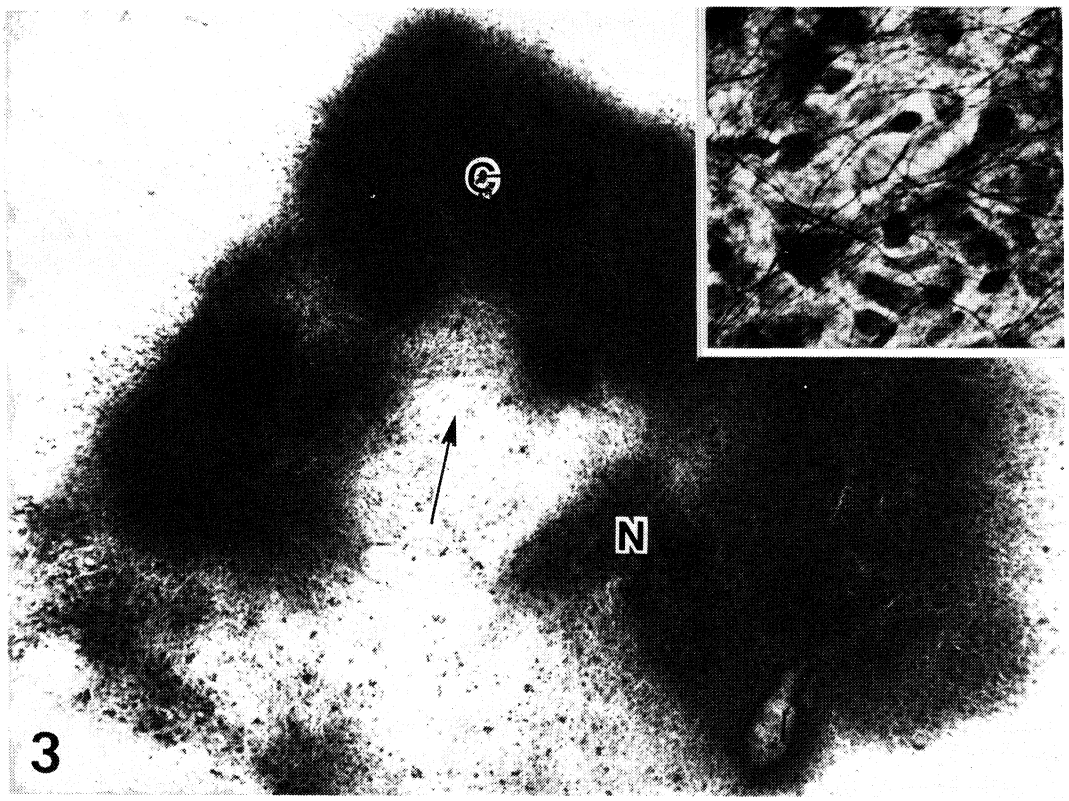

Fig. 3. Ara C treated mouse cerebellar explant, 15 DIV, that had been transplanted at 9 DIV with dissociated purified rat oligodendrocytes cultured for 23 DIV. Myelinated axons, inset, are concentrated in the area between cortex (C) and intracerebellar nucleus (N). The arrow designates the location of the fibers shown in the inset. The fibers are in an area equivalent to the white matter zone *in vivo*. Luxol fast blue/cresyl violet stain, x57; inset, x615.

white matter zone, and was comparable in amount to that seen in Ara C treated cerebellar explants transplanted with kainate exposed cultures. That axons within the host explants were indeed myelinated was supported by observations of whole mount culture preparations stained for myelin (Fig. 3), and by ultrastructural observations. Myelin was only infrequently observed in the outgrowth zone, as is the case with normal cerebellar cultures /16/.

Most of the applied oligodendrocytes appeared as small aggregates of cells in the outgrowth zone, where axons are far less dense than in the explants. Thus most of the transplanted oligodendrocytes did not myelinate axons. These relatively isolated oligodendrocytes did, however, stain positively for galactocerebroside and myelin basic protein, indicating that oligodendrocyte maturation had proceeded *in vitro*, consistent with previous studies of isolated oligodendrocytes cultured without axons /8, 15/.

Ultrastructurally, mature oligodendrocytes and myelinated axons were present well below the surfaces of Ara C treated mouse cerebellar explants transplanted with dissociated rat oligodendrocytes. Compact myelin of normal periodicity was observed in both longitudinal and cross sectional planes. Also present were compact myelin membranes surrounding empty spaces devoid of axons. These structures appeared only in cross sectional planes, and were therefore interpreted as being spherical in shape. The myelin spherules occurred frequently, and were observed in the vicinity of both myelinated and unmyelinated axons. The thickness of the myelin membranes was comparable to that of myelin around axons.

Oligodendrocytes Derived From Optic Nerve

Wolf and co-workers used Ara C treated B6C3 F1 hybrid mouse cerebellar cultures to test the myelinating capability of oligodendrocytes in immature and mature normal mouse optic nerve /24/ and in immature shiverer mouse optic nerve /22/. They found that 57% of host Ara C exposed explants transplanted with postnatal day 7–11 (P 7–11) normal optic nerve became myelinated /24/. The myelin was visible by light

microscopy 5–7 days after transplantation. These observations are consistent with our own with Ara C treated Swiss-Webster mouse cerebellar cultures transplanted with Swiss-Webster mouse optic nerve fragments, in which 53% of the host cultures had light microscopically visible myelin within the explants by 7 days after transplantation (previously unpublished observations). In our experience, the myelin was less abundant in optic nerve transplanted host cerebellar explants than in Ara C treated cerebellar cultures transplanted with either kainic acid exposed explants or dissociated oligodendrocytes, as described above. In both Wolf's /24/ and our studies, the myelin within the host explants was always found in apposition to cut surfaces of the transplanted optic nerve fragments.

Wolf *et al.* /24/ noted that myelination of Ara C treated cerebellar cultures occurred as frequently with optic nerve transplants from mature mice, but oligodendrocytes from the older optic nerve fragments required 6–8 days more *in vitro* to myelinate axons than glia from immature optic nerve. Myelin was also less abundant in cultures transplanted with mature optic nerve. By computer generated reconstructions of transplanted cerebellar cultures, Wolf *et al.* determined that some oligodendrocytes migrated to the most remote parts of the explants away from the optic nerve transplants to myelinate axons. The distance of such migrations was at least 0.6 mm.

In the study with Ara C treated normal mouse cerebellar cultures transplanted with P 7–12 shiverer optic nerve, Stanhope *et al.* /22/ found that the phenotype of the formed myelin corresponded to the genotype of the optic nerve. That is, shiverer oligodendrocytes produced shiverer type myelin around normal host axons, as indicated by the ultrastructural characteristics of absent major dense lines and abnormally dark intraperiod lines. This was in contrast with a previous study /3/ in which transplantation of shiverer cerebellar cultures, which contain no ultrastructurally normal myelin, with normal mouse optic nerve resulted in the appearance of normal as well as abnormal myelin in the host cultures. Together these studies strongly suggested that the myelin defect was associated with mutant oligodendrocytes rather than axons.

DISCUSSION

There are two possible sources of myelin in Ara C treated cultures transplanted with oligodendrocytes from various origins: 1) the myelin is formed around host axons by intact oligodendrocytes that migrated into the host cultures; 2) the myelin is formed by residual host oligodendrocytes whose maturation was induced by diffusible factors emanating from the transplanted tissue of cells. The evidence is overwhelmingly in favor of the former possibility, and includes: 1) the failure of myelination in cultures other than those with directly superimposed tissue or cells, as opposed to side by side placement of host explants and transplants; 2) the virtual absence of myelin within explants upon which pellets of oligodendrocytes were superimposed, whereas myelinated host axons were present within the aggregates of applied oligodendrocytes; 3) the formation around host axons of myelin whose phenotype corresponded to the genotype of the transplanted oligodendrocytes.

That transplanted oligodendrocytes can migrate within host tissues has been demonstrated in *in vivo* studies /1, 9/. Earlier *in vitro* transplantation studies /2, 4, 17, 20, 25/ and the study with computer-generated reconstructions of cerebellar cultures by Wolf *et al.* /24/ provide evidence for oligodendrocyte migration *in vitro*. The difference between our results /19/ and those of Nishimura *et al.* /11/ with regard to application of dissociated oligodendrocytes to host cerebellar cultures is probably related to migration of the applied cells. It is likely that oligodendrocytes compacted into pellets may not be able to migrate from the pellets, thus explaining the virtual absence of myelin or mature oligodendrocytes in Nishimura's explants. Oligodendrocytes applied as cell suspensions are free to migrate into the host cultures, and thus the presence of mature oligodendrocytes and myelin within our explants.

The difference in the percentage of transplanted Ara C treated cultures that are myelinated and the time of the initial appearance of myelin when optic nerve is used as a source of oligodendrocytes versus other sources may also be related to oligodendrocyte migration. Only 57% of cultures myelinated when transplanted with immature optic nerve, and the myelin appeared 5–7 days after transplantation /24/, as opposed to 78% and 3–5 days for cultures transplanted with kainic acid exposed explants /17, 20/ and 92% and 2–5 days for cultures transplanted with cultured dissociated oligodendrocytes /19/. It is quite possible that oligodendrocytes have greater difficulty migrating out of the optic nerve, which is usually covered with meninges, than from the other transplant sources. This notion is supported by the consistent appearance of myelin at the cut end of the optic nerve fragments, as well as the presence of some myelinated fibers at

uncut portions of optic nerves where meninges have been incised /24/. Also consistent with the concept of the differences being due to oligodendrocyte migration is the earliest appearance of myelin and the highest percentage of transplanted cultures myelinated when cell suspensions of oligodendrocytes are used as the source /19/, these oligodendrocytes being presumably the most free to migrate.

The appearance of empty spherules of myelin membranes along with myelinated axons in our Ara C treated cerebellar explants transplanted with cultured dissociated oligodendrocytes /19/ is of interest in light of previous reports of such membranes in cultures of isolated oligodendrocytes /7, 8, 12–14, 23/. The presence of myelin-like membranes in isolated oligodendrocyte cyltures has led to speculations that the formation of myelin membranes is an intrinsic property of oligodendrocytes, and does not require induction by axons. We have not, however, noted spherules of myelin membranes in Ara C treated cerebellar explants transplanted with kainate exposed cultures /4/. In this latter preparation, the transplanted oligodendrocytes did appear somewhat hyperreactive, as the dendrites of some large neurons as well as a more than usual number of granule cell somata were myelinated. We therefore interpreted the formation of myelin spherules as a more extreme form of oligodendrocyte hyperreactivity induced by culture in isolation, and concluded that while the formation of myelin or myelin-like membranes may be a property intrinsic to oligodendrocytes, it is expressed only under special circumstances, and not when normal axon-glia interrelationships are present /19/.

The Ara C treated cerebellar culture/oligodendrocyte transplantation system has been useful not only as an assay for the capability of oligodendrocytes to myelinate central axons, but has also served to demonstrate some additional biological properties of oligodendroglia. Oligodendrocyte migration, development, genotypic expression, intrinsic properties and reactivity are all subject to analysis with this system. Information such as increased percentages of cultures myelinated by dissociated cells versus a tissue source of oligodendrocytes might be a useful guide for *in vivo* studies, including the ultimate application of such technology to man.

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