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Sources of Human Schwann Cells and the Influence of Donor Age

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We evaluated several tissues as possible sources for culturing human Schwann cells. The average cell yield (total cell number/mg of nerve fascicle) obtained from adult autopsy cases and transplant organ donors was similar (2×10^4 and 2.9×10^4, respectively), but significantly higher yields were obtained from dorsal roots of pediatric patients undergoing selective dorsal rhizotomy (6.1×10^4). Fresh tissue was not essential since cells isolated from 0 to 20 h postmortem were equally viable. However, we found evidence that donor age affects the intrinsic growth rate of Schwann cells and perineurial fibroblasts in culture. © 1994 Academic Press, Inc.

Human Schwann cell cultures are useful for investigating the pathogenesis of peripheral neuropathies, and effective culture techniques would be necessary if autografts of human Schwann cells were to be used clinically to promote regeneration in the nervous system. We previously reported a method for isolating large numbers of human Schwann cells from adult sural nerves obtained from patients undergoing graft repair of a brachial plexus injury (6). The isolation procedure involves maintaining nerve fascicles as floating explants for 7–10 days before complete enzymatic dissociation into a single cell suspension. The focus of these initial studies was optimizing cell yield, viability, and attachment. Large numbers of Schwann cells were easily obtained from relatively small amounts of tissue ($>2 \times 10^4$ cells/mg of nerve), which was a significant improvement over successive reexplantation methods (1, 7, 8, 10). Although sural nerve grafting affords the opportunity to obtain fresh, normal tissue, it is an infrequently performed procedure. We have since identified alternative tissue sources to make the isolation of human Schwann cells more widely applicable.

Portions of the lumbar plexus and/or proximal sciatic nerves were obtained at autopsy (4–20 h postmortem interval) or from transplant donors with no history of neuromuscular disease. Dorsal roots were obtained from pediatric patients with cerebral palsy undergoing selective rhizotomy for spasticity. Nerve fascicles or dorsal roots were maintained in culture for 7–10 days before enzymatic dissociation as previously described (6). Large amounts of tissue could be removed at autopsy without loss of viability for at least 20 h postmortem, although the average donor was over 60 years of age (Table 1). Bacterial contamination due to nonsterile conditions could be controlled with antibiotic prophylaxis since only one specimen was lost to bacterial contamination. Transplant organ donors were 10 years younger on average than autopsy donors, but the difference in cell yield was not significant. The average age of dorsal root donors was 7 years, and, although less than 100 mg of tissue was obtained from each patient, the cell yield was threefold higher than from adult tissue ($P < 0.001$, Student's t test).

Age and tissue-related factors have previously been shown to affect the growth properties and replicative life span of human fibroblasts in culture (3–5, 9, 11). Accordingly, we found a significant inverse relationship between age and the rate of perineurial fibroblast growth ($P < 0.001$, Spearman rank correlation). Fibroblasts from pediatric donors grew rapidly, multiplying more than sixfold within 8 days of culture (Fig. 1A). However, a precipitous fall in the growth rate occurred after approximately 40 years of age, and fibroblasts from donors older than 60 years grew only slightly.

The influence of age on the growth rate of the Schwann cells could not be measured directly due to the presence of contaminating fibroblasts. Nevertheless, the relative rate of decline in the Schwann cell population (identified with antibodies to S-100) over time between cultures from adult and pediatric donors was similar even though the pediatric fibroblasts were growing twice as fast as the adult fibroblasts (Fig. 1B). This implies that pediatric Schwann cells were also growing faster, otherwise the fibroblasts would more rapidly overtake the cultures. Moreover, the initial cell yields were two to three times greater from pediatric than adult donors. Together, these results imply that Schwann cells from younger donors have a greater mitotic rate and life span in culture. A better means for controlling or eliminating fibroblasts will enable further characterization of the growth properties of the human Schwann cells.

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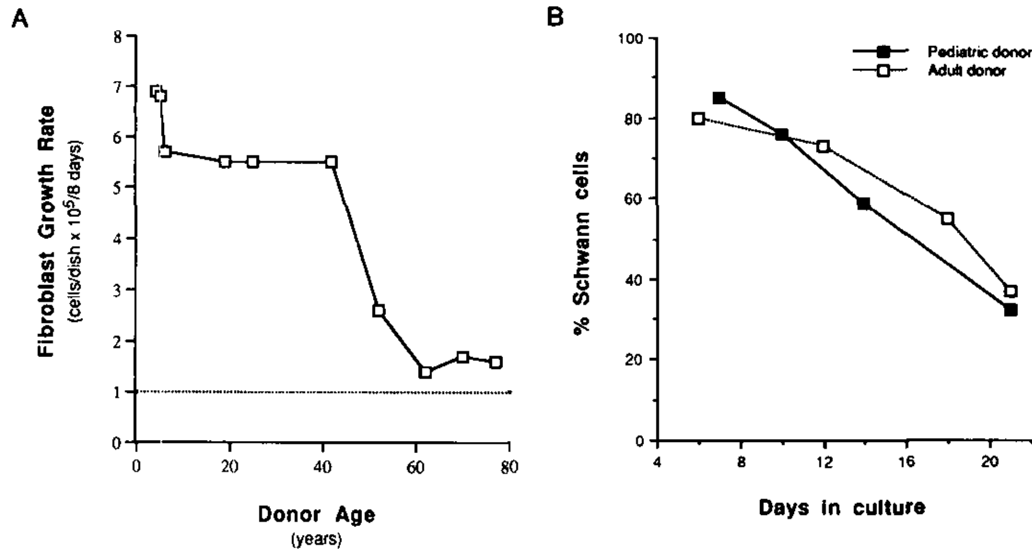

FIG. 1. Influence of donor age on cell growth. (A) Perineurial fibroblasts were purified by serial passage on uncoated plastic dishes in DMEM with 20% FBS. Growth studies were initiated after four to six passages in culture by seeding 1×10^5 cells/35-mm dish in medium with 10% FBS. After 8 days of culture, cells were released from triplicate wells with trypsin and counted in a hemocytometer. Dotted line indicates the initial plating density. Each point represents the mean of triplicate determinations from a single donor. Standard deviations for replicates were < 5% of the mean. (B) Schwann cells were seeded on glass coverslips coated with 1 mg/ml of poly-L-lysine. Feeding medium consisted of DMEM with 10% FBS, 5 μg/ml of bovine glial growth factor (6), and 0.1 mg/ml of cholera toxin. After various times in culture, cells were stained by indirect immunofluorescence using antibodies to S-100 and nuclear counterstained with Hoechst 33342 (1, 4). At least six visual fields to total over 300 cells were counted under a Leitz fluorescent microscope using fluorescein and uv filters to determine the ratio of Schwann cells to the total cell number. Standard deviations for replicate determinations were < 5% of the mean.

This study demonstrates that large numbers of viable human Schwann cells can readily be cultured from peripheral nerves obtained at autopsies, after transplant organ donation or from selective dorsal rhizotomy. Although certain disease states may preclude nerve harvest, autopsy probably provides the most readily accessible source of peripheral nerve tissue, and neither the postmortem interval before harvest nor bacterial contamination provided a significant obstacle to successful culture. The availability of nerve specimens from organ donors or rhizotomies is more limited, though these sources typically provided younger tissue with a greater proliferative potential. Moreover, this study underscores the importance of age differences on normal and pathological responses in human cells.

TABLE 1

Cell Isolation from Human Peripheral Nerves

Source	N	Age (years)	Tissue weight (mg)	Cell yield (cells/mg tissue)	Viability (%)
Autopsy	5	62	1350	2.0×10^4	81
		(51–77)	(502–2006)	($1.0–3.6 \times 10^4$)	(74–87)
Organ donor	4	49	1675	2.9×10^4	88
		(37–64)	(600–2450)	($2.3–3.8 \times 10^4$)	(79–96)
Dorsal rhizotomy	9	7	76	6.1×10^4*	81
		(3–12)	(23–109)	($4.3–7.8 \times 10^4$)	(68–95)

Note. Nerve fascicles or dorsal roots were cultured for 7–10 days in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). Because of the inherent risk of microbial contamination, the medium for autopsy tissue was supplemented with 200 U/ml of penicillin, 0.2 mg/ml of streptomycin, and 50 μg/ml of gentamicin as a precaution. The tissue was digested with 100 U/ml of collagenase and 0.8 U/ml of dispase and the total cell yield and viability were determined by fluorescent staining with propidium iodide as previously described (1). Mean values are given with the range in parentheses.

* Significantly greater than the other two groups ($P < 0.001$, Student's t test).

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