Human fetal cerebellar and cortical tissue transplanted to the anterior eye chamber of athymic rats: Electrophysiological and structural studies

(human neuroblast development/xenograft/cerebellum/cortex cerebri/intraocular transplantation)

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ABSTRACT Human fetal tissue fragments from cortex cerebri and cerebellum were grafted to the anterior chamber of the eye of adult athymic nude rats. The grafts were obtained from tissue fragments recovered after elective routine abortions, performed in weeks 8–11 of gestation. Both cerebellar and cortex cerebi grafts survived and developed in the anterior chamber of the eye for 1–4 months. The transplants slowly became vascularized from the host iris. The grafts developed blood vessels with laminin-immunoreactive walls and contained relatively high amounts of glial fibrillary acidic protein and neurofilament-immunoreactivity in the neuropil after 4 months in oculo. Recordings of extracellular action potentials from the grafts revealed spontaneously active neurons with action-potential waveforms similar to those observed in immature rodents. Morphologically, the grafts showed no signs of rejection. Clusters and bands of large neurons resembling Purkinje cells and dense aggregates of smaller granule-like cells could be found in the cerebellar grafts. Large neurons were also seen in the cortex grafts. Taken together, these data suggest that the athymic rat may serve as a useful tool for studies of central nervous system tissue from otherwise immunologically incompatible species.

Syngeneic grafting of brain tissue has emerged over the last decade as a valuable approach to studying the development and regeneration of neural connections in the central nervous system of mammals (1–3). Recently, interest has focused on the developmental properties of xenogeneic grafts of central nervous system tissue (4–6).

A major problem with xenogeneic brain tissue grafting has been poor or variable survivability. This problem has been overcome in part by daily treatment with immunosuppressive agents (7–11). The most widely used immunosuppressive agent, cyclosporin A, is thought to suppress both humoral and cell-mediated immunity (12). However, the precise action of cyclosporin A and other immunosuppressive agents is unknown, as is the extent to which such agents might interfere with graft survival and development. In this perspective, the use of a host/graft system that does not require any immunosuppressive agents would be a better tool for studies of development and regeneration of xenogeneic grafts.

The athymic rat has been shown to lack T-cell function (13, 14). Because of its inability to react immunologically to foreign tissue, it has been used extensively as a host to successfully support transplants of various malignant human tumor cell lines without the need for immunosuppressive therapy (15, 16). Therefore, such a host may be the ideal recipient for xenogeneic brain grafts.

The anterior chamber of the eye has been used extensively for studies of xenogeneic brain transplants (see refs. 2 and 3). This technique offers unique advantages over other transplantation sites because the survival and growth can be monitored without invasive procedures (2, 3). Here we report studies of human fetal cerebellar and cortical brain tissue grafted to the anterior chamber of the eye of athymic nude rats. Survival and growth of these grafts was examined by electrophysiological and morphological techniques.

MATERIALS AND METHODS

Fetal material to be grafted was obtained after termination of first-trimester pregnancies. Healthy women with an apparently normal pregnancy in weeks 8–11 of gestation and admitted to the hospital for elective abortion were informed both orally and in writing about the aim of the study and the procedure to be used, and they gave their consent. Anonymity was strictly maintained. The abortion was performed by using paracervical blockade followed by premedication. After dilatation of the cervical canal, fetal fragments were removed by forceps after which the abortion was completed by vacuum aspiration. The fetal tissue fragments were collected and kept in isotonic saline until further processed. The study was approved by the Regional Ethical Committee of the Karolinska Hospital, and all experiments conformed to guidelines of the Swedish Medical Research Council and the U.S. Public Health Service.

Tissues were examined using a stereomicroscope, and small pieces measuring 1–3 mm3 were prepared for grafting. The medial portion of the cerebellar anlage and small pieces of cerebral cortex were dissected free from pial membrane and inserted into the anterior chamber of the eye of 2-month-old nude rats by methods described earlier for syngeneic rat/rat transplants (see ref. 2). Vascularization and growth of the grafts was followed by repeated measurements through the translucent cornea.

Spontaneous activity and responses to electrical surface stimulation were measured by extracellular recordings with single-barrel micropipettes. Electrophysiological recordings were performed on two cortex cerebri grafts and four cerebellar grafts that were grown for 6–8 weeks in oculo and from two cerebellar grafts after 4 months in oculo. Thirty-eight neurons from the six cerebellar grafts and 12 neurons from the two cortex grafts were suitable for analysis. The host animals were anesthetized (1.25 g of urethane i.p. per kg

Abbreviations: GFA, glial fibrillary acidic protein; NF, neurofilament.
of body weight), and the cornea overlying the graft was removed. A Plexiglas perfusion chamber was placed over the eye, and the graft was perfused with Earle's balanced salt solution at 37°C throughout the experiment. Single-unit activity was recorded as described (17). For each cell, 10–20 spikes were averaged by digital computer, and 95% confidence limits and mean waveforms were displayed. All electrophysiological experiments were initiated in a darkened room after a sufficient time had elapsed for recovery from retinal bleeding during surgery. When recording from cortex cerebri grafts, electrical activity was augmented by addition of 50 mM sodium glutamate to the electrolyte solution in the pipette or 2000 units of sodium penicillin per ml to the perfusion fluid. Electrical stimulation of parallel fibers in cerebellar grafts was performed with a bipolar electrode of two twisted wires having a tip separation of 0.1 mm placed on the surface of the graft. Monophasic 0.5- to 1.0-msec square-wave pulses of 1–60 V were utilized.

Grafts were processed for immunohistochemical localization of glial fibrillary acidic protein (GFA), neurofilament (NF), and laminin by using the indirect immunofluorescence technique of Coons (18) as described (11).

RESULTS

Cerebellar and cortical grafts survived well in the anterior chamber of the eye and became vascularized from the host iris. Vascularization occurred over the first weeks. Fetal cerebral cortex began to increase in size a few days after grafting and grew rapidly between weeks 2 and 6 after grafting. Cerebellar grafts initially decreased in size before resuming growth after 1 week in oculo. They then grew progressively but at a slower rate than did cortex cerebri, reaching a size about one-third that of the cortex cerebri grafts at 6 weeks.

Cresyl violet-stained sections through cerebellar grafts, sampled at 1.5–2 and 4 months after transplantation, revealed immature brain tissue. Clusters and bands of larger neurons, presumably Purkinje neurons, were often seen. Somewhat smaller and more densely arranged neurons, presumably granule cells, formed an internal granular layer on one side of the Purkinje neurons, with a cell-poor area, probably corresponding to the molecular layer, on the other side (Fig. 1a and b). Dense clusters of small neurons, presumably corresponding to remaining patches of the external granular layer, were also found. In addition, pigmented melanocytes migrated into graff neurons.

Laminin immunohistochemistry paralleled the in vivo observation of a relatively slow vascularization of the cerebellar grafts. Thus, at 6 weeks in oculo, the cerebellar graft shown in Fig. 1d was still not vascularized despite good growth. Eventually all grafts became vascularized, although laminin immunohistochemistry revealed abnormally thick walls (Fig. 1e). Cerebellar transplants at 4 months after transplantation contained a dense plexus of NF-immunoreactive material. This consisted mainly of nerve-fiber bundles, but also included groups of small- or medium-sized cell bodies (Fig. 1c). However, the larger neurons, presumably corresponding to Purkinje cells, were devoid of NF immunofluorescence.

GFA-like immunoreactivity was found in all transplants. The density of GFA-immunoreactive glial processes was somewhat higher than in normal adult cerebellar tissue. In patches of residual external granular-layer cells, a less dense plexus of GFA-immunoreactive material was seen (Fig. 2). Many areas of the cerebellar transplants contained long, straight GFA-positive fibers running in various directions, suggesting the presence of disorganized Bergmann glia fibers (Fig. 2b). The amount of GFA in the cerebellar transplants increased with time during the period studied (Fig. 2c and d).

Grafts of cortex cerebri contained large scattered neurons with one or two processes as well as groups of smaller cells. There were no signs of degenerative processes or rejection in either the cerebellar or the cortical grafts examined.

Action potentials were recorded from 28 neurons from four cerebellar grafts at 6–8 weeks in oculo. Initially negative or positive waveforms were observed with an average duration of 1.9 ± 0.1 msec. The neurons tended to discharge in doublets. The discharge rates were slow, and the pattern of discharge was irregular. The interspike-interval histograms usually consisted of two modal peaks; one at 5-msec, representative of intradoublet intervals, and a later peak at 150 msec that reflected long periods between doublets. A typical cerebellar Purkinje neuron is illustrated in Fig. 3a. Electrical stimulation of the granule-cell parallel fibers with an electrode placed on the surface of the graft was unable to elicit Purkinje-cell discharge in three neurons tested in these grafts.

A total of 12 cells were recorded from the two cortex cerebri grafts. Fig. 3b illustrates the discharge of one of these neurons. The pattern of firing was intermittent, and neurons tended to discharge when initially recorded and then to stop. Perfusion of penicillin (3000 units/ml) increased discharge rates; under these conditions, cell discharge could be followed for a period of several minutes. The majority of action-potential waveforms were initially negative, but some neurons displayed an initial positivity. The average action-potential duration was 2.5 ± 0.2 msec.

Two cerebellar grafts were allowed to develop for 4 months in oculo. Ten cerebellar neurons were recorded from these grafts. These neurons demonstrated firing patterns that were more mature than those recorded from the four cerebellar grafts grown in oculo for 2 months. Action-potential durations were significantly shorter (1.14 ± 0.1 msec; P < 0.01, two-tailed Student's t test). Sustained discharge was frequently seen, and spontaneous firing rates were faster than those observed in the younger transplants. Electrical stimulation of the cerebellar cortical surface activated parallel-fiber afferents to the Purkinje cells. Surface stimulation was capable of eliciting evoked discharge in one of three neurons tested (Fig. 4), demonstrating that intrinsic excitatory circuitry was developing in these cerebellar grafts.

DISCUSSION

The present results demonstrate that human fetal cerebellar and cerebral cortex grafts survive in athymic nude rat recipients, and continue their development in the anterior chamber of the eye.

The growth of the grafts and their histological appearance suggests that good viability was maintained in oculo. Neurons of various sizes and glial elements could be visualized readily, as could vascular elements. The spatial distribution and appearance of the various cellular elements clearly indicated an immature organization, even in grafts that had been in oculo for 4 months.

Grafts of both brain types that developed for 6–8 weeks in oculo expressed spontaneous electrical activity, with long duration action potentials and occasional multiple firing, typical for immature cortical and cerebellar neurons in rodents (19). The cerebellar grafts at 2 months in oculo did not respond to surface electrical stimulation, which suggests that functional intrinsic circuitry had not yet been established. Cerebellar grafts allowed to develop for 4 months in oculo had characteristics of more mature neurons in that the waveforms were significantly shorter, and interspike-interval histograms consisted of mainly shorter intervals (19). Purkinje neurons in the older grafts responded to electrical surface stimulation, suggesting that intrinsic parallel-fiber circuitry was beginning to develop. These grafts still had
FIG. 1. Cresyl violet-, NF-, and GFA-stained sections of human fetal cerebellar grafts. (a) Cresyl violet-stained overview of the central two-thirds of the graft at 4 months in oculo. Toward the bottom is seen the heavily pigmented host iris. Three dense aggregations of nuclei midway between the iris and free surface of the graft may represent residual patches of the external granular layer. Large neurons, presumably Purkinje cells, are seen both scattered throughout the graft neuropil and in layers and groups close to the iris. Note also infiltration of host iris...
Fig. 2. GFA immunoreactivity in human cerebellar tissue grafted to nude rats. (a) and (b) Consecutive sections of the same area of a cerebellar transplant after 4 months in oculo. (a) When the tissue is stained with cresyl violet, the free surface of the transplant is seen at the top with a patch of presumed residual external granule layer cells (G) below. Host iris blood vessels are seen at the bottom. (×260.) (b) When the tissue is labeled with GFA-antibodies, the patch of external granule cells can be shown to contain relatively little GFA immunoreactivity. Longer and relatively straight GFA-positive fibers in the upper half of the transplant may represent disorganized Bergmann glia. (×260.) (c) Overview of part of a human cerebellar graft after 6 weeks in oculo. The transplant contains many GFA-immunoreactive cells and processes suggesting a moderate gliosis. The iris is to the left. (×100.) (d) Cerebellar transplant from a 10-week-old fetus after 4 months in oculo. The amount of GFA-positive structures has increased as compared with c. In addition, a certain degree of organization of GFA-positive processes can be seen. (×100.)

Some characteristics of immaturity; therefore, longer periods of growth in oculo may be necessary for maturation to take place.

Recent studies have shown that human fetal tissues can also survive and develop in cyclosporin A-treated host rats. Thus, human fetal substantia nigra dopamine neuroblasts can functionally reinnervate the dopamine-denervated adult rat striatum (7, 10) provided that the host animals are given continuous daily cyclosporin treatments. Similarly, it has been shown that several different human fetal central nervous system areas can survive and develop when grafted to the anterior chamber of the eye of cyclosporin A-treated host rats (11). However, in spite of rigorous immunosuppression, only a limited proportion of the grafted material survived intraocularly (19). It is not known to what extent cyclosporin treatment, in itself, interferes with brain development. As shown in the present experiments, the athymic nude rat offers an alternative solution to xenogeneic brain tissue grafting with several advantages. There is robust growth and development with no signs of rejection, suggesting that all transplants that are viable at the time of grafting will survive.

Several examples of the juxtaposition of human and rat cellular elements were observed in the present study. The human transplants became vascularized from the rat host iris. Moreover, melanocytes with many pigmented processes invaded the transplants and became integrated with the graft neuropil.

We have previously studied syngeneic grafts of cerebellar melanocytes in the human brain-tissue neuropil. (×100.) (b) Close-up of the transplant showing a band of larger neurons, presumably Purkinje cells, surrounded by small cells, presumably granular cells. (×220.) (c) NF-like immunoreactivity in a human cerebellar transplant at 4 months in oculo. Many nerve fiber systems express NF immunoreactivity, particularly at the transplant surface. Note also a few NF-positive cell bodies (e.g., at the arrow). In general, most larger neurons, presumably Purkinje neurons, are NF negative. (×150.) (d and e) Laminin immunoreactivity 6 weeks (d) and 4 months (e) after transplantation. At 6 weeks the host iris is rich in laminin immunoreactivity (at the bottom of d), whereas the transplant neuropil is laminin negative. After 4 months in oculo (e), the transplant neuropil now contains many blood vessels with abnormally thick laminin-immunoreactive walls and thin laminin-immunoreactive sprouts. (d and e, ×100.) (f) Normal rat host cerebellar cortex shown in the same magnification as d and e, illustrating the thinness of normal brain capillaries visualized by laminin immunohistochemistry. (×100.)
In conclusion, we have shown that human fetal brain tissue can survive and grow in the anterior chamber of the eye of the athymic nude rat. This technique uniquely permits study of human brain development, connectivity, and pharmacological properties in an otherwise immunologically incompatible species.

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