Transplantation of Fetal Nerve Cells from Insects to the Brain of Amphibians and Mammals

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Cells of the neural primordia of the Notch mutant of *Drosophila melanogaster* are transplanted to the neural tube of four amphibian species and two mammalian species. It is shown that cells of the neural primordia of drosophila survive and differentiate in the amphibian and mammalian brain. Differentiated cells of the transplant establish axon-dendrite junctions with cells of the recipient and penetrate into the structures of its brain. Tailed amphibians die several days after xenotransplantation. Transplantation of drosophila cells to the nervous system of tailless amphibians and mammals does not cause developmental abnormalities or death of the animals.

Key Words: *transplantation; nervous system; fetal tissue; xenografts; insects; amphibians*

Xenoplastic transplants are successfully used for the elucidation of some important problems in biology and genetics. The most interesting results have been obtained when a transplant has been performed at the embryonal stages of development [1,4,14]. Transplants of embryonal nervous tissue to the brain ventricle are possible in the presence of the major histocompatibility barrier. After transplantation of fetal nervous tissue of Sprague-Dawley rat fetuses to the vascular bed of the anterior thalamus of adult Wistar rats, donor cells survived for 3 months [17]. These first studies laid the basis for an entire trend in experimental transplantology. Cell transplants between species are now used routinely in investigations of the developmental determination and differentiation of animal and human nerve cells [3,5,18]. The most widespread method of experimental xenotransplantation is the transplantation of embryonal and fetal human cells to the brain in rats, rabbits, and mice [19]. Such an approach is well grounded, since it makes it possible to assess the potential of fetal human cells transplanted to the brain of experimental animals. Xenotransplantations of nervous tissue between mice and rats [13] and rabbits and mice [11] have also proved informative. These studies have shown that after xenotransplantation, the donor tissues preserve their characteristic properties and pattern of differentiation.

Successful transplantations of nerve cells across different mammalian species suggest the possibility of transplanting neural primordia across animals of different classes. The feasibility of this has been demonstrated by embryonic-stage transplantations of fragments of murine ovarian cylinder to the early gastrula of the toad [10]. Amphibian embryos with murine tissue fragments have survived for 3 days. Amphibian and mammalian cells have aggregated, but differentiation has not been observed in the murine fragments. Functional and structural compatibility of the nervous systems of vertebrates and

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invertebrates has been demonstrated for the first time in tissue culture [9]. These experiments have shown that synaptic junctions and functional relationships are formed between neurons of animals of different classes. However, no attempts have been made to combine the nervous systems of insects and vertebrates *in vivo*. We have studied the possibility and results of transplantation of nerve cells of insects to the brain of amphibians [6-8,16]. The results of transplantation of cells from the neural primordia of *Drosophila* to the brain of the amphibian embryo are analyzed in the present study.

MATERIALS AND METHODS

The neurogenic primordium of Notch mutants of *Drosophila melanogaster* was used as the donor tissue. In the homozygous mutants used for xenotransplantation the entire ventral ectoderm is transformed so that it develops as neural tissue. After mechanical removal of the chorion, embryos at stage 9 of development [12] were selected and divided with preparation needles, and portions of the ventral neurogenic primordium were isolated for transplantation. Groups of cells were sucked into a glass capillary and introduced to the neural tube of amphibians with a micromanipulator (Fig. 1). Embryos of *Salamandrella keyserlingii*, *Pleurodeles waltlii*, *Xenopus laevis*, *Rana arvalis*, and noninbred laboratory mice were used as the recipients. In the amphibians the transplant was performed immediately after closure of the neural tube. Transplants were performed on young (less than 2 months) mice and neonatal noninbred rats. The recipients were fixed after 2-20 h and on days 2, 3, 5, 14, 49, 50, and 180 posttransplant. For light microscopy the material was treated as described previously [6,8], stained after Mallory, with hematoxylin-eosin, and shadowed with silver nitrate. For scanning and transmission microscopy the material was treated routinely and examined under Jeol 100 CX II and Hitachi S-500 microscopes [16]. The transplants were identified by cell labeling with tetramethylrhodamine β isothiocyanate (TRITC)- and fluorescein isothiocyanate (FITC)-dextran [15]. Unlabeled donor tissue was identified by *in situ* hybridization on sections with DNA of mutable drosophila genes (MDG). MDG-4 of drosophila, which is absent in amphibians, was used [2]. *Xenopus laevis* were subjected to behavioral testing in a vertical water maze. The total time of exploratory activity, the number of periods of active exploration, and the number of successful tests were recorded [6]. After the experiments the animals were narcotized and sacrificed, and the isolated brains were studied by the above-described methods.

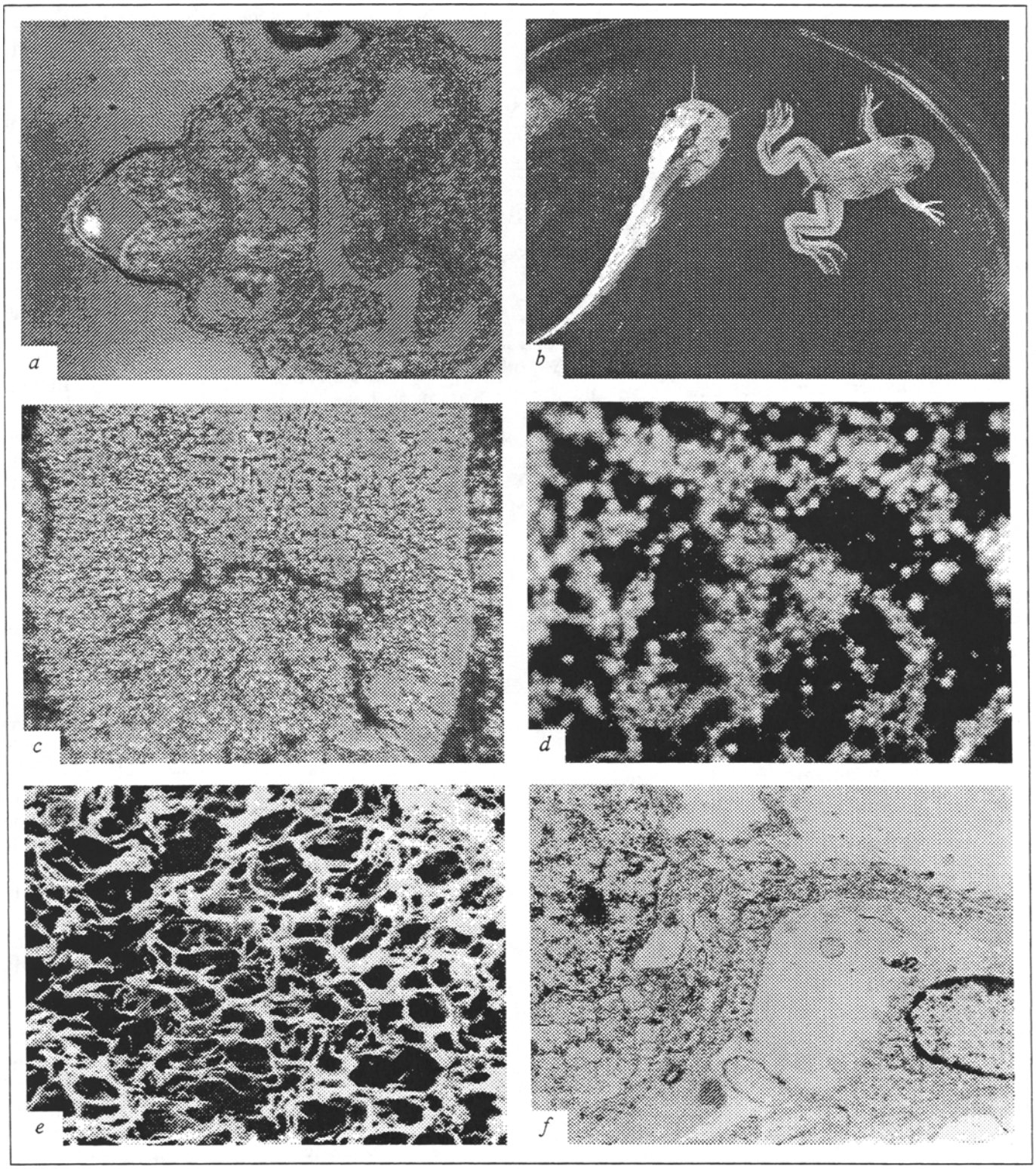
RESULTS

The results of transplantation of neural primordium of Notch *Drosophila* mutants were different in different species. Histocompatibility between donor and recipient tissues proved to be low in tailed amphibians. The embryos of tailed amphibians developed without pronounced morphological changes during the first 2 days. During this period, the transplant attached to one of the walls of the recipient brain. Drosophila cells differentiated, forming a neuropil, ganglia, and proliferative zones. Donor neurons established relationships with recipient cells. However, by the end of day 3 changes occurred in the morphological and histological organization of the tailed amphibian embryos. Solitary amoeba-like cells with lobopodia emerged at the surface of the cephalic ectoderm and brain. These cells departed from the embryonal tissue and migrated into the incubation medium. Histological investigation demonstrated that the amoeba-like cells are dedifferentiated neurons and cells of the neural crest and ectoderm. Marked changes occurred in the chromatin of the nucleus. The cell nuclei became rounded; the chromatin extended in one direction, and then spread amorphously over the enlarged nucleus. Disperse granulation of chromatin, becoming transformed into apoptoid packing of chromatin and death of cells, then started.

During combined culturing of several embryos we observed embryo fusion. Active disintegration of cells was not observed in this case. Histological examination demonstrated that the merging of the nervous systems of different recipients was occur-

Fig. 1. Transplantation of cells of neural primordia from Notch mutant of *Drosophila melanogaster* to neural tube of amphibians. *a*) localization of FITC-labeled tissue of drosophila neural primordia in cavity of fourth brain ventricle of *Xenopus laevis* 24 h posttransplant. Fluorescent microscopy, phase contrast, ×30. *b*) differences in tempo of development of control (left) and experimental (right) animals. Individuals with xenotransplant complete metamorphosis, whereas the control group after sham operation is at the start of metamorphosis. *c*) xenotransplant in cavity of third brain ventricle of *Rana arvalis* 50 days posttransplant. Ganglia-containing neuropil forms contacts with walls of recipient brain. Phase contrast, unstained section, ×120. *d*) radioautograph of drosophila cells in neural tube of *Xenopus laevis*. Cells are detected 6 months posttransplant by *in situ* hybridization on sections with DNA of mobile genetic elements of drosophila. Dark field, ×480. *e*) scanning electron microscopy of neuropil of drosophila in cavity of lateral forebrain ventricles of *Xenopus laevis* 6 months posttransplant. ×800. *f*) transmission microscopy of zone of ganglion containing cells of drosophila and *Xenopus laevis*. Nuclei of drosophila neuron marked by an arrow.

ring. Drosophila cells were found at the boundaries of the integrated nervous systems. These data provide evidence that insect cells can not only integrate with the nervous system of amphibians, but also stimulate integration of the nervous system between different individuals. In 2-3% of cases we observed survival of the tailed amphibian larvae on days 14-20 postoperation. These animals differed from the controls in various abnormalities of the head and pectoral girdle. The follow-up of the behavior of such animals showed abnormalities in the motor and exploratory activity. The animals



were incapable of feeding properly and swam in circles.

This series of experiments allowed for some conclusions to be drawn. Insect cells can be transplanted to the brain of tailed amphibians, where they survive and differentiate into bona-fide neurons. The effect of drosophila cells on the brain of tailed amphibians is negative. Long-term development of the chimeric animal leads to dedifferentiation of neurons, and developmental abnormalities emerge.

Transplantation to the neural tube of tailless amphibians demonstrated that drosophila cells may be found in any of the brain ventricles. However, at the late stages they grouped preferentially in the third ventricle or around the choroid plexus. Evidently, the cells of the transplant readily adapted to the composition of the cerebrospinal fluid, since we did not observe their death. Analysis of the development of tailless amphibian embryos uncovered differences between the rates of growth of experimental and control animals. After xenotransplantation the embryos developed faster. They went through metamorphosis 10-11 days earlier and were larger. In the sixth month of development the differences between the weight of animals in the experimental and control groups were 1.5 g. It should be mentioned that the compared groups of animals were taken from the same parents. The survival of transplants was similar in both species of tailed amphibians (Fig. 1). After transplantation of drosophila cells to *Xenopus laevis* the maximal time of the recipient survival was 6 months. In both tailless and tailed amphibians neural primordial cells of drosophila differentiated on day 3-4 posttransplant. They formed a neuropil with bipolar and multipolar neurons. Ganglia, localized both inside and outside the neuropil, formed there. These formations were of three kinds. The ganglia formed by transplanted cells contained the neuropil inside, while the outer cells formed processes between transplant and recipient. Ganglia of the mixed type consisted of donor and recipient cells. All types of ganglia were integrated with the neuropil of the transplant or with the walls of the recipient brain. Along with the ganglia, reticulate structures consisting of differentiated drosophila neurons formed at the inner walls of the brain ventricles. Axons and dendrites of drosophila neurons penetrated the wall of the *Xenopus* brain, using the ependymal cells which compose the lining of the walls of the ventricles as a substrate. Processes of drosophila neurons moved along the cilia of the ependymal cells overhanging the ventricular cavity. The neuronal processes, penetrating the wall of the recipient brain, formed synaptic junctions, which testifies to functional integration of the nervous systems. Integration of donor and recipient nerve cells was attended by active vascularization of the transplant and of the adjacent parts of the amphibian brain.

On days 50 and 180 posttransplant we observed a more complete integration of the nervous systems of insects and amphibians. Penetration of drosophila cells in the recipient brain was characterized by the formation of ganglia of the mixed type inside the secondarily formed small cavities. A multipolar neuron of the amphibian was usually localized in the center of such a ganglion, and drosophila cells closely encircled it. The recipient neuron usually had processes terminating in the surrounding cerebral structures. The processes of drosophila nerve cells, which also penetrated into the recipient brain, were arranged along them. Similar cell associations formed in the ventricular cavities, although a melanin-containing amphibian cell often occupied the center of such a group. It is worthy of note that the number of melanocytes was much lower in the skin of recipients than in the animals of the control group. In the animals surviving for a long time posttransplant we discovered small zones with dying drosophila cells. The death of cells was compensated for by proliferation of small numbers of drosophila cells occupying the central part of the neuropil in the subependymal zone of the brain walls.

A study of the behavior of animals with xenotransplants showed a number of specific features. The total time of motor activity was two times lower in such animals than in the control group undergoing a sham operation. Since the number of times when the animals came to the surface was the same in the control group as in the experimental group, the efficacy of seeking the way out of the water maze was twice as high in the frogs with a chimeric brain. It should be noted that this result is characteristic only of animals with unlabeled drosophila cells in the brain. In individuals with fluorescent-labeled drosophila cells the total activity was close to that in the control. However, in such animals the number of exploratory attempts was only half that in the control animals. Evidently, the differences between the two experimental groups stem from the toxic effect of fluorescent markers. It should be mentioned that we revealed no marked differences in the learning of animals as they gained individual experience. Histological investigation of the brain of animals, which followed behavioral testing, demonstrated the presence of drosophila cells in the

brain ventricles and walls. Transplants were largely localized in the telencephalon and mesencephalon.

A study of the mammalian brain at different times posttransplant showed that differentiation of drosophila cells occurred within 24 h, indicating that the rate of differentiation in this case is markedly higher than that in the experiments on amphibians. The results of morphological integration of the nervous systems were similar to the above-described events. However, we noted some specificities characteristic only of mammals. Drosophila cells did not migrate considerable distances from the site of injection. During the first 3-4 days a 20-30% mortality of cells was observed in the transplant. The situation then stabilized, and appreciable mortality of cells was not observed during 2 months posttransplant. In mammals a cicatrix did not form at the boundary of the transplant, and the drosophila cells did not proliferate. This is due to the fact that one day posttransplant all drosophila cells injected in the mammalian brain were differentiated. As in amphibians, donor cells stimulated vascularization of the transplant and of the surrounding structures of the recipient brain. The transplant exhibited the ability to form contacts with the recipient brain and to activate intercalation of the processes of mammalian nerve cells into the donor neuropil. However, we did not discover any nondifferentiated cells still capable of proliferating. Two months posttransplant viable drosophila cells were found in both young rats and adult mice.

Our findings lead to the conclusion that it is actually possible to integrate the nervous systems of vertebrate and invertebrate animals. However, the nerve cells of insects are more readily engrafted in tailless amphibians or mammals. In tailed amphibians histocompatibility with nervous tissue of insects is low. Evidently, after differentiation the cells of the transplant start producing peptides which cause dedifferentiation of brain cells and surrounding tissues in tailed amphibians. Changes in the structure of DNA in the nuclei of tailed amphibian neuroblasts suggest that the metabolic products of drosophila nerve cells directly act upon the genetic apparatus of the recipient cells.

In tailless amphibians and mammals a xenograft did not cause pathological changes in development. Morphological and functional contacts which were established between drosophila neurons and the brain of tailless amphibians affected the tempo of development and the behavior of recipients. Probably, integration of the nervous systems of animals of different classes can be used as a technique of predictable action upon the brain. This conclusion is based on several findings.

First, nerve cells of insects can "take" in the brain of vertebrates. Second, morphological and functional contacts are established between transplant and recipient. Third, drosophila cells preserve their viability for a rather long time, which shows promise of their long-term effect on the recipient brain.

However, the cells of the drosophila neural primordium have another advantage which makes remote xenotransplants a most attractive method of directed action upon the brain. This is the fact that the *Drosophila* genome is well studied, and there is a possibility of obtaining mutant strains of insects with nerve cells exhibiting the necessary type of metabolism, which can be used in medical practice.

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