IMPLANTATION OF EMBRYONIC PANCREAS IN THE ANTERIOR CHAMBER OF THE EYE IN NORMAL AND DIABETIC RATS

V. P. Zharkov, V. N. Yarygin, and L. L. Gol'tsman

UDC 616.379-008.64-089:616.379-089:843-031:611.84

KEY WORDS: implantation, diabetes, islets of Langerhans, anterior chamber of the eye.

Progress in the treatment of experimental and clinical diabetes mellitus is linked with transplantation of the pancreas on a vascular pedicle or free grafting of fragments of the fetal gland, of separate islets, or of dissociated and precultured β-cells [1, 5]. The last method is preferred because implantation of cultures consisting of β-cells enables their long survival in the recipient [4, 6]. However, the method of culture is expensive and unsuitable for widespread introduction; the main difficulty consists of the collection and preservation of a large number of cells for transplantation. Deficiency of β-cells evidently explains why complete insulin independence of patients is not found after successful implantation of endocrine cells. The cardinal problems have not been solved: tissue incompatibility between graft and recipient, choice of the optimal site for transplantation. In this connection reports have been published of successful implantation of pancreatic endocrine tissue into the cerebral ventricles and the anterior chamber of the eye (ACE), regions considered to be protected against control by the immune system [2, 7-9].

This paper gives the results of allografting of fragments of embryonic pancreas into ACE of normal rats and rats with alloxan diabetes.

EXPERIMENTAL METHOD

Experiments were carried out on 49 mature Wistar rats of both sexes weighing 200-250 g. The animals were divided into three groups: 1) 22 normal rats into whose ACE fragments of embryonic pancreas were implanted; 2) five rats with untreated alloxan diabetes; 3) 22 rats into whose ACE fragments of embryonic pancreas were transplanted 2 weeks after induction of diabetes. Experimental diabetes was induced by subcutaneous injection of a 5% aqueous solution of alloxan (Chemapol, Czechoslovakia) in a dose of 200 mg/kg. The blood glucose concentration was determined by the orthotoluidine method. Animals whose blood sugar level on the eve of the operation exceeded 300 mg % were used as recipients. Material was obtained from the donor and implanted in the recipient's ACE by a technique based on the method described previously [2]. The pancreas was removed from 17-18-day rat embryos of the same strain. Under a stereoscopic microscope the pancreas was removed from the embryos, cut into pieces measuring 0.3-0.5 mm^3, and immersed in Eagle's medium. After anesthesia of the eye and under a stereoscopic microscope, the cornea of normal rats and rats with alloxan diabetes was opened. Using an insulin syringe with micrometric delivery, on which a glass cannula (internal diameter 0.7-0.9 mm) was fitted, an implant with a volume of 2.5-3 mm^3 was introduced through the corneal incision into ACE. A 1% solution of atropine and a 20% solution of albucid were instilled into the conjunctival sac. No immunosuppressors were used. Every day the volume of water consumed was measured, and once a week the animals were weighed and the glucose concentration in their urine determined. The experiments continued for 30 days in group 1 and 30 and 45 days in group 3, after which the eye together with the implant was enucleated. Material was fixed in Ruge's solution and embedded in paraffin wax. Histological sections 8 μ thick were stained with hematoxylin-eosin and with aldehyde-fuchsine by Gomori's method in the writers' modification to determine the content of specific granules in the β-cells [3].

Department of Histology, Kursk Medical Institute. Department of Biology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 216-219, February, 1988. Original article submitted April 3, 1987.

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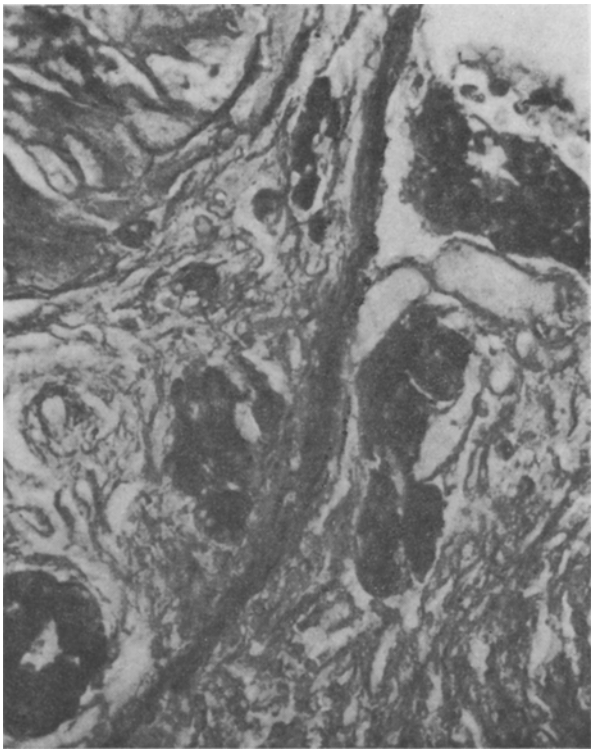

Fig. 1. Endocrine tissue in implant one month after transplantation into ACE of rats with alloxan diabetes. Islets of Langerhans consist of β-cells. Here and in Figs. 2 and 3: aldehyde-fuchsine. 280 ×.

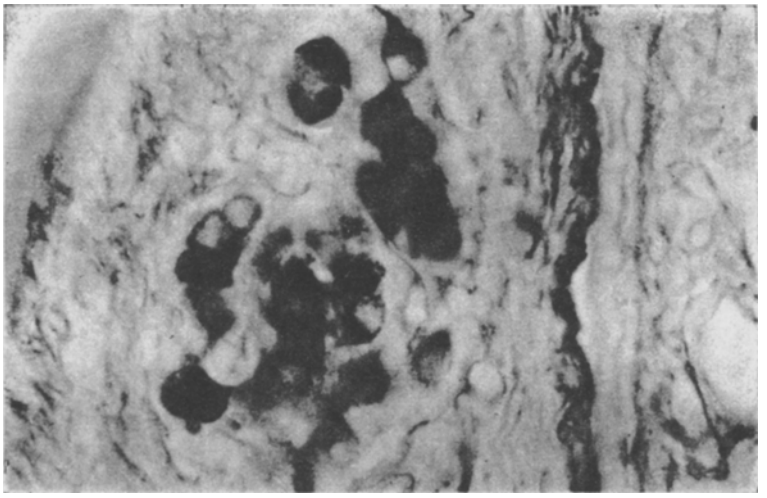

Fig. 2. IL in implant one month after transplantation into ACE of normal rat. β-Cells at different stages of secretory cycle. 630 ×

EXPERIMENTAL RESULTS

On the 3rd day after injection of alloxan the animals of groups 2 and 3 developed signs of diabetes: polyurea, polydipsia, polyphagy, hyperglycemia, and glucosuria. Rats of group 2, into which fragments of the pancreas were not transplanted, died from cachexia with signs of diabetic coma within the next 6 weeks. The blood glucose level in the rats of group 1 remained within normal limits (78-118 mg %) until they were removed from the experiment. Implantation was ineffective in three of the 22 animals of group 3. Their body weight continued to decline, their daily water consumption was 93 ± 6.4 ml, and throughout the

Fig. 3. Implant in ACE of rat with alloxan diabetes, 45 days after transplantation. Solitary islet cells scattered in the stroma. 280 ×.

period of observation (until 1 month) their glucosuria amounted to 2.5 ± 0.5%. In the remaining 19 rats of this group, by the end of the 1st week after implantation the hyperglycemia and glucosuria had started to diminish. During the 2nd and 3rd weeks the blood glucose concentration of seven animals fell to 135-145 mg%. The blood glucose level of 12 rats was 160-200 mg %, and the urinary glucose 0.5-1.0%. Enucleation invariably led to elevation of the blood and urinary glucose levels in all 19 rats, and also to return of the clinical manifestations of chronic diabetes. However, the survival period of these rats was longer than that of the rats of group 2.

The small implants in the rats of groups 1 and 3 were located peripherally in ACE. Blood vessels from the iris and ciliary body grew into the implant and broke up into capillaries of sinusoidal type. In 14 of the 22 rats of group 1 and in eight of the 11 animals of group 3, 1 one month after transplantation endocrine tissue formed by islets of Langerhans (IL) and by tubular epithelial glandular structures and single β-cells could be identified. The IL were round or irregular in shape, they varied in size, and were not surrounded by a clearly defined capsule (Fig. 1). The number of cells in IL varied within wide limits, from 3-5 to 84-97, and the principal cellular forms of IL were insulin-producing cells. These cells were oval or elongated in shape and were in close contact with the capillaries. On staining with aldehydefuchsine, specific granules were revealed in the cytoplasm of the β-cells. The number of granules varied in endocrine cells in the animals of group 3, evidence of desynchronization of the secretory cycle (Fig. 2).

The endocrine tissue was still present in ACE of seven of the 11 rats on the 45th day after transplantation. Besides organotypical structures (IL), tubular glandular formations were identified in the implants, in which individual β-cells had differentiated and contained Gomori-positive granules in their cytoplasm. Many typical islet cells were scattered also in the stroma of the graft (Fig. 3). This was formed by loose connective tissue, and besides fibers it also contained fibroblasts, macrophages, and neutrophilic granulocytes and lymphocytes.

A cellular response was observed in all cases. Solitary lymphocytes and neutrophils were found in implants with differentiated and vascularized IL. They were not concentrated around the IL, nor did they make contact with endocrine cells. In some cases allogeneic

tissue had been absorbed and replaced by connective tissue. Many IL were at different stages of destruction and only individual β-cells remained functionally active. The stroma of the graft consisted predominantly of lymphocytes, macrophages, and fibroblasts.

These investigations indicate the possibility of survival, development, differentiation, and function of an embryonic endocrine pancreas in ACE of adult control and diabetic rats. In some animals with experimental diabetes, complete or partial compensation of the diabetes was observed during the period of observation. Histological investigation of ACE of these animals revealed morphologically mature and functionally active endocrine β-cells. Despite the obvious specific difficulties, implantation into ACE is free from one fundamental difficulty, namely the problem of rejection of foreign tissue to the degree to which this occurs with implantation into other regions.

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