MORPHOLOGY AND PATHOMORPHOLOGY

β-CELL MIGRATION FROM FETAL CALF PSEUDOISLETS IN VITRO

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UDC 616.379-018.1-039-092.4-07

KEY WORDS: pancreas; islet cell culture; insulin; cell migration.

Cultures of fetal calf pancreatic islet cells have attracted attention as an alternative source of β-cells for transplantation into type I diabetics [1, 2, 5]. Microfragments resembling islets of Langerhans in adult mammals, or so-called pseudoislets, which are rich in β-cells and which secrete insulin, can be obtained by the method described by the writers previously [3]. The aim of this investigation was to study the morphological and functional changes in the pseudoislets during culture.

EXPERIMENTAL METHOD

The pancreas from 12 calf fetuses with crown-rump length of 27-35 cm was used. The pancreas was removed subtotally under aseptic conditions, transferred into a Petri dish, and 5-6 ml of Hanks' solution containing collagenase (0.3 U/ml, from "Serva," Germany) was injected into the parenchyma. The pancreas was then cut into four or five parts and incubated in a flask on a water bath (37°C) for 40 min. Digestion was stopped by the addition of cold Hanks' solution, and the contents of the flask were vigorously shaken and filtered through a sieve with mesh of 0.6 mm, after which the microfragments were washed by centrifugation (100*g*, 15 sec). Tissue obtained from one pancreas was resuspended in 40 ml of MEM medium containing a double set of amino acids and vitamins, 10% fetal calf serum, 2 mM L-glutamine, 20 mM HEPES, and 80 μg/ml gentamicin. The suspension was introduced 2 ml at a time into wells of a 24-well planchet (3524, from "Costar," USA) and cultured in a humid atmosphere containing 5% CO_2 [3]. The pseudoislets were fixed in Bouin's fluid and embedded in paraffin wax after culture for 18, 42, 66, and 140 h. Sections 5-7 μ thick were stained with hematoxylin and with Gomori's aldehyde-fuchsine. Insulin-containing cells on the bottom of the wells were detected by the indirect immunofluorescence test [7], using monoclonal antibodies to insulin D4V8 (Research Institute of Genetics and Selection of Microorganisms, Moscow) and goat antibodies to mouse IgG, labeled with FITC ("Ortho Diagnostic Systems," USA). After culture for 18, 66, and 140 h the floating pseudoislets from two wells were collected, and transferred to a nitrocellulose filter (pore diameter 2.5 μ) into a chamber through which passed MEM medium, containing successively: 1) 5.5 mM glucose (MEM), 2) 16.5 mM glucose (MEM-G); 3) 16.5 mM glucose and 5 mM theophylline (MEM-GT); 4) 16.5 mM glucose, 5 mM theophylline, and 0.4 μM glucagon (MEM-GTG), at the rate of 1 ml/min. Samples of 0.25 ml were taken at the exit from the chamber every 3 min and frozen for subsequent determination of insulin by radioimmunoassay [4]. In the series of experiments on the 6th day the wells were washed with fresh medium and insulin secretion per hour was determined directly in the wells [6], which were filled initially with MEM and later with MEM-GTG.

EXPERIMENTAL RESULTS

After culture for 18 h the pseudoislets were compact formations, most of which was composed of polygonal cells with translucent homogeneous cytoplasm and round nuclei. Aldehyde-fuchsine-positive β-cells were numerous

Research Institute of Transplantology and Artificial Organs, Ministry of Health of Russia, Moscow. (Presented by Academician of the Russian Academy of Medical Sciences A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 658-660, June, 1992. Original article submitted October 30, 1991.

 0007-4888/92/0006-0886$12.50

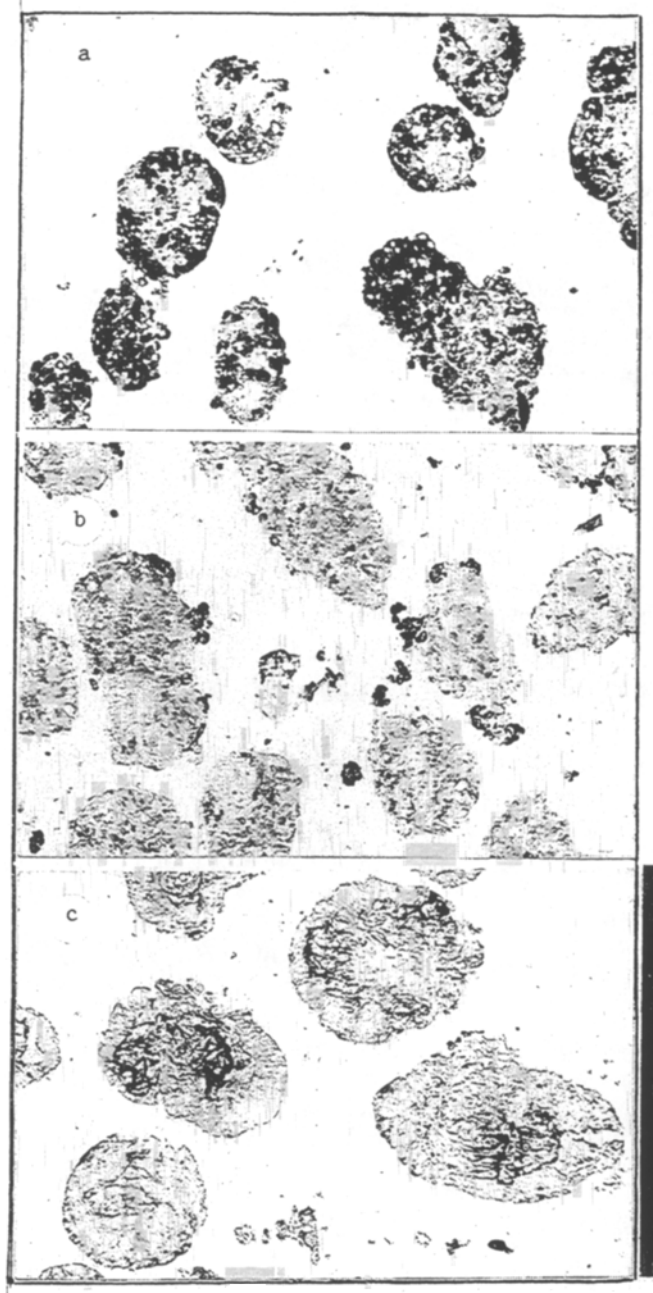

Fig. 1

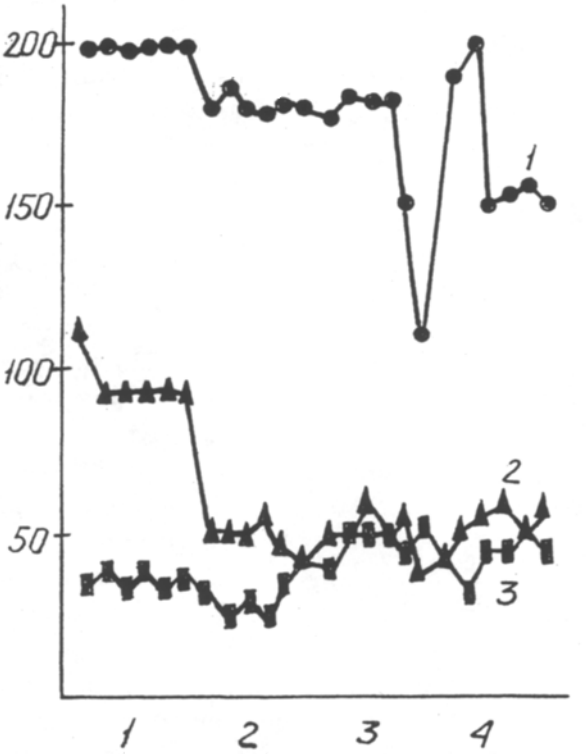

Fig. 2

Fig. 3

Fig. 1. Changes in structure of pseudoislets during culture: a) after 18 h, b) 42 h, c) 140 h (intensively stained elastic fibers), Aldehyde-fuchsine, 130×.

Fig. 2. Insulin secretion by pseudoislets in perfusion system. Abscissa: 1) in MEM, 2) in MEM-G, 3) in MEM-GT, 4) in MEM-GTG. Ordinate, rate of secretion (in μU/min); after 18 h (1), 66 h (2), and 140 h (3) of culture Representative experiment.

Fig. 3. Insulin-containing cells on bottom of well. Indirect immunofluorescence test, 1000×.

and were distributed relatively uniformly throughout the thickness of the pseudoislets (Fig. 1a). After culture for 42 h the morphological picture changed suddenly. The pseudoislets became uneven in outline, due to the appearance of projections and outgrowths on their surface, formed by concentrations of small epithelial cells. Staining with aldehyde-fuchsine revealed significant redistribution of the β-cells in the pseudoislets As a rule a few or single β-cells could be seen in the central part, but most of them were found at the periphery of the pseudoislets, in the form of projections or bands running in a centrifugal direction (Fig. 1b). After 66 h only single β-cells could still be found in the pseudoislets, and after 140 h they could no longer be seen. The central zone of the pseudoislets in this case had signs of cellular degeneration, whereas the periphery was formed by cylindrical cells, resembling the epithelium of ducts (Fig. 1c).

The study of the insulin-producing activity of the pseudoislets in a continuous flow system showed that they did not respond by increased secretion of insulin to high glucose concentrations (MEM-G), even in combination with theophylline (MEM-GT), but additional loading with glucagon (MEM-GTG) on the 1st day of culture led to a small increase of secretion (Fig. 2). On the 3rd and 6th days a considerable lowering of the level of secretion and disappearance of the reaction to MEM-GTG were observed, and coincided with disappearance of aldehyde-fuchsine-positive cells from the pseudoislets, detected morphologically.

On stimulation of the cultures in the wells on the 8th day (140 h of culture) basal insulin secretion in MEM amounted to 18.3 ± 3.2 μU/well h, and secretion stimulated by MEM-GTG amounted to 33.1 ± 4.8 μU/well·h ($n = 4$, $p < 0.05$). This suggested that disappearance of the β-cells, first in the center and later at the periphery of the pseudoislets, is not simply death or dedifferentiation, and that the cells responding to loading with MEM-GTG migrate from the floating microfilaments to the bottom of the well.

Immunofluorescence investigation of cells fixed on the bottom revealed many cells containing insulin, forming concentrations of different shapes in some places (Fig. 3). We concluded that we were observing a spontaneous process of differential migration of β-cells from the floating pseudoislets and their subsequent reassociation on the bottom of the wells; the insulin-producing activity of the pseudoislets themselves was significantly depressed.

These results must be taken into account when microfragments of the fetal calf pancreas are used for transplantation. The possibility cannot be ruled out that many precursors of β-cells persist for a longer time in the pseudoislets. Nevertheless, to prevent otherwise unavoidable losses of β-cells due to their migration, there would seem to be a stronger case for using roller cultures.

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