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IMMUNOLOGIC TYPING OF SPLENIC LYMPHOCYTES OF INTRAUTERINE HUMAN FETUSES USED AS DONORS OF PANCREATIC ISLET CELLS

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Free grafting of pancreatic islet tissue has been used in recent years for the treatment of patients with diabetes. A suspension of freshly isolated pancreatic tissue from cadavers of adults, children, and stillborn infants, and also pancreatic islet cells (PLC) from human fetuses, cultured beforehand, are used for this purpose [5, 6, 7]. This problem is under active investigation in the Soviet Union [2-5]. A matter of great importance for its further successful development is that of immunologic typing of fetal donors for the selection of PIC cultures antigenetically most compatible with the recipient.

The aim of the investigation described below was to study this problem.

EXPERIMENTAL METHOD

To determine the HLC phenotype of a potential donor of PIC, splenic lymphocytes of 52 human fetuses at the 18th-25th week of intrauterine development were used (spontaneous abortions, termination of pregnancy on medical grounds). Lymphocytes were isolated from splenic pulp by centrifugation in a Ficoll-Verografin density gradient. HLA antigens were detected by the two-stage NIH method, using a kit of anti-HLA-sera. Antigens of the A series: A1, A2, A3, A9, A10, A11, A19, A23, A24, A25, A26, A28, A29, A32; and antigens of the B series: B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, B21, B22, B27, B35, B40, were determined. The time from removal of the spleen from the fetal cadaver to the beginning of typing was 3-8 h.

Cultures of human fetal PIC were obtained by the method described previously [1]. Growth of the culture was evaluated 3-8 days after seeding of the cells in 100-ml flasks, on the basis of cytophysiological data (morphologic study of the cultures, determination of the immunoreactive insulin concentration in the culture medium). In 7 cases the results of immunologic typing were compared with those of cytophysiological investigation of PIC cultures.

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TABLE 1. Viability of Splenic Lymphocytes from Human Fetuses Used as Donors of PIC Cultures

Number of specimens of lymphocytes studied	Viability of lymphocytes, %
6	До 10
2	11—20
2	21—50
3	51—95
39	96—100

TABLE 2. Detection of HLA Antigens on Splenic Lymphocytes of Human Fetuses Used as Donors of PIC Cultures

Number of antigens discovered	Number of specimens of lymphocytes studied	
	absolute	%
4	26	66
3	9	24
2	4	10
0	0	0

EXPERIMENTAL RESULTS

The HLC phenotype was definitely established in 39 of the 52 cases. In 13 cases HLA-A and HLA-B histocompatibility antigens could not be detected because of the poor viability of the isolated lymphocytes, determined by supravital staining with 0.3% trypan blue solution. In six of these cases the proportion of viable cells did not exceed 10% (Table 1). The investigations showed that a complete set (full house) of antigens in series A and B could be established in 66% of cases, three antigens were discovered in 24% of cases, and two antigens in 10% of cases (Table 2).

Histocompatibility antigens were distributed with a frequency corresponding to that of the distribution of antigens among the adult population of Moscow. For instance, antigens A2 (47.5%) and A1 (22.5%) were found most often, antigens B14 (5%) and B16 (5%) infrequently. A tendency was observed for the frequency of discovery of antigens A3 (17.5%) and B12 (10%) to fall, possibly due to inadequacy of the sample studied.

The next step was to look for correlation between the abundance of HLA antigens in the fetal donors and the cytophysiological characteristics of the PIC cultures. Five PIC cultures with a marked HLA phenotype were studied. All these cultures were formed from the structural point of view in the course of 72 h. Two fractions could be distinguished in them: settling and floating. The settling fraction consisted of small foci of epithelial cells, adherent to the bottom of the flask. Later, a circular monolayer zone of growth formed around these foci of attachment, and it was shown by light and electron microscopy to consist mainly of β-cells of the islets of Langerhans, in different stages of the secretory process. The second, larger part of the culture consisted of cell complexes floating in the medium. Among the floating cultures, in turn, cytotypical cultures and cultures of organoid character were distinguished. The cytotypical floating cultures were compact spherical complexes measuring 0.1-0.3 mm, consisting entirely of epithelial cells, most of which were β-cells. The organoid floating cultures were regularly spherical or ovoid in shape and 0.1-0.7 mm in diameter. Their surface was covered with a thin layer of fusiform fibroblasts, the "stroma" consisted of collagen, lysed in the course of enzymatic treatment, and remnants of destroyed exocrine pancreatic cells, whereas the "parenchyma" consisted of compact groups of islet cells of different sizes, predominantly β-cells. All these cultures actively secreted insulin into the growth medium; its concentration reached 12,000-15,000 μU/ml by the 5th-8th day of culture.

After seeding of a suspension obtained from the pancreas of two fetuses with undetectable HLA antigens, adhesion of the cells to the bottom of the flask did not take place and, consequently, the settling fraction of the culture in these cases was completely absent. In addition, the overwhelming majority of microfragments obtained during micing and enzymatic treatment of the pancreas of these fetuses did not become regularly spherical in shape and their

surface remained uneven ("shaggy") and was not covered by a layer of fibroblasts. Only a very small proportion of microfragments floating in the growth medium changed into organoid floating cultures as described above. By the 5th-8th day of culture very small quantities of insulin could be detected in the growth medium (not more than 200 μU/ml).

The investigation thus showed that HLA typing of splenocytes from fetuses is possible in principle starting from the 18th week of intrauterine development.

These first observations are evidence of a possible parallel between the formation of the antigenic structure of the splenic lymphocytes of fetal donors, detectable by typing, and the cytophysiological characteristics of the cultures obtained from the pancreas of these fetuses and, in particular, with their insulin-producing activity. If this hypothesis is confirmed by future investigations on a more extensive material, the results of HLA typing and parameters of viability of the splenic lymphocytes of the donors (human fetuses) can possibly serve as a unique prognostic sign for use when determining the morphological and physiological value of an islet cell culture obtained from the donor's pancreas.

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