Neo-Islets Obtained from Rat Pancreas Do Not Contain Passenger Leukocytes

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UDC 616.379−018.1:576.086.83.089.67

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 5, pp. 502−504, May, 1993
Original article submitted January 26, 1993

Key Words: *allotransplantation; neo-islets; passenger; leukocytes*

The rejection of pancreatic islets (PI) is the main obstacle to their successful transplantation in the treatment of type I diabetes mellitus. The rejection is thought to be determined by the PI-residing leukocytes (so-called passenger leukocytes) bearing class II antigens of the major histocompatibility complex (MHC) [6].

Several methods have been described for decreasing the immunogenicity of the islets prior to transplantation: culture in an atmosphere of 95% O_2 [5], in acidified Hanks solution [7], or at an increased temperature [9], γ-irradiation [3], treatment with antibodies to class II antigens plus complement [2], and cell sorting [12].

This paper presents data concerning the cellular composition of neo-islets formed as a result of the reaggregation of the islet cells cultures under stationary conditions [1,11]. We show here that the neo-islets contain no passenger leukocytes.

MATERIALS AND METHODS

The pancreases were obtained from male Wistar rats weighing 180-220 g. The islets were isolated by intraductal administration of collagenase (Serva) in Hanks solution (0.3 U per ml), followed by digestion without stirring (37°C, 40 min) and stepwise density gradient centrifugation in Ficoll-Paque (Pharmacia) at 400 g for 5 min. The islets were washed by centrifugation in Ca^{2+}, Mg^{2+}-free Hanks solution (HS) and consecutively incubated in 0.04% EDTA in HS for 5 min at 20°C and in 4 ml of 0.25% trypsin solution in HS for 15 min at 37°C. Then 50 μl of DNAase (Serva) solution (1 mg per ml) were added, and the mixture was carefully pipetted with a siliconized Pasteur pipette until a single-cell suspension was obtained. The action of trypsin was stopped by adding 6 ml of cold Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum, 20 mM HEPES, 2 mM L-glutamine, and 80 mg per liter gentamycin. The cells were washed three times in MEM and counted. 10^4 cells in 0.1 ml MEM were placed in the wells of a 96-well tissue culture plate (Costar 3596) and cultured for 7 days in a humidified atmosphere with 5% CO_2 at 37°C. On the 1st, 3rd, 5th, and 7th day the contents of the wells were harvested, washed three times by brief centrifugation (100 g, 10 sec) and stained using the immunofluorescence technique.

Mouse monoclonal antibodies (MAb) OX1, OX6, and OX21 recognizing rat common leukocyte antigen, monomorphous determinant of class II MHC antigens, and human C3B inactivator, respectively, were provided by Dr. J. Austin (John Radcliffe Hospital, Oxford). MAb OX21 served as a control of nonspecific binding. Affinity-purified MAb D4B8 recognizing rat insulin (provided by Dr. V. Yurin, Institute of Genetics and Selection of Microbes, Moscow) diluted 1:200 were used for detection of β-cells, Cells were treated first with MAb at room temperature for 90 min, with subsequent washing, and then with affinity-purified FITC-conjugated goat polyclonal

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0007-4888/93/0005-0549$12.50

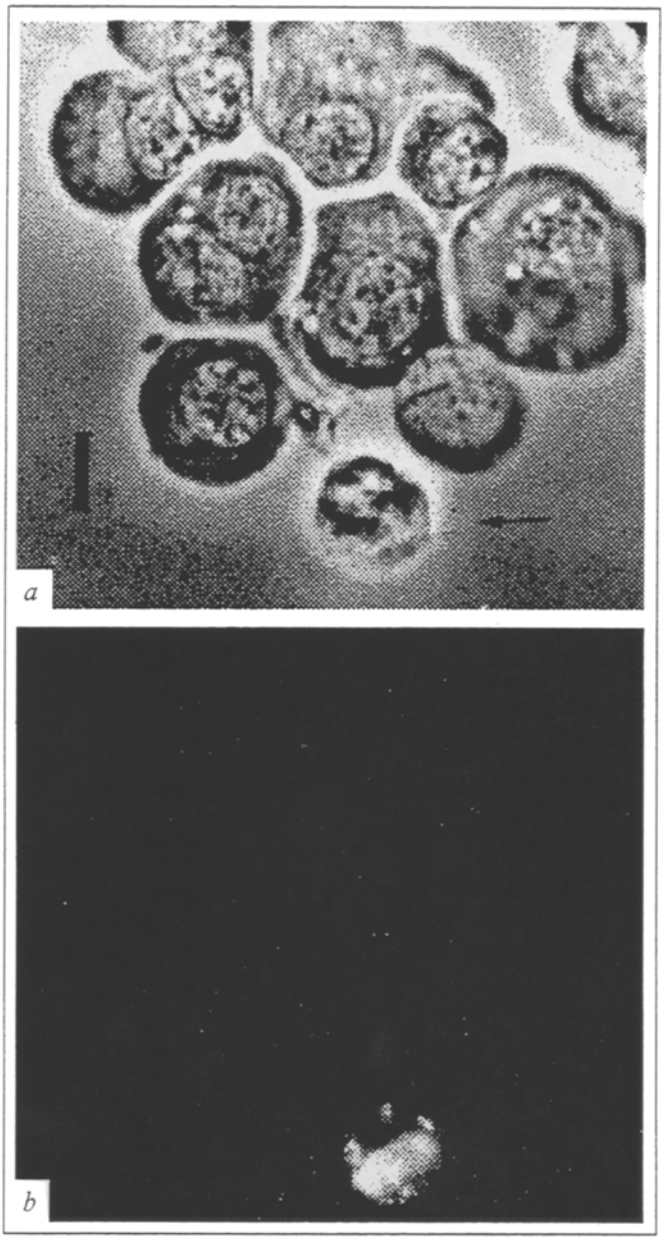

Fig. 1. Phase–contrast (*a*) and fluorescent (*b*) microscope analysis of initial suspension of islet cells reacting with MAb OX1. Arrow points to the cell bearing the common leukocyte antigen. Here and in Fig. 2: magnification 950, scale 10 μ.

antibodies to mouse IgG diluted 1:20 for 60 min. Preparations were made using cytorotor, fixed with methanol, and embedded in glycerol. Rat blood mononuclear cells were used as a positive control of the MAb OX6 reaction. In the course of the immunofluorescent study of established neo-islets as well as before the staining of insulin, cell aggregates and suspension were cytocentrifuged and fixed with 2% formalin in permeabilizing isotonic solution and then treated with MAb. The preparations were studied using phase-contrast and fluorescent microscopy. Insulin secretion was determined on the third day of culture. The cells in 8 wells were washed 5 times with medium, after which fresh MEM supplemented with either 5.5 mM glucose or 16.5 mM glucose and 5 mM theophylline was added. The aliquots of medium were aspirated before and after a one-hour incubation for the subsequent determination of the basal and stimulated insulin secretion, respectively, in radioimmunoassay.

RESULTS

The mean number of islets obtained from one rat was 642±149 ($M\pm m$, n=8), The contamination with exocrine tissue was negligible. The number of cells per islets was $(11.0\pm0.33)\times10^5$ (n=20). Cell viability exceeded 90%. The initial suspension of islet cells

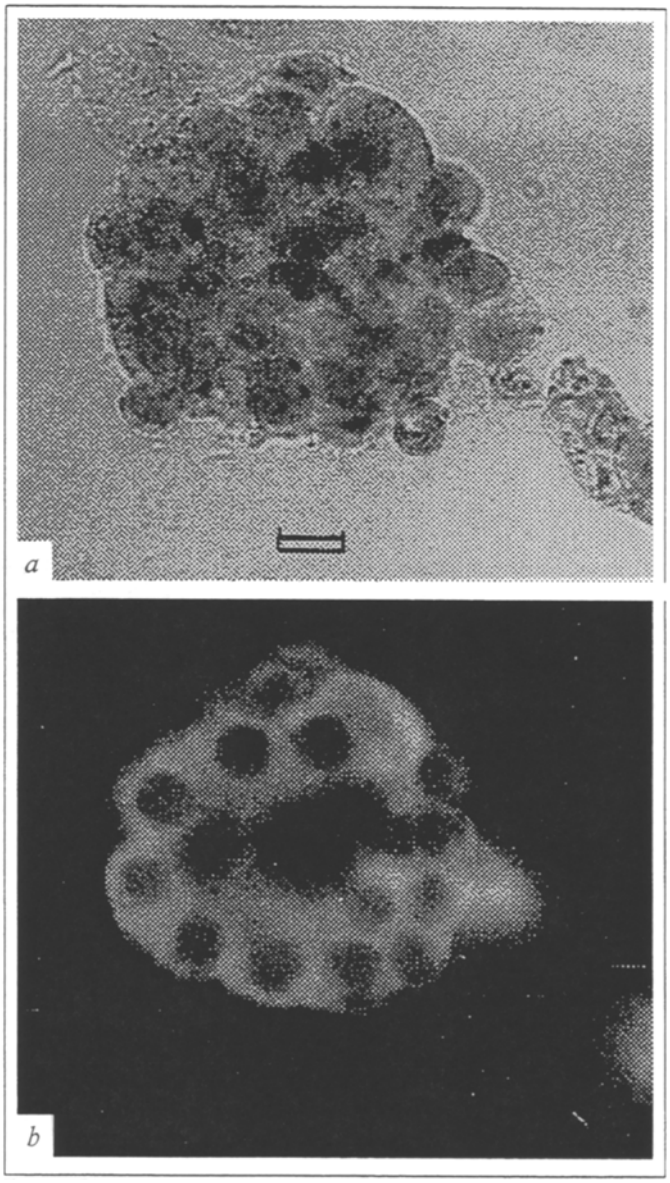

Fig. 2. Phase–contrast (*a*) and fluorescence (*b*) microphoto of a neo–islet reacting with antibodies to insulin. Most of the cells in the neo–islet are β–cells.

includes 4.1±1.1% OX1+ cells (Fig. 1), 3.2±1.3% OX6+ cells, and 59.2±4.2% insulin-positive cells (n=20). We did not observe any nonspecific fluorescence in the control experiments using MAb OX21.

In the stationary culture the endocrine cells aggregated spontaneously, forming clusters - neo-islets. The first signs of reaggregation appeared after 6-8 hours of culture, when the cells formed couples and triplets. This process continued up to the fifth day, when the aggregates acquired a round form and resembled the initial islets, though differing from them in their size (50-100 μ); each well contained 10-15 neo-islets. Beginning from the 7th day no further growth of aggregates was observed; the aggregates adhered to the bottom of the well, thereby complicating their harvest. However, on the 5th day the neo-islets could be easily harvested from the wells; in addition, the majority of fibroblasts and some macrophages remained attached to the bottom, while non-attached single cells could be removed by washing in brief centrifugation.

The total staining revealed that the aggregates consisted largely of β-cells (Fig. 2) and contained neither OX1+ nor OX6+ cells. A single-cell suspension prepared by treatment of neo-islets with trypsin was free of OX1+ and OX6+ cells in 11 out of 12 cases. At the same time, this suspension consisted 69.4±4.4% of insulin-positive cells (n=12).

The study of insulin secretion revealed the functional integrity of the neo-islets. In the presence of MEM supplemented with 5.5 mM glucose they secreted 96.6±7.0 pm insulin per well in one hour, while in response to the glucose + theophylline load the output of insulin rose to 270.7±16.6 pm per well in one hour (n=6, p<0.001).

The reaggregation of pancreatic islet cells in tissue culture was first described by Takaki [11]. Later, Shizuru and Kakizaki [10] showed that the islet cells link anew in the rotating cultures. The aim of our work was to study whether the phenomenon of islet cell reaggregation in stationary cultures can be used as a method of eliminating passenger leukocytes.

The data presented show that in the course of neo-islet formation in stationary cultures the leukocytes are not incorporated in the islet structure and can be easily withdrawn by brief centrifugation. The procedure of washing seems to be a significant step, as the leukocytes (at least up to the 5th day) remain viable in the culture and some of them are loosely attached to the surface of the neo-islets.

Due to their small size, neo-islets have an advantage over the native islets in the sense of the supply with nutritive substances in the absence of a capillary network, and are preferable for intravessel transplantation, e.g., via the portal vein. The neo-islets are more efficient producers of insulin than the single-cell suspension, as the secretory activity of β-cells depends on the intercellular junctions [8].

Thus, the neo-islets present a variant of functioning β-cells meeting the requirements for allografts: low immunogenicity and high functional activity.

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