Intracerebral xenotransplantation of semipermeable membrane-encapsulated pancreatic islets

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INTRODUCTION

Studies have shown that porcine ICCs xenotransplanted into rat’s brain could survive for only eighteen days\cite{1,2}, suggesting that the immuno-privileged function of brain cannot ensure the long-time survival of heterogeneous islets. Immunity-isolation is an effective way to lessen the immunological rejection damage\cite{3,4}. Better effect can be achieved through the combination of different immunity-isolation techniques\cite{5}. Rat’s pancreatic islet is easy to be separated, transgenic rats are also developed, and the cell line secreting insulin has been established\cite{6}. However, the exact effects of apoptosis on the death of xenotransplanted islets cannot be shown\cite{7-9}. In this study, BSM-encapsulated SD rat’s ICCs were xenotransplanted into a normal dog’s brain to identify their decreasing effect on rejection and death of implanted islets.

MATERIALS AND METHODS

Separation, purification and culture of rat ICCs\cite{6}

SD rats, weighing 50-200 g were used in this study. Pancreas and its amiculas and vessels were washed twice in Hank’s solution and then minced. The fragments were digested with collagenase V (1 g/L, Sigma) at 37 °C for 15-20 min, ground and filtrated through an 80-mesh steal net to remove connective tissue, washed in cold Hank’s solution to stop the activity of collagenase. Then the filtrates were filtrated with a 400-mesh steal net to remove the small exocrine cells, red and white blood cells with the bigger ICCs kept on the net. The cell clusters on the net were collected and put into the Ficoll fluid (Euro-Collin) at the concentrations of 25 g/L, 23 g/L, 20 g/L and 11 g/L. The mixed liquors were centrifuged at 3 500 r/min at 4 °C for 15 min. ICCs were washed three times. The purity of ICCs obtained in this way was (88.2±7.6) %. These ICCs were cultured in non-insulin DMEM supplemented with 10% fetal bovine serum, 2 mmol/L glutamine, 10 mmol/L nicotinamide, 5 mmol/L hydroxyethyl methylmethane sulfonic acid, 100 000 U/L penicillin and 1 g/L streptomycin, and maintained at 37 °C in a high-humidity incubator with 50 mL/L CO$_2$. A total of 400-1 000 purified ICCs were obtained from each rat. These ICCs were stained with trypan blue and the activity was 90%.

Lymphocytes diffusion

Five semipermeable membrane encysts were filled with 1 mL of fresh blood plasma, put into 200 mL of blood and maintained at 37 °C in a high-humidity incubator with 50 mL/L CO$_2$ and kept agitated with magnetic force. Fluid
in encysts was suctioned respectively 5 h later, then smeared and observed under a light microscope by Wright’s staining.

**Glucose diffusion**

Eight semipermeable membrane encysts were filled with 1 mL of isotonic sodium chloride, put into 400 mL of isotonic sodium chloride containing 29.1 mmol/L glucose and kept agitated with magnetic force. The encysts were taken out at 1, 4, 8, 12, 16, 20, 24 and 28 min, respectively, and washed with isotonic sodium chloride. The fluid in the encysts was obtained and the concentration of glucose was detected.

**In vitro culture of BSM-encapsulated ICCs**

About twelve thousands separated ICCs were divided into ten parts, five were encapsulated by BSM, five were routinely cultured. The culture medium was changed every three days. The concentration of insulin in the medium was detected by aseptic BSM. The volume of encysts was 1.5 cm×1.5 cm×1.5 cm. Each of them contained 6 000 to 10 000 ICCs. After ICCs were cultured for 72 h, they were encapsulated by BSM. The recipients were normal male local hybrid dogs weighing 7-14 kg. The dogs were intravenously anesthetized after anesthesia. Penicillin and streptomycin were used. The first antibody was mouse anti-human insulin used at 1:100 dilution and stained according to the avidin-biotin compound method (ABC), and then counterstained with hematoxylin to identify B cells in the grafts. The DNA fragmentation of transplanted B cells was identified by insulin-TUNEL double staining.

**RESULTS**

**In vitro experiments of BSM**

The BSM was not damaged during the whole process of the experiment *in vitro*. In lymphocyte-diffusion experiment, lymphocytes and any other blood cells were not immersed into the semipermeable membrane capsule, but glucose could rapidly diffuse into the capsule. The concentration of glucose in the capsule was 14.3 mmol/L after 8 min, which was 50% of the concentration out of the capsule. After 28 min. The concentration of glucose in the capsule reached the same environmental level. The concentration of insulin on d 3, 6 and 15 was (139.0±3.4) mU/L, (78.7±3.9) mU/L and (66.2±2.9) mU/L, respectively in the experimental group, and (145.4±4.2) mU/L, (79.4±2.1) mU/L and (48.2±4.1) mU/L, respectively in the control group. There was no conspicuous variability (*P*>0.05) between the two groups at various time points. Insulin secreted from pancreatic islet cells could diffuse rapidly out of the capsule.

**ICCs xenotransplanted into dog’s brain**

The dogs of both groups had no conspicuous behavior disorder. No dog in the experimental group was infected. The graft was deep gray and homogenous in the experimental group. It was connected with the ventricle at the inner side and adhered to the thickening dura mater at the lateral side as well as in contact with the cerebral parenchyma. The boundary was clear. Brain tissue around the graft had no obvious hemorrhage, necrosis and inflammatory abscess. Only residual semipermeable membrane was observed in the control dogs.

Under light microscope, the graft was consisted of epithelial cells and loose connective tissue, including epithelial cells, fibroblasts, collagen fiber, and small vessels. In the middle of them, there were lymphocytes and other kinds of white blood cells. Epithelial cells mostly adhered to the fibroblasts, collagen fiber or small vessels, and were arranged loosely in the connective tissue along the collagen fibers. The structure of small vessels was normal, while that of some parts of the semipermeable membrane was different, but there was no clump-like infiltration of lymphocytes.
Slight hyperplasia and hydropsia of glial cells were observed in the brain tissue around the graft, but severe infiltration of inflammatory cells was not observed.

Under electron microscope, secreting granulae were observed in the endochylema. The granulae were not only different in size but also different from those of pancreatic islet cells. Intact and contracted caryons, aggregated chromosome and typical apoptotic bodies were found in some cells (Figure 1).

No xenotransplanted cell disappeared in the control group. Cytoplasms of many epithelial cells in the grafts of EG were stained yellow, indicating that they were islet B cells (Figure 2). The implanted B cells showed typical apoptosis by insulin-TUNEL double staining.

These facts suggest that in vivo xenotransplantation of BSM in combination with the privileged site can inhibit the rejection of implanted heterogeneous ICCs.

In the present study, cells displayed different morphological features in different environments to adapt themselves to the microenvironment. The culture medium was gradually absorbed after grafting and replaced by loose connective tissue until solidification. The appearance of connective tissue was a kind of compensatory reaction, giving support and nutrition to islet cells and keeping them stable. Islet cells were arranged loosely in the connective tissue along the collagen fibers, while the in situ compact cell clumps were observed under the pressure of exocrine division.

It is easier to transplant pancreatic islets when they are capsuled and to dislodge them when the rejection is serious during the early period of transplantation. This is very important when a clinical trial is carried out. According to the in vitro experiments and transplant experiments, the semi permeable membrane used in our study had a favorable permeability, some degree of rigidity, lighter foreign body reaction and toxicity.

It was reported that cerebrospinal fluid (CSF) can ensure islet survival with sufficient oxygen and nutrients[10]. We made a tunnel into the ventricle, which could connect capsules with CSF immediately. Accordingly, islets were supplied with nutrients in the earlier period, which is beneficial for their survival. However, how many islet nutrients can be obtained in this way remains unclear. We also found that the implanted B cells showed classic apoptosis by insulin-TUNEL double staining and electron microscopy. The death of implanted heterogeneous B cells is associated with apoptosis. Why apoptosis occurs and how to delay or block this apoptosis are the subjects to be studied[11-14].

Surgical procedures injure brain tissue and integrality of the blood-brain barrier. Wet compressing with dexamethasone on the injured brain can decrease the blood-brain barrier permeability and non-specific exudation of inflammatory cells[15].

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