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FK 506: An Effective Immunosuppressant for Islet Xenotransplantation

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REPLACING the damaged insulin-producing tissue by transplantation of pancreatic islets is considered the most physiological approach to diabetes treatment and has the potential to prevent the development of chronic complications. Due to the large number of potential diabetic recipients, it would be impossible to obtain sufficient supply of donor organs to fulfill the demands. One solution to resolve donor organ shortage is the use of xenogeneic pancreatic islets. FK 506, a new immunosuppressive agent, is very effective in rat islet allotransplantation across the major histocompatibility (MHC) barrier.[1,2] Use of this agent in islet xenotransplantation has not been studied extensively.

The present study was conducted to determine the efficacy of FK 506 on the survival of human islet xenograft in discordant rats.

MATERIALS AND METHODS

Human Islets

Human islets were isolated from a 12-year-old donor with the automated digestion–filtration methods as described earlier.[3] Islets were then purified with Euro-Collins Ficoll gradient in a Cobe 2991 cell separator. Purified islets were placed in culture medium and transported from Pittsburgh to Vancouver for xenotransplantation.

Transplantation and Immunosuppression

Male ACI rats (Harlan Sprague-Dawley, Indianapolis, Ind) of 200 to 225 g body weight were used as recipients. Human islets stained with dithizone were hand-picked under dissection microscope. A total of nine rats were transplanted with 500 human islets each under the left renal capsule. Five transplanted rats received FK 506 (Fujisawa Pharmaceutical Co, Osaka, Japan) treatment at 2 mg/kg/d intramuscularly for 2 weeks and then at weekly intervals until they were killed, starting immediately after transplantation. The remaining four rats received saline injection.

Immunohistochemical Study

Rat recipients of human islet xenografts treated with FK 506 were killed on days 16, 36, 74, 100, and 124 after transplantation. Equal halves of the kidney containing the graft were snap frozen in LN2 or fixed in Bouin's fixative. Paraffin sections were stained for insulin, glucagon, and somatostatin with immunoperoxidase staining (ABC staining kit, Vectastain, Dimension Laboratories, Mississauga, Ontario); frozen sections were stained for cellular markers with monoclonal antibodies against rat leukocyte common antigen, endothelial cell (OX43), class II MHC antigen, and CD4 and CD8 lymphocyte subsets (Cedarlane Laboratories, Hornby, Ontario).

Perfusion

The kidneys containing the islet graft for 36, 74, and 100 days were perfused in vitro to assess the function of the islets before being processed for immunohistochemistry. The animal was anesthetized with halothane. The abdominal cavity was opened and the renal artery cannulated with a PE60 catheter according to the method of Korsgren et al.[4] The cannulated kidney was removed and placed in a moist chamber at 37°C, the catheter connected to a peristaltic pump, and the organ perfused at 0.1 mL/min with 37°C M199 supplemented with 25 mmol/L HEPES and 0.4% bovine serum albumin and continuously gassed with 5% CO_2/95% O_2. The experiment started with 60 minutes of perfusion with a medium containing 100 mg/dL glucose followed by a 60-minute perfusion with medium containing 450 mg/dL glucose, and finally 60 minutes with medium containing 100 mg/dL glucose. The samples collected were stored at −20°C until insulin assay by radioimmunoassay (Pharmacia RIA kit, Pharmacia, Montreal, Quebec). The graft site was divided for histology as described earlier.

RESULTS

In nonimmunosuppressed rats, by day 5 after xenotransplantation nearly all the insulin-containing cells had disappeared and the bulk of the graft was replaced by fibrotic tissue. Some of the ductal elements were still detected and normal in appearance. CD4 and CD8 T-cell subsets had infiltrated the graft tissue.

The transplanted kidney with human islet graft removed from an FK 506–treated recipient 16 days after xenotransplantation contained human islets that appeared healthy and well vascularized by the host. Insulin-containing cells were present in abundance. In some areas occasional lymphoid cells were seen.

Figure 1 shows that the human islet xenograft still contained insulin after 124 days in the xenogeneic rat recipient. The general appearance of the human islets was similar to that of the 16-day specimen. Again, the amount of lymphoid cells in the vicinity of the graft varied with the location. Although both CD4 and CD8 cells were present around the graft, they were rarely seen within the islets.

Figure 2 shows that the human islets that had survived in xenogeneic recipients responded to glucose stimulation when perfused in situ in the graft bed. Although insulin secretion after glucose challenge was delayed, a distinct increase of insulin response was detected. In contrast, no

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0041-1345/92/$3.00/+0

Fig 1. An area of human islet xenograft tissue 124 days after xenotransplantation under the kidney capsule of an FK 506 (2 mg/kg/d for 2 weeks then weekly IM)-treated ACI rat. Lymphoid cells of both CD4 and CD8 subsets are present in the vicinity of the graft, but not within the islets (×560).

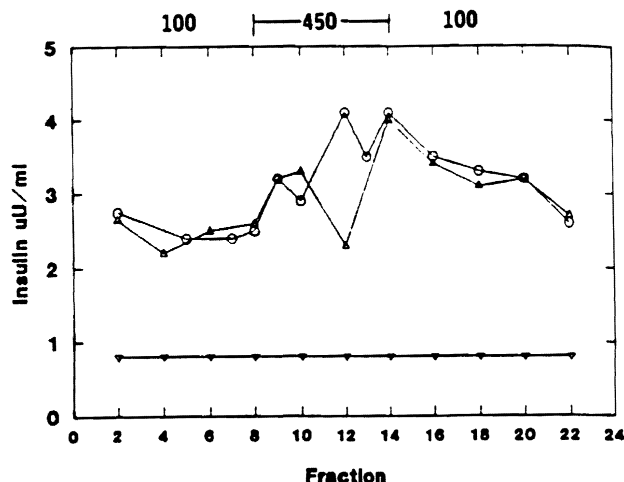

Fig 2. Insulin concentrations in effluent medium collected from human islet-bearing kidneys of FK 506-immunosuppressed ACI rats. Kidneys were perfused with medium containing 100 or 450 mg/dL glucose as indicated. (Δ—Δ) 36 days; (O—O) 100-day xenograft; (∇—∇, mean of n = 4) control kidney without graft tissue. The human islets responded to glucose stimulation. In contrast, no insulin was detected in the perfusates from normal control kidneys without islet transplant.

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DISCUSSION

In an earlier report we demonstrated that FK 506 was effective in the prolongation of fresh rat islet allograft across the MHC barrier.[1] The present findings confirmed that this drug was effective for the marked prolongation of human islet xenograft survival in rat recipients. Despite the presence of lymphoid cell infiltration in some areas, the graft tissues appeared to be healthy and contained cells with demonstrable insulin and glucagon by immunohistochemical stainings up to >4 months after transplantation, when the experiment was terminated. These human islets were also found to be functional as demonstrated by significant insulin response to glucose stimulation in two thirds of the cases. Histologic examination of the graft that did not respond to glucose challenge had many fewer islets in the graft bed than the others, and may account for the poor results.

The important finding of this study is that FK 506 alone and given intermittently was effective in the islet prolongation in this discordant xenograft model. In most discordant islet xenograft transplantation models, the tissues were rapidly rejected.[5,6] The present findings support the view that with the use of a new generation of immunosuppressive agents, islet xenotransplantation in humans is a future possibility.

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