Gamma Irradiation and Donor Antigen Injection Prior to Xenografting of Pig Islets Into the Thymus of Diabetic Rats

V.H. de Villa, J.A. Cienfuegos, F. Pardo, J.L. Hernandez, J. Gonzalez, J. Torramadé, F. Regueira, and L. Montuenga

GREATER interest has been put in xenografting that could solve the worldwide problem of donor scarcity should graft acceptance be achieved. The pig is a promising donor organ source in pancreatic islet xenografting owing to the similarity of porcine to human insulin and the ready availability of this species.[1] Moreover, an animal source allows programming of the transplantation and pretreatment of the graft or the donor or both.

As breaking the immunologic barrier between disparate species such as the pig and the rat is difficult without any form of immunosuppression, we have looked into the possibility of immunomodulation or immunoalteration of both the graft and the recipient to see whether islet survival could be achieved.

Low-dose gamma irradiation has been reported to enhance graft survival by reducing immunogenicity in allograft models without detriment to islet cell function.[2] In the recipient the thymus gland has been reported to be a privileged site where, again, allografted rat islets survived without chronic immunosuppression.[3,4] Early rejection or primary nonfunction between discordant species is believed to be antibody mediated,[1,5] hence, donor antigen presentation to the recipient before transplantation, which induces host unresponsiveness or binds circulating natural antibodies, could also ameliorate graft acceptance. This form of pretreatment has been shown to work in cardiac[6,7] and renal[8] allograft models, although almost always in combination with an immunosuppressive drug.

In the cure of diabetes with islet transplantation, success lies not only in being able to suspend an exogenous source but also in obviating the need for continuous immunosuppression. We have therefore tried to look into a combined immunomodulation of the graft and alteration of host immune response, without pharmacologic immunosuppression to determine whether intrathymic islet xenograft survival would improve.

MATERIALS AND METHODS

Male Wistar rats weighing 180 to 220 g, made diabetic with 70 mg/kg of streptozotocin, were used as recipients. Only animals with nonfasting blood glucose >350 mg/dL for at least 3 weeks before transplantation were considered for the study.

Pancreases were obtained from Large-White pigs weighing 20 to 25 kg after cold preservation with Euro-Collin's solution through the aorta. The gland was distended by intraductal injection of collagenase using a peristaltic pump, followed by enzyme activation in a water bath at 39°C. After disintegration of the gland, tissue digest was filtered serially and subjected to density gradient purification with bovine serum albumin (BSA), obtaining variable grades of purity (40% to 95%). The tissue preparation was suspended in enriched RPMI medium and radiated with 250 cGy using a linear accelerator. Around 1000 to 1200 islets were injected into the thymus gland of each rat anesthetized with a ketamine-diazepam-atropine preparation given intraperitoneally.

Animals were divided into three groups: the first receiving untreated islets, the second receiving gamma-irradiated islets, and the third group receiving both treated islets and 10^7 freshly prepared pig spleen cells through the portal vein 7 days prior to transplantation. In this group a splenectomy was also performed.

Serial blood glucose monitoring using tail vein samples was performed daily during the first 10 days and thrice weekly thereafter with ≤200 mg/dL considered as normal. Once rejection or primary nonfunction was diagnosed, thymus glands were removed and processed for microscopic examination.

RESULTS

In no instance was there a return to normal levels in the nontreated group, as shown in Table 1, with blood glucose levels remaining >350 mg/dL throughout the study. In the second group, one animal had normal blood glucose for only 1 day followed by a gradual rise. Interestingly in the third group, two animals showed a return to normal glucose level for 1 day and remained so for 8 days in a third animal. However, microscopic sections of the removed thymus glands did not show any recognizable endocrine tissue. Residual histologic changes in the form of fibroblast and epithelioid cell infiltrates were seen.

Table 1. Graft Function Expressed as Days of Normoglycemia in Each Animal Per Treatment Group

Graft Immunomodulation	Host Pretreatment	n	Normoglycemia (no. of days)
None	None	11	0,0,0,0,0,0,0,0,0,0,0
Gamma rays	None	5	0,0,1,0,0
Gamma rays	Pig splenocyte injection	9	0,1,0,0,0,8,0,0,1

From the Departments of Surgery and Histology, University of Navarre, Pamplona, Spain.

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Address reprint requests to J.A. Cienfuegos, MD, Department of Surgery, Clinica Universitaria de Navarra, Apartado 192, 31080 Pamplona, Spain.

0041-1345/92/$3.00/+0