Extended Allograft Survival of Islets Grafted into Intra-abdominally Placed Testis

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SUMMARY

Isolated islets of ACI donor rats were cultured for 4 days at 37°C and then grafted into male diabetic Wistar-Lewis rats into three different organ sites without adjuvant immunosuppression. None of 6 recipients of the intraportal injection of 10 islets per gram of body weight became normoglycemic. Similarly, six rats that received islets injected under the renal capsule remained diabetic. None of six rats that received an intratesticular islet allograft became normoglycemic while these organs remained within the scrotum. By contrast, six rats that were transplanted with an identical number of islets into the testis, which were then surgically placed into the abdominal cavity, promptly became aglycosuric and have remained so for more than 50 days. DIABETES *33*:405–406, April 1984.

Although several studies have indicated that the testis is a privileged site for the grafting of a variety of tissues,[1–3] the mechanisms responsible for the protective effects of intratesticular transplantation against rejection have not been established. At least two theories have been proposed including the lack of lymphatics from this organ,[4] and the inhibitory effects of the male hormone, testosterone, on lymphocyte activity.[5] Because grafted tissues survive relatively longer in the testis compared with most other organ sites,[6] no one, to our knowledge, has explored the effects of the testis as an abdominal organ on either long-term survival and/or function of an islet graft. The goal of this study was to compare the survival times of islet allografts in three different organ sites, namely, the liver, under the renal capsule, and intratesticular with the testis either in the scrotum or in the abdominal cavity. For this purpose the Wistar-Lewis rat, which has been shown to be quite resistant to islet transplantation,[7] was selected as the recipient of islet allografts of ACI donor rats and the survival times of the grafts in the three different organ sites were investigated. The results will show that islets remained functional only in those testicles that had been surgically implanted in the abdominal cavity after transplantation.

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MATERIALS AND METHODS

Two highly inbred strains of rats, Wistar-Lewis (RT1-1) and ACI (RT1-a), that have been maintained in our laboratory at the Veterans Administration for the past 4 yr were used throughout this study. Male Wistar-Lewis rats weighing between 150 and 200 g were made diabetic by the intravenous injection of 65 mg/kg streptozotocin and were used as the recipients of islet allografts. ACI rats of either sex, weighing between 130 and 160 g, were used as islet donors.

Islet isolation. Islets were isolated according to the method of Lacy and Kostianovsky[8] and purified by means of Ficoll gradient centrifugation as described recently by our laboratory.[7] The islet preparations were then transferred to biologic grade Petri dishes in groups of 300 per dish. Each Petri dish contained 6.0 ml CMRL-1066 medium supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), and glucose at 200 mg/dl. The islets were culture maintained at 37°C in a humidified atmosphere of 5% CO_2 for 4 days before transplantation.

Islet transplantation. Two weeks after the injection of streptozotocin only those male diabetic Wistar-Lewis rats with a plasma glucose concentration in excess of 400 mg/dl were used as recipients regardless of the site of islet implantation. The method used for the intraportal injection of islets has been described in detail elsewhere.[9] For the injection of islets under the renal capsule the rat was anesthetized with ether and a small incision made in the left flank area immediately over the left kidney. A total of 10 islets per gram of body weight was collected under a stereomicroscope with a drawn-out Pasteur pipette, centrifuged, and the pellet transferred to a small tuberculin syringe in a total volume of approximately 0.25 ml of Hanks' buffer solution (HBBS). The islets were then injected under the left renal capsule. The

TABLE 1
Response of diabetic Wistar-Lewis rats to 4-day cultured islets of ACI donors grafted into three different organ sites

Group (no. of rats)	Organ site	No. of islets per gram of body weight	Graft survival (days)
1 (6)	Liver	10	All <3
2 (6)	Under renal capsule	10	All <3
3 (6)	Intratesticular in scrotum	10	All <3
4 (6)	Intratesticular intra-abdominal	10	30,* >50, >55, >55, >60, >63

Orchidectomy.

syringe was flushed once with a small volume of HBBS in order to inject as many of the islets as possible. By using this technique, fewer than 50 islets remained in the syringe after implantation. For the intratesticular transplantation of islets a similar procedure for the collection of the cells was used. Under light ether anesthesia a small incision was made in the scrotum and the islets were then injected into the testis through a 26-gauge needle. Each testis received 50% of the total number of islets required by the recipient based on its body weight, i.e., if the rat weighed 180 g, a total of 1800 islets were selected and 900 were grafted per testis. For the surgical placement of the testis into the abdominal cavity, a lower abdominal incision exposing the urinary bladder was made. The testicles were then pulled into the abdominal cavity through two small openings in the inguinal area and these openings were sutured with 4-0 chromic to prevent the movement of the testicles back into the scrotum. After surgery the rats were immediately transferred to metabolic cages and the urine volume, urine glucose content, and body weights were obtained at daily intervals. Plasma glucose levels were determined at weekly intervals. A rat was considered transplant improved if the plasma glucose concentration was $\leq$ 170 mg/dl and it was aglycosuric. It was considered transplant failed if the urine glucose content equaled the amount of glucose secreted per 24 h before transplantation.

RESULTS

The mean ± SEM of plasma glucose concentrations of the recipients of intraportal (group 1); under the renal capsule (group 2); intratesticular, scrotum (group 3); and intratesticular, intra-abdominal (group 4) allografts were 484 ± 9, 463 ± 18, 478 ± 14, and 467 ± 20 mg/dl, respectively, immediately before transplantation. These values were not significantly ($P > 0.3$) different from each other. Table 1 shows that all of the Wistar-Lewis rats grafted with islets of ACI donors into the three conventional organ sites, namely groups 1–3, remained hyperglycemic. By contrast, all of six Wistar-Lewis recipients of intratesticular allografts with these organs inside the abdominal cavity became aglycosuric within 48 h after transplantation and have remained so for more than 50 days. The testicles of one of these aglycosuric rats were removed 30 days after transplantation. The animal reverted to the diabetic state within 48 h after orchidectomy.

DISCUSSION

The organ site selected for the injection of islets has been shown to profoundly affect allograft survival. Thus, according to a recent study by Bobzien et al.,[6] islet xenografts were shown to survive significantly longer in the testis compared with other organ sites such as the liver, spleen, or under the renal capsule. The observations made in this study are in agreement that the testis appears to be the most suitable site for the survival of islet allografts,[6] but only after this organ had been grafted into the abdomen after islet implantation. None of the rats became aglycosuric while the testis remained in the scrotum, although in three of these animals the total urine glucose content decreased somewhat and the 24-h urine volume dropped from an average of 70 to 35 ml for a brief period after transplantation. However, not one of these rats became aglycosuric even after 1 mo of observation. By contrast, the response of the rats in which the testes were made an abdominal organ was quite dramatic. The return to aglycosuria occurred within 24–48 h after transplantation and remained so for more than 50 days. In addition, all of these animals rapidly gained weight after transplantation. That the islets were injected into the testis rather than into the general circulation at the time of transplantation was evidenced by the abrupt return to a diabetic state in one of these rats after an orchidectomy 30 days after the onset of normoglycemia. In conclusion, our data suggest that at least two components are critical for islet function and/or survival after intratesticular implantation: first, that a factor such as the hormonal milieu protects the islets against rejection and second, that a higher temperature is required for islet function. A combination of these factors is found only when the testis becomes an abdominal organ.

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