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Successful Intra-Splenic Transplantation of Syngeneic and Allogeneic Isolated Pancreatic Islets*

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Summary. Adult rat islets harvested by the collagenase digestion/Ficoll separation technique were injected into the splenic pulp in 9 syngeneic (Lewis→Lewis and WaG→WaG) and 13 allogeneic [(DA X Lewis) F1→Lewis] experiments. Normal serum glucose levels and 24 hour urine volumes were restored in all 9 syngeneic recipients and in 11 out of the 13 allogeneic recipients in a mean of 3.3 days. Splenectomy performed on 3 of the 9 syngeneic recipients 110–178 days after transplantation resulted in a prompt return to the diabetic state. In all the remaining syngeneic recipients, normal values have persisted for the current period of observation of 6 months. In 5 untreated allogeneic recipients, rejection occurred in a mean of 5.2 days. The administration of a short course of ALS (1 ml I. P. days −1, 1, 3 and 5) to the remaining 6 animals greatly prolonged graft survival with all animals remaining normoglycaemic for at least 4 weeks. These results were not significantly different from those recorded in comparable groups of intra-portal allogeneic islet recipients.

Key words: Pancreatic Islets, transplantation, spleen, rejection, normoglycaemia.

The results of human whole or segmental pancreatic transplantation remain disappointing. Although 51 such transplants have been performed throughout the world to date, only one patient is at present alive and with a functioning graft [1]. In contrast, the early results of experimental free islet cell transplantation have been more encouraging. Reversal of experimentally induced diabetes in animals has been achieved by implanting islet tissue into a variety of sites, including the peritoneal cavity [2, 3, 4, 5], the portal vein [6, 7, 8, 9 10], beneath the renal capsule [11], and at the root of the mesentery [9]. However, only the intra-portal method of administration has consistently resulted in the permanent restoration of normoglycaemia. Unfortunately the liver carries the inherent disadvantage that it is a vital organ and, as such, cannot be removed if problems arise following transplantation and, indeed, we have recently drawn attention to the possible association between long-term successful intra-portal transplantation and the development of multiple biliary cysts of the liver [12]. It is thus possible that this site will be unsuitable for clinical use.

The spleen, on the other hand, is not a vital organ and can be removed without seriously endangering the patient's life. Like the pancreas it possesses a portal venous drainage and, as with the liver, it is a highly vascular organ and likely to provide a similar environment for the successful nutrition of implanted islet tissue. Furthermore, it should be possible in clinical practice to implant tissue into the spleen by transfemoral catheterisation of the splenic artery thus avoiding the need for laparotomy under general anaesthesia – a procedure that could be repeated many times as more islets became available from culture, cytopreservation, or multiple donors.

We have therefore studied the effect of transplantation of isolated islet tissue into the splenic pulp in both syngeneic and allogeneic strain combinations, and compared the results of allogeneic transplants with those obtained with the intra-portal method of administration.

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Experimental Design

To study the metabolic effects of the intra-splenic transplantation of isolated rat islets without the problem of immunological rejection, 600–800 adult islets were implanted into the splenic pulp of streptozotocin-diabetic syngeneic recipients in 9 consecutive experiments (3 Lewis→Lewis, 6 WaG→WaG). In a further 23 transplant experiments performed to study the effect of the rejection response on islets situated in the spleen compared with those in the liver, allogeneic islets from (DA X Lewis) F1 donors were transplanted into Lewis recipients. In 13 recipients, the islets were transplanted into the splenic pulp and in the remaining 10 animals were injected into the portal vein. To compare the ease with which the rejection response could be modified by immunosuppression, half of the successful transplant recipients in each group were treated with a short course of rabbit anti-rat lymphocyte serum (1 ml I.P. days −1, 1, 3, and 5), the remaining animals in each group receiving no form of immunosuppression and acting as controls.

Splenectomy was performed on 3 of the 9 syngeneic recipients 110–180 d after transplantation to study the histological appearance of the transplanted islets and the metabolic effects of graft removal.

Materials and Methods

Rats

All rats were virgin females between 12 and 16 weeks old and weighed between 150 and 225 g. Da (AgB^4), Lewis (AgB^1) and the (DA X Lewis) F1 Hybrid were bred and reared in the animal house of the Radcliffe Infirmary, Oxford. WaG (AgB^2) rats were supplied by Bantin and Kingman Ltd. of East Grimston, Yorkshire.

Induction of Diabetes

Diabetes was induced in recipients by the intravenous injection of streptozotocin (65 mg/kg body weight) given into a tail vein. Only those animals with non-fasting serum glucose levels > 400 mg/100 ml and 24 h urine volumes of > 50 ml on two consecutive occasions at least ten days after injection were considered to have established diabetes for the purposes of these experiments.

Islet Isolation and Transplantation

Islets were isolated by a modification of the Collagenase digestion/Ficoll separation technique [2]. This has previously been described in detail [10].

The abdominal cavity of intra-portal recipients was opened under ether anaesthesia and the islets, suspended in 1 ml of Hanks solution in a Iml disposable syringe, were injected into the portal vein through a 25 FG needle. Repeated aspiration and injection of small quantities of portal blood was necessary to transfer all islets from the syringe into the vessel.

For intra splenic recipients, the islets were suspended in 150 µl of Hanks solution and aspirated into a 250 µl Hamilton's syringe into which a bolus of 100 µl of Hanks solution had previously been drawn. The needle of the syringe was passed through the length of the pulp space of the spleen in the long axis of the gland, and the islets slowly injected as the needle was withdrawn − the 100 µl of Hanks solution ensuring transfer of all islets out of the dead space of the needle.

Post Transplantation Measurements

Following transplantation all animals were confined to individual metabolic cages and allowed free access to food and water. Blood samples were obtained by amputating the tip of the tail. Non-fasting serum glucose levels, 24 h urine volumes, urine glucose and body weights were recorded daily for the first 14 d, on alternate days from 16–40 d, and less frequently thereafter. Serum glucose estimations were performed on an Analox GMT4 Glucose analyser (Analox Instruments Ltd., Oxford). Urinary glucose was estimated with Clinistix (Ames Co., Slough).

Anti-Lymphocyte Serum

Rabbit anti-rat lymphocyte serum was prepared in a single batch in our laboratory by the intraperitoneal injection of 10^8 rat thoracic duct lymphocytes on three occasions at weekly intervals into an adult rabbit. The serum was collected at weekly intervals over the next seven weeks and pooled. A further booster dose of 10^8 lymphocytes was given at the end of the fourth week of collection. The serum was stored in 1 ml aliquots at −40°.

Each recipient received 1 ml of ALS intraperitoneally 24 h before transplantation, and a similar volume was given on the first, third and fifth days after transplantation.

Results

1. Syngeneic Intra-Splenic Recipients

Of the 9 syngeneic intra splenic recipients, 6 were normoglycaemic within the first three days of transplantation, and the remaining three became nor-

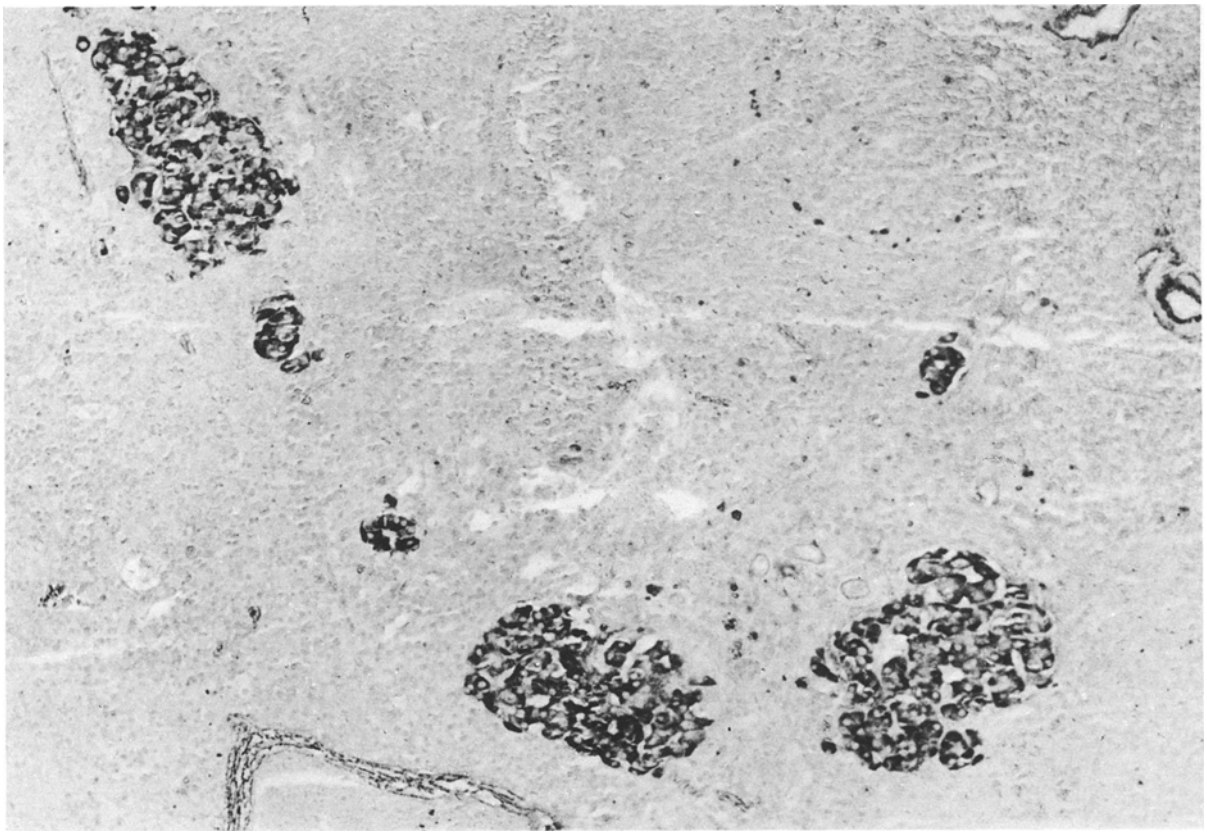

Fig. 1. Syngeneic pancreatic islets seen in the splenic pulp 110 days after transplantation. Aldehyde Fuchsin. Mag. × 160

moglycaemic on the 4th, 5th and 11th days. Splenectomy was performed on three recipients 110, 165, and 178 d after transplantation with a return of the diabetic state (serum glucose > 400 mg/100 ml) in all three animals within 24 h. Histological study of the spleen excised at 110 d showed well granulated beta cells lying within the splenic pulp along the implantation tract (Fig. 1). All remaining animals have remained normoglycaemic with normal 24 h urine volumes and in good health for the current period of observation (6 months) (Table 1).

2. Allogeneic Intra-Splenic and Intra-Portal Recipients (Table 2).

In two of the 13 intra-splenic recipients normoglycaemia was not reached before rejection occurred. The results of the 11 successful intra-splenic transplants and of 10 intra-portal allogeneic transplants in the same donor-recipient strain combination are shown in Table 2.

Rejection of intra-splenic islets in recipients not receiving immunosuppression occurred in a mean of 5.4 d compared with 4.8 d when the intra-portal route of administration was used. There is no significant difference between these two values ($p > 0.05$).

The addition of a short course of anti-lymphocyte serum greatly prolonged the survival of intra-splenic islets with all recipients remaining normoglycaemic for at least 4 weeks. Administration of ALS to intra-portal recipients produced a wider range of results (20 – > 100 d) but these results were not significantly different from those obtained in the intra-splenic group ($p > 0.05$).

Discussion

Previous reported attempts to correct streptozotocin induced diabetes in experimental animals by the intra-splenic transplantation of islet tissue have met with only limited success. Ziegler et al. [13] transplanted both fetal pancreata and isolated adult islets under the splenic capsule of syngeneic recipients without success. Colin and Baillat [14] demonstrated significant but incomplete regression of diabetes by the administration of isolated adult islets into the splenic pulp of recipients of the same inbred strain, although subsequent transplants by this group have

Table 1. Summary of results of body weight, serum glucose, 24-h urine volume and urine glucose in syngeneic intra-splenic islet recipients (Mean ± SEM). n = 9 to 110 days, n = 8 thereafter (see text)

	Body wt. (g)	Serum Glucose (mg/100ml)	24 hr Urine Vol. (ml)	Urine Glucose (Clinistix)
Pre-Streptozotocin	179±12	121±9	5±1	–
Pre-Transplant	163±6	538±35	73±10	+++
Post-Transplant (Days)				
10	175± 7	133±12	4±1	–
20	183± 8	118±12	4±1	–
30	187± 9	114±10	3±1	–
40	193±11	117±11	4±1	–
60	202±10	132±11	4±1	–
80	213± 9	118± 5	4±1	–
100	218±12	132± 6	5±1	–
120	217±11	141±16	5±1	–
140	220±11	133± 3	4±1	–
160	221±12	130± 5	6±1	–
180	222± 9	134± 6	5±1	–

Table 2. Survival of intra-splenic (I. S.) and intra-portal (I. P.) allogeneic islets

	Strain	Method of Administration	Immuno-suppression	Day of Rejection Serum glucose > 200 mg/100 ml
A	Fl→Lewis	I. S.	Nil	5, 5, 5, 6, 6
B	Fl→Lewis	I. P.	Nil	4, 5, 5, 5, 5
C	Fl→Lewis	I. S.	ALS[a]	28, 29, 31, 33, 33, 38
D	Fl→Lewis	I. P.	ALS[a]	22, 34, 50, 55, > 100

A versus B : p = > 0.05 n. s.
C versus D : p = > 0.05 n. s.
[a] 1 ml I. P. days − 1, 1, 3, 5

been more successful [15]. More recently Koncz et al. [16] have also produced amelioration of the diabetic state, but failed to produce normoglycaemia. Using similar techniques we have consistently achieved restoration of normoglycaemia. The failure of other workers to obtain similar results may have been due to the difficulty experienced in transferring all the islets harvested into the splenic pulp. In two preliminary experiments, not reported in this series, we attempted to implant islets into the spleen using the standard 1 ml disposable syringe and needle which had proved entirely satisfactory for intraportal administration. However, when used for intrasplenic implantation it was obvious that a large number of islets were remaining in the deadspace of the needle. These two animals did not become normoglycaemic until 18 and 20 d after transplantation and reverted to the diabetic state 67 and 95 d later. However, once the complete yield of islets was transferred to the splenic pulp by the introduction of the technique already described, identical results to those seen in syngeneic intra-portal recipients were achieved. The demonstration of a prompt return to the diabetic state in all three of the syngeneic recipients submitted to splenectomy 110–178 d after transplantation suggests that it is unlikely that the maintenance of normal values in our long term survivors is due to regeneration of the recipients' own islet tissue. Spontaneous recovery from streptozotocin induced diabetes does undoubtedly occasionally occur, but in our experience generally manifests itself within the first few weeks of injection.

Our allogeneic results are particularly encouraging. It has been suggested that the liver may play an important role in suppressing immune responses to histocompatibility on sheep red cell antigens [17, 18], and that the intra-hepatic transplantation of free endocrine allografts confers upon them an immunological advantage compared to other sites. Barker [19] reported prolonged survival of a series of intra-portal islet transplants compared with intraperitoneal transplants, and Pfeffermann et al. [20] reported significant prolongation of survival of parathyroid allografts when placed in the liver compared with the thigh hamstring muscle. However, in both series prolonged survival was only seen when the tissue was implanted across a minor histocompatibility barrier. In contrast, the implantation of antigenic tissue directly into lymphoid tissue might be expected to evoke a more rapid rejection process which would be more difficult to suppress. Our allogeneic experiments, carried out across a strong histocompatibility barrier, have failed to demonstrate any significant difference between intrasplenic and intra-hepatic allograft survival, and islets in both sites appear equally susceptible to immunosuppression with ALS. This finding is surprising and at present inexplicable. Local suppression of antigen sensitive cells by the high local concentration of antigen, or direct stimulation of suppressor T cells, which are present in the spleen in abundance, are theoretical explanations. Furthermore as the spleen functions as a trap for antigen in the circulation, the placing of islets in the pulp space of the spleen rather than intra-arterially might expose the islets to a different population of lymphocytes in the pulp space to that around arteries.

Thus the splenic pulp may prove a more suitable site for the clinical transplantation of pancreatic free grafts than other sites hitherto evaluated. Certainly, in experimental animals, results comparable to those obtained with the intraportal method of administration can be achieved in both syngeneic and allogeneic recipients.

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