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Treatment of diabetic rats with encapsulated islets

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Abstract

Immunoprotection of islets using bioisolator systems permits introduction of allogeneic cells to diabetic patients without the need for immunosuppression. Using TheraCyte™ immunoisolation devices, we investigated two rat models of type 1 diabetes mellitus (T1DM), BB rats and rats made diabetic by streptozotocin (STZ) treatment. We chose to implant islets after the onset of diabetes to mimic the probable treatment of children with T1DM as they are usually diagnosed after disease onset. We encapsulated 1000 rat islets and implanted them subcutaneously (SQ) into diabetic biobreeding (BB) rats and STZ-induced diabetic rats, defined as two or more consecutive days of blood glucose >350 mg/dl. Rats were monitored for weight and blood glucose. Untreated BB rats rapidly lost weight and were euthanized at >20% weight loss that occurred between 4 and 10 days from implantation. For period of 30–40 days following islet implantation weights of treated rats remained steady or increased. Rapid weight loss occurred after surgical removal of devices that contained insulin positive islets. STZ-treated rats that received encapsulated islets showed steady weight gain for up to 130 days, whereas untreated control rats showed steady weight loss that achieved >20% at around 55 days. Although islet implants did not normalize blood glucose, treated rats were apparently healthy and groomed normally. Autologous or allogeneic islets were equally effective in providing treatment. TheraCyte™ devices can sustain islets, protect allogeneic cells from immune attack and provide treatment for diabetic-mediated weight loss in both BB rats and STZ-induced diabetic rats.

Keywords: diabetes • islets • bioisolator • BB rats • STZ

Introduction

Diabetes mellitus is a common disorder with a prevalence of 4–5% [1]. Type 1 diabetes mellitus is an immune-mediated disease that is associated with a near complete loss of the insulin secretory function of pancreatic β cells that results in insulin-dependence for life [2]. Tight blood glucose control is required to delay or prevent the onset of late complications that are debilitating and associated with fivefold increased mortality [1, 3–5]. Almost 90% of type 1 diabetes mel litus (T1DM) patients are sporadic rather than familial, requiring general population screening for multiple autoantibodies in order to detect disease onset. Because this is difficult [6] most patients are diagnosed after the onset of hypoglycaemia and the ability to reverse their diabetes will be highly significant. The failure to develop successful islet transplantation and inadequacy of current insulin replacement therapy has resulted in a focus on pancreatic islet β-cells for much cell and gene therapy research towards the treatment of diabetes [7–10].

Immunoprotection of islets using bioisolator systems permits the introduction of allogeneic cells to patients without the need for immunosuppressive treatment. Towards this goal we investigated two rat models of type 1 diabetes, BB rats and rats made diabetic by STZ treatment. We chose to implant islets after the onset of diabetes to mimic the probable treatment of children with T1DM as they are usually diagnosed after disease onset. The noninvasive monitoring of encapsulated islets in bioartificial membranes has shown the importance of oxygen tension to cell survival and metabolism [11–13]. Encapsulation devices have been used to deliver islet cells to dogs [14] and mice [15]. However, these initial studies have not led to general clinical application. TheraCyte™ immunoisolation devices made by Baxter have been studied in nonobese diabetic (NOD) mice to deliver insulin [16] and in nude rats to deliver human growth hormone [17]. These devices have been used to implant allogeneic parathyroid tissue to patients [18]. The vascularization [19] and diffusion properties [20] of Theracyte™ devices have been studied. TheraCyte™ immunoisolation

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devices have an inner immunoisolation membrane and an outer vascularization membrane. Islet cells are particularly prone to necrosis under low oxygen levels [21] which is likely due to the high dependency on adenosine-5′-triphosphate (ATP) generated from aerobic metabolism. We, and others, have shown that islets maintained at high density do not survive well [22]. Due to the inherent need for the diffusional supply of oxygen to match the consumption of oxygen by the islet, immunoisolation devices must maximize the rate of diffusion of oxygen per islet [12, 13, 23]. The TheraCyte™ is optimally suited for the maintenance of islets *in vivo* by allowing cells to be loaded into the chamber at controlled densities and spatial configurations and the promotion of vascularization by the outer membrane of the device. Recently, we have shown that TheraCyte™ encapsulation devices seeded with allogeneic vascular smooth muscle cells transduced to express rat erythropoietin (EPO) provided sustained elevations of hematocrit (HCT) [24].

We monitored the effect of islets implanted in two models of type 1 diabetes, the BB rat and rats made diabetic by streptozotocin (STZ). The spontaneous type 1 diabetes in the BB rat shares a number of important characteristics with the human disease, such as weight loss, polydipsia, polyuria, glucosuria, hyperglycaemia and hypoinsulinaemia [25]. Inbred diabetes prone (DP) BB rats all develop diabetes without gender differences and exogenous insulin is needed for survival [25]. BB rats with the lymphopenia gene (lyp) region on chromosome 4 develop diabetes between 45 and 60 days of age. The lyp/lyp genotype is associated with an islet inflammatory infiltration which is detectable about 3–5 days before the onset of hyperglycaemia [26]. The lyp gene has recently been identified as a novel immune-associated nucleotide related gene (Gimap5, also known as IAN 5) [27]. The recessive mode of inheritance [28] is explained by a deletion of a base-pair resulting in the truncation of the lyp protein, now recognized as a novel IAN-related gene (Gimap5) [27]. The BB rats are kept specific pathogen free which we have shown is associated with a complete penetrance of diabetes in the BB DR lyp/lyp rats [28]. High-dose streptozotocin (STZ)-treated rats lose pancreatic β cells and the ability to secrete insulin and provide an alternative model of T1DM [29].

Materials and methods

BB rats and STZ-induced diabetic rats

BB rats were generated in Seattle from an established breeding colony [27]. Male and female BB rats were studied. The standard cross–intercross breeding was used to generate lyp/lyp which all develop diabetes between 45 and 60 days. Surgery was performed after two consecutive days of blood glucose >350 mg/dl. Normal male Fischer 344 and Wistar rats received streptozotocin intraperitoneally (IP) at a dose of 60 mg/kg to induce loss of pancreatic β cells and development of diabetes. Diabetes was assessed by daily blood glucose measurements and by daily weighing. STZ-treated rats were considered diabetic when two consecutive blood glucose measurements of >350 mg/dl were recorded.

Isolation and evaluation of islets

Fischer 344 or Wistar rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (35 mg / 230 g rat) and islets were isolated from pancreas using enzyme digestion (Liberase, Roche; Indianapolis, IN, USA) as described [30]. Islets were suspended in Roswell Park Memorial Institute (RPMI) media 1640 containing 11.1 mM glucose supplemented with 10% heat-inactivated foetal bovine serum (Gibco, BRL, Grand Island, NY, USA). Islets prepared with this methodology have been shown to respond normally to glucose and other secretagogues with respect to both insulin secretion, oxygen consumption and calcium [31–33].

Subcutaneous implantation of bioisolator devices

Bioisolator devices obtained from TheraCyte™ Inc. Irvine, CA were loaded with 1000 islets and implanted subcutaneously in anaesthetized animals. Sterilized 20 μl volume devices were flushed with tissue culture medium and loaded with freshly isolated islets *via* the access port that was then sealed with silicone glue. Two-month-old Fischer or BB Wistar rats weighing 190–230 g were anaesthetized with an IP injection of ketamine (44 mg/kg) plus xylazine (6 mg/kg) and acepromazine (0.75 mg/kg). All rats received 0.04 mg dexamethasone IP before surgery. Skin was shaved from the abdomen, swabbed with betadine and a midline incision made in the skin and a small pocket created by blunt dissection with a haemostat. A device seeded with islets is placed into the pocket and the incisions closed with sutures. Mock surgery consisted of anaesthesia, blunt dissection to create a pocket under the skin and suture of the skin wound without placement of an encapsulation device. Rats were weighed daily and blood glucose levels determined. All procedures were approved by the University of Washington Internal Animal Care and Use Committee.

Histology

TheraCyte™ devices harvested from treated rats were fixed in phosphate-buffered 10% formalin and embedded in paraffin. The sections were stained with haematoxylin and eosin and immunostained using monoclonal anti-smooth muscle actin, clone 1A4 (Dako, Carpinteria CA, USA), and guinea pig anti-insulin (Dako), followed by appropriate biotinylated secondary antibody, ABC-Elite (Vector Laboratories, Burlingame CA, USA) and 3,3′-diaminobenzidine [34] by the Department of Pathology Histology Core, University of Washington (Dr. Charles Alpers and Kelly Lee Hudkins).

Results

Treatment of diabetic BB rats with encapsulated islets

Five untreated diabetic BB rats exhibited rapid weight loss and were killed at 3–11 days after onset when >20% weight loss achieved (Fig. 1). There was no difference in weight loss between untreated diabetic rats and rats that were subjected to mock

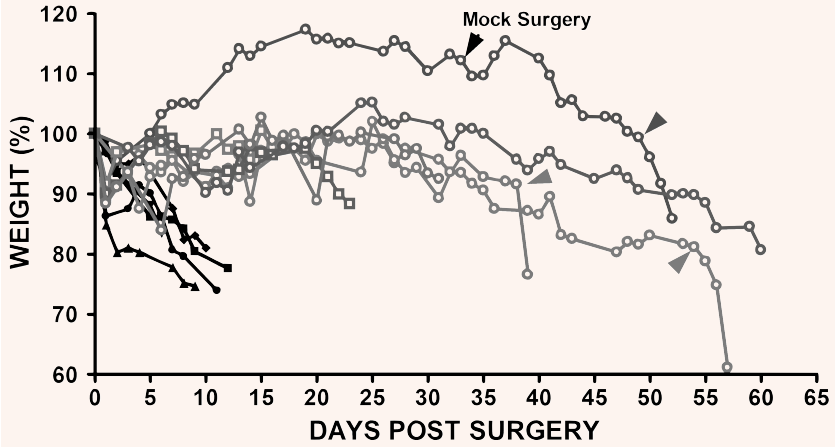

Fig. 1 Weight profiles of lyp/lyp BB rats treated with encapsulated islets. Five control rats received mock or no surgery; black symbols (four females and one male). Seven BB diabetic rats (six females and one male) received 1000 islets. Five rats received autologous islets (open circles) and two rats received allogeneic islets (square symbols). Weight of rat at surgery is 100%. Arrow heads indicate when Theracyte-encapsulating devices were removed. Mock surgery (black arrow) indicates treated animal subjected to anaesthesia and blunt skin dissection without device removal. Control rats were killed when >20% weight loss reached.

surgery. Untreated control rats never retained weight or showed any weight gain. Weight at the time of surgery that followed 2 days of elevated blood glucose >350 mg/ml is recorded as 100%.

In contrast, all seven rats that received encapsulated islets showed low weight loss of 5–10% that was steady for 35–50 days and one animal showed prolonged weight gain (blue symbol) (Fig. 1). The mean weights of treated animals were significantly elevated over untreated controls ($P < 0.04$). One animal died at 15 days during surgery to remove device (gold symbol) and one died spontaneously at 23 days (purple symbol). In three animals, the encapsulated islets were removed (coloured arrow head) and all animals experienced rapid loss of weight and were killed when weight loss was >20% and this occurred within 1–4 days after device removal (Fig. 1). Despite our inability to generate normoglycaemia in treated diabetic BB rats, the treated animals survived for up to 60 days. Blood glucose on all rats was elevated (>450 mg/dl) (data not shown). However, in contrast to untreated BB rats, the animals receiving encapsulated islets appeared healthy, were free of diarrhoea and continued to groom. Two animals received allogeneic islets (squares) and five animals autologous islets (circles) and all were successful in maintaining weight (Fig. 1). In one treated animal mock surgery was performed and this did not lead to weight loss (black arrow) suggesting that surgery alone was not responsible for weight loss, but was due to removal of encapsulated islets. Cross-sections of devices removed from treated animals stained positive for insulin reflecting the presence of functional islets (Fig. 2A and B). Islets from a normal rat provided a positive control for insulin staining (Fig. 2C). Islets were arranged contiguously in a sheet that was part of a three-dimensional structure of cells that had grown out of the seeded islets. Cross-sections of encapsulation devices stained with haematoxylin and eosin showed the presence of islets and cells that have expanded around the islet layer (Fig. 2D–F). Capillaries containing red cells were present around the device adjacent to the immunoisolation barrier. By staining for smooth muscle cell actin, we determined that the majority of cells that have grown around the layer of islets were smooth muscle cells (Fig. 2G–I). A positive control was provided by vascular smooth muscle cells in the walls of capillaries, as these structures showed positive staining for smooth muscle cell actin (Fig. 2G–I). Implants are inserted subcutaneously and the majority of cells that proliferate around the membrane, especially immediately around the circular support structures, are fibroblasts [24]. Fibroblasts around the implant did not stain positive for smooth muscle cell actin, providing a negative control (Fig. 2G–I).

Treatment of STZ-treated diabetic rats with encapsulated islets.

Rats that were made diabetic by STZ received 1000 allogeneic islets and all initially lost weight through 15–20 days but then gained weight ranging from 5–20% of the weight at surgery and this was sustained for at least 130 days, with the exception of one animal that died spontaneously at 78 days (Fig. 3). In contrast, untreated controls showed continued weight loss (Fig. 3). The mean weights of treated rats was significantly elevated over untreated controls ($P < 0.001$). Rats that received encapsulated islets appeared healthy and were free of diarrhoea. Two animals that received STZ without islet implants showed mean blood glucose levels 420.1 ± 27.1 and the mean value for two treated animals was 308.8 ± 2.5 ($P < 0.05$). We restricted the data to values collected after 5 days after surgery as all animals showed elevated blood glucose over this time period. The glucose meter we used in these studies had a maximum value of 450 mg/dl and because of this we believe the actual blood glucose levels from untreated

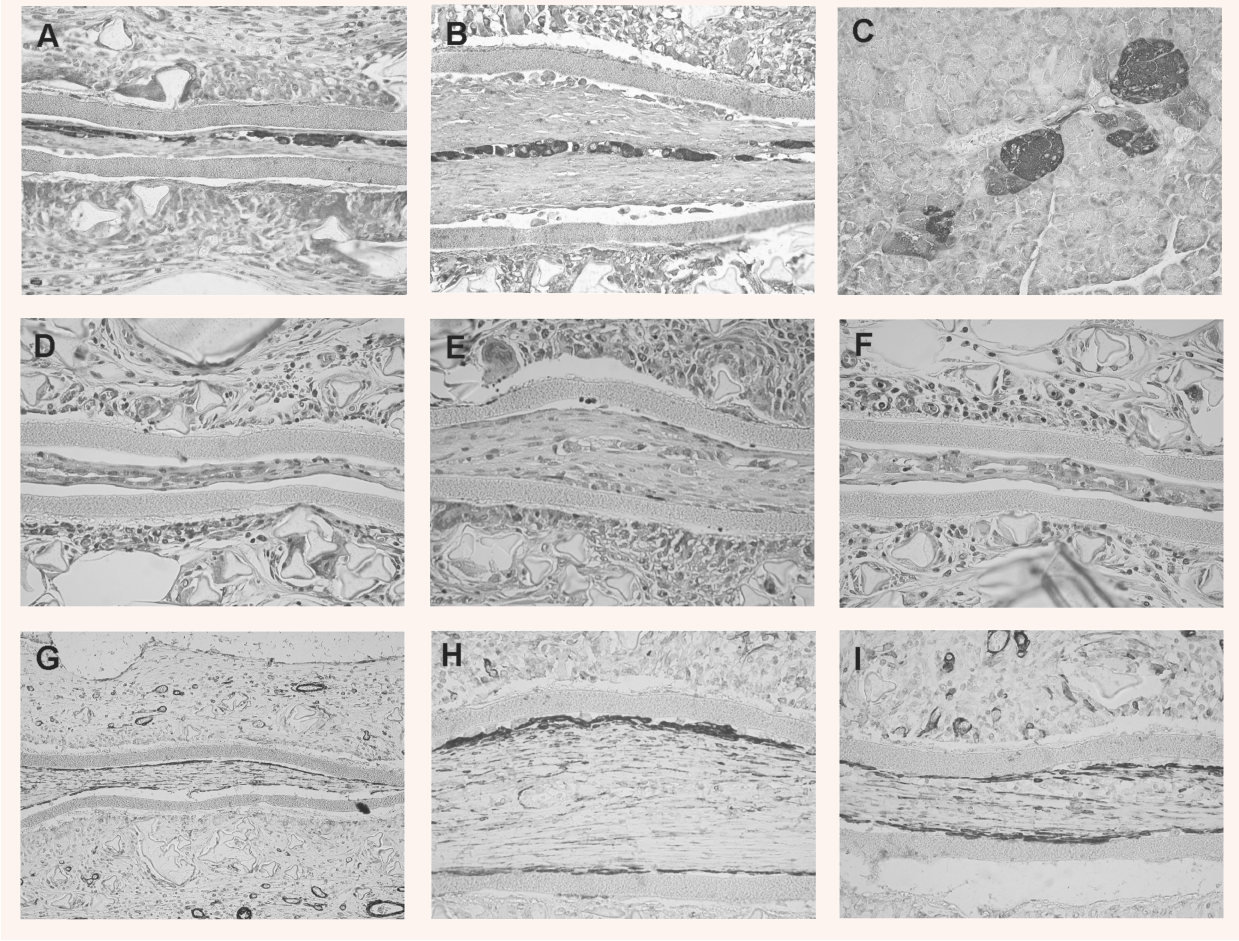

Fig. 2 Cross-sections of Theracyte™ encapsulation devices removed from treated diabetic BB rat. The device shown was loaded with 1000 islets and removed from a treated rat 49 days after implantation (blue symbols in Fig. 1). (**A**, **B**) Insulin immunostaining shows islets in layers in a matrix of other cells. A control rat pancreas shows islets staining positive for insulin (**C**). Sections **D**–**F** were stained with haematoxylin and eosin and show cell distribution and blood vessels around the encapsulation device. Sections **G**–**I** were immunostained for smooth muscle cell actin and show smooth muscle cells around the islets. Lower magnification in **G** shows capillaries around the immunoisolation membrane that stain positive for smooth muscle cell actin. All magnifications 40× except section G that was 20×.

animals were greater than the mean of 420.1 mg/dl that we computed. In one control animal following STZ treatment (black triangles), all of the blood glucose values were >450 mg/dl. These data show that although we did not normalize blood glucose levels, some control of glucose was achieved in STZ-induced diabetic rats.

Discussion

Although both treated diabetic BB rats and rats made diabetic by STZ treatment did not achieve normoglycaemia, there was considerable therapeutic benefit from implanted islets. STZ-treated diabetic rats showed significant lowering of blood glucose, although normoglycaemia was not achieved. This suggests that implanted islets provide therapeutic benefit despite sustained elevated blood glucose. Our data confirmed that diabetes induced by STZ treatment is a markedly less severe disease than that associated with diabetic BB rats. Untreated BB rats rapidly lost weight and were killed at 3–9 days after surgery when >20% weight loss occurred. This contrasts with untreated STZ diabetic rats that achieved the same weight loss at about 55 days. Delivery of islets to STZ diabetic rats provided a gain in weight over 130 days that was a large improvement over the same number of islets delivered to diabetic BB rats. However, treated BB rats

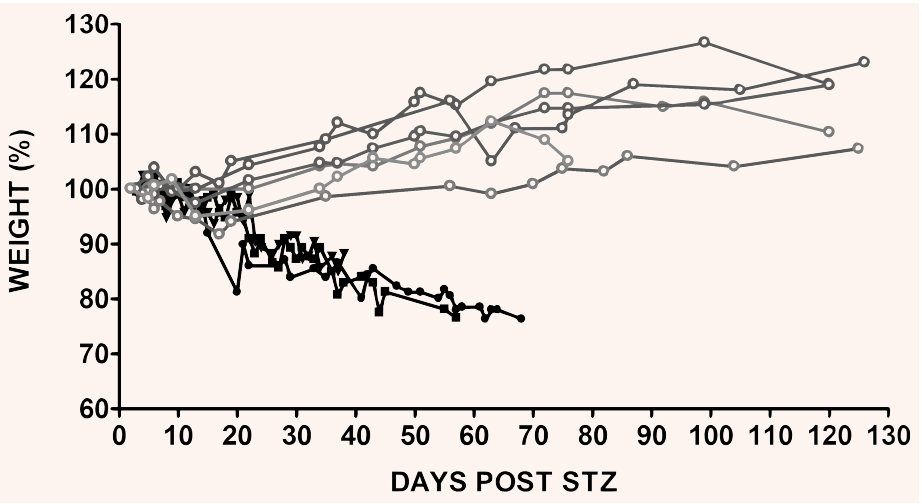

Fig. 3 Weight profiles of STZ-induced diabetic rats treated with encapsulated allogeneic islets. Weight at STZ administration is 100%. Control rats receiving mock or no surgery (black closed symbols). Six diabetic rats receiving 1000 encapsulated islets (open symbols). Control rats were killed when >20% weight loss reached.

showed weight control over 60 days and this is encouraging given the severe nature of diabetes in these spontaneously diabetic animals. We chose to deliver islets to rats after the onset of diabetes as this more closely represents the potential treatment of T1DM children that are usually diagnosed after disease onset. That both autologous and allogeneic islets can be delivered to diabetic rats in an encapsulating device and retain biological function is clinically significant. Recently, treatment of STZ-induced diabetic rats employing 3000 Islet Equivalent (IEQ) per animal in a subcutaneous device achieved normoglycaemia [35]. In this study, autologous islets were implanted and this is not applicable to patients. In a conservative approach, we chose to deliver 1000 islets to rats (5000 IEQ/kg), that is about half the number of islets (12,000 IEQ/kg) typically transplanted into T1DM patients [36].

The finding that encapsulated islets continued to secrete insulin and were surrounded by predominantly smooth muscle cells that had grown out of the seeded islets is encouraging. Not only were these islets functional, but the finding that cells from the islets proliferated to form a three-dimensional tissue around the islets suggest that coseeding islets with such cells transduced to secrete hormones may improve the survival and function of encapsulated islets. We have shown that vascular smooth muscle cells transduced to secrete EPO and seeded in TheraCyte™ devices provided sustained increases in haematocrit in rats for at least 17 months [24]. Hormones, such as GLP-1 [37, 38] or EPO [39, 40] promoted increases in islet function and viability and may confer an increased ability to survive the encapsulation process. Prolonged bioactivity of islets coencapsulated with polymerized synthetic GLP-1 has been described [41]. Recently, it was shown that inclusion of HbC with encapsulated islets to enhance oxygen-transporting capacity increased cell viability and insulin secretion [42]. Similarly, vascular endothelial growth factor (VEGF) has been used as a supplement to islet grafts to increase blood supply [43]. Diabetic mice receiving 1000 islets with VEGF showed decreased blood glucose that was sustained for 28 days in contrast to controls without VEGF that only showed transient changes in blood glucose [43]. In preliminary studies, we increased the insulin secretion of islets that were transduced with lentivirus to secrete GLP-1 (data not shown). We believe that delivering encapsulated GLP-1 transduced cells to diabetic rats offers the promise of increased and prolonged therapeutic efficiency. Similarly, islets transduced with lentivirus to express Bcl-2 may have increased survival *in vivo* [44]. These data show that TheraCyte™ devices can sustain islets, protect allogeneic cells from immune attack and provide treatment for diabetes-mediated weight loss in both BB rats and STZ diabetic rats, suggesting their use to treat T1DM.

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