Syngeneic transplantation of rat pancreatic islets into the spleen. Light and electron microscopical findings

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(Received 2 October 1986; Accepted 24 March 1987)

Key words: Lewis rat; Streptozotocin diabetes; Syngeneic islet transplantation; Spleen; Histochemistry; Ultrastructure

Summary

An islet transplantation model was established with the two congenic Lewis rat strains LEW.1W and LEW.1A, which were made diabetic by a single i.p. injection of 50 mg/kg b.w. streptozotocin. Isolated neonatal islets of the two strains served as a graft. Syngeneic transplantation into the spleen resulted in permanent graft survival and in normoglycemia of streptozotocin diabetic rats of the two strains. 200 days after transplantation functionally intact islets were demonstrable in the spleen by histological, histochemical and electron microscopical investigations. Histochemical findings indicate an activation of interdigitating reticular cells in the white pulp and of macrophages in the red pulp of the spleen as consequences of the islet transplantation. In addition a few electron microscopical findings concerning possible interactions between host plasma cells, lymphocytes and transplanted beta cells of islets are described and discussed.

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Presented in part at the Second European Congress on Cell Biology, July 6–11, 1986, Budapest, Hungary. Dedicated to Professor Dr. Z. Lojda on the occasion of his 60th birthday.

Introduction

This study describes for the first time light (histochemical and immunohistological) and electron microscopical findings in an islet transplantation model, in which donor and recipient came from the congenic Lewis rat strains LEW.1W and LEW.1A and in which the islet graft was cultured and functionally characterized in vitro before transplantation. This defined rat islet transplantation model allows the morphological investigation.

Materials and Methods

Animals and induction of diabetes

The two congenic rat strains LEW.1W/MaxK (RT1^u) and LEW.1A/MaxK (RT1^a) were bred in our breeding colony and kept under constant conditions (12 hr light–dark regime, 22 ± 2°C, 60 ± 5% relative humidity, standard pellet chow and tap water ad libitum).

Femal congenic LEW rats weighing 150–180 g were rendered diabetic with a single dose of streptozotocin (Upjohn Co., Kalamazoo, U.S.A.) (50 mg/kg body weight, i.p.). All rats remained diabetic without any further treatment for one week. Thereafter they were treated with a daily injection of insulin (2.0 IU) until they served as recipients. Only animals with a nonfasting plasma glucose concentration of 17 mmol/l were used for transplantation.

Islet isolation

Pancreatic islets were isolated using a modification of the collagenase digestion/Ficoll separation technique [6]. Under sterile conditions the pancreata were rapidly excised from the donor and connecting tissue and fat tissue were removed from the gland. Twenty pancreata were placed in a vial containing 5 ml collagenase solution (Serva, Heidelberg, F.R.G.) (0.6 mg/ml). After vigorous shaking by hand (over 5 min), the partly digested pancreatic tissue was transferred into a second vial containing 5 ml collagenase solution and was shaken by hand for about 5–7 min. The crude undigested pancreatic tissue was then transferred into a third digestion vial with 5 ml collagenase. Both vials were vigorously shaken until the supernatant contained almost only small exocrine particles and free islets. Crude undigested tissue particles were removed, the supernatant containing exocrine cells and islets were separated from the exocrine cells by centrifugation on a Ficoll (Pharmacia, Uppsala, Sweden) gradient (10 ml 25%, 4 ml 23%, 4 ml 21%, 4 ml 12% Ficoll solution) for 20 min at 600 g. Free islets were hand-picked with a finely drawn Pasteur pipette under a dissecting microscope.

Islet culture

The isolated islets were washed two times with tissue culture medium 199 from Parker, supplemented with 10% newborn calf serum (both from Institut für Immunpräparate und Nährmedien, Berlin, G.D.R.), 4.5 mmol/l Mg^{++} , 20 mmol/l HEPES, 50 mg/l gentamycine and glucose to a final concentration of 10 mmol/l. Batches of 1000–1300 islets were cultured for 4 or 5 days in a humidified atmosphere of 5% CO_2 in air at 37°C in petri dishes containing 3 ml of the medium described above [14]. After 24 h the culture medium was changed.

Viability test in vitro

Freshly isolated islets and cultured islets were functionally characterized by incubating 5 islets per tube in 200 μ l of a supplemented Krebs-Ringer-bicarbonate buffer [9] at 1.5, 10 or 20 mmol/l glucose in the presence of 370 kBq 3H -leucine (spec. radioactivity: 2.164 TBq/mmol; Amersham, UK) for 2 h. Methodological details have been described previously [9,11]. The 3H -leucine incorporation into (pro)insulin was measured by immunoprecipitation of insulin and proinsulin with a specific antibody according to the method of Jahr et al. [4]. Insulin release and insulin content were determined radioimmunologically [3].

Islet transplantation

Islets (800–1100) were implanted into the spleen of diabetic recipients by injecting the islets with a disposable scalp vein set. The spleen was exposed via an abdominal incision during hexobarbital anesthesia (0.1 g/kg b.w.) of the diabetic animals. The recipient rats were treated with insulin in a decreasing dosage (2.0–0.8 IU/day) for the first three days after transplantation. Plasma glucose levels were determined daily for three weeks and then weekly up to 200 days after transplantation. Blood was taken from the tail vein of the animals at approximately 7 a.m. The rats were considered as successfully transplanted if the plasma glucose concentration was 11 mmol/l or less. Plasma glucose was measured using the Beckman glucose analyzer (model ERA 2001).

Histochemical and immunohistological investigations

200 days after islets had been transplanted the spleen was removed under hexobarbital anesthesia, fixed in Bouin's solution for 20 hr at room temperature, dehydrated in graded alcohol solution and embedded in paraffin. Serial sections were stained with hematoxylin/eosin and for insulin reactivity with Victoria blue 4 R [12], pseudoisocyanine chloride [8], aldehyde fuchsin [2] and colloidal iron [5]. Immunofluorescence of insulin was histochemically determined with guinea pig anti-porcine

insulin antiserum (1:5) and fluorescein-labelled immunoglobulin [1]. Control sections were incubated with fluorescein-labelled immunoglobulin alone. Additionally some other reactions were tested (alcian blue; pH 1.5, toluidine blue, pH 4.5; methylene blue; PAS-reaction) in connection with the characterization of noninsulin containing splenic cells, which are reactive with victoria blue 4 R.

Electron microscopical investigations

Splenic tissue was separated within 30–90 s of removal into 1 mm³ pieces and subsequently, immersed in ice-cooled glutaraldehyde (pH 7.2) for 2 hr. After washing in 0.2 mol/l saccharose solution the pieces were postfixed for 2 hr in 1% isotonic OsO₄ solution (pH 7.2). They were hydrated in Tyrode's solution and dehydrated in an ascending acetone series, with adding of 0.5% phosphotungstic acid and 0.5% uranyl acetate at the 70% acetone stage. Embedding in Mikropal with polymerization at 60°C. Ultrathin sections of transplanted islets and from the borderline between the islets and splenic tissue were prepared with the aid of semithin sections stained with Giemsa's method, using an OmU₂ ultramicrotome (Reichert Co., Vienna, Austria). Sections were post-contrasted with lead nitrate and sodium citrate [7] and examined in a SEM 3-2 type 100 kv electron microscope (FSE-works, Berlin-Oberschöneweide, G.D.R.).

Results

In vitro function of islets

Freshly isolated neonatal islets of the two congenic rat strains LEW.1W and LEW.1A did not differ in their beta cell specific functions, which were characterized by measuring the ³H-leucine incorporation into (pro)insulin, insulin release and insulin content. The glucose-stimulated ³H-leucine incorporation into (pro)insulin showed a nearly seven fold and the glucose-stimulated insulin release an almost twelve fold increase, when the glucose concentration was enhanced from 1.5 to 20 mmol/l.

Neonatal pancreatic islets cultured for 4 days in tissue culture medium 199 at a glucose concentration of 10 mmol/l in the presence of 5.3 mmol/l Mg⁺⁺ accumulated insulin during the culture period up to 130% in comparison to the insulin content of freshly isolated islets.

As a result of culture, the islets showed an increased sensitivity to glucose demonstrated by higher absolute rates of ³H-leucine incorporation and insulin release at 10 mmol/l glucose.

The only difference between the beta cell functions of neonatal islets of LEW.1W and LEW.1A rats was observed in the glucose-stimulated ³H-leucine incorporation

Fig. 1. Plasma glucose values of streptozotocin diabetic LEW.1W rats after syngeneic islet transplantation.

into (pro)insulin and insulin release. At the glucose concentration of 20 mmol/l ^3H -leucine incorporation into (pro)insulin was higher and the insulin release was lower in islets of LEW.1A rats after culture compared to LEW.1W islets.

Transplantation model

The transplantation of 800–1100 isolated islets from LEW.1A rats and LEW.1W rats resulted in a normalizing of plasma glucose levels within 1–8 days in the syngeneic system (Fig. 1). Allogeneic islet transplantation across the strong histocompatibility barrier ($\text{RTI}^a \rightleftharpoons \text{RTI}^b$) did not result in a normalizing of hypoglycemia.

Light microscopically, a nonaltered border was observed between transplanted islet tissue (Fig. 2A, below) and splenic tissue (Fig. 2A, top). In some cases around the implanted islets few lymphocytic cells were observed in close contact with the implanted islet cells (Fig. 2B). The demonstration of insulin in beta cells by the indirect immunofluorescence technique and the histochemical reactivity with the basic dye victoria blue 4 R showed identical results (Figs. 2C, D). Individual cells, however, are also reactive in orthologic structures of the spleen (red pulp, macrophages; white pulp, nonlymphoid cells at the inner border of the marginal zone and in the periarteriolar lymphocytic sheaths (marginal 'metallophil' macrophages, interdigitating reticular cells)) only following preoxidation of sections and staining by victoria

Fig. 2. Section from the transplantation site with intact islet cell regions. (A) Histologically unchanged interface of transplanted islet cells (below) and splenic tissue with follicular artery (top). Hematoxylin-eosin. $\times 210$. (B) Lymphocytes (arrows) at the periphery of transplanted islet tissue. Hematoxylin-eosin. $\times 450$. (C) Insulin staining by victoriablue 4 R. Abundant insulin containing beta cells are to be seen. $\times 100$. (D) Indirect immunofluorescence with guinea pig anti-insulin serum. The islet graft shows a high fluorescence of insulin containing beta cells. $\times 200$.

Fig. 3. (A) The islet graft (left and right) show a high fluorescence of insulin containing beta cells (IC). Perifollicular no reactive cells are seen. F = follicle. $\times 145$. (B) Perifollicular reactive cells (IDC) (interdigitating reticular cells; macrophages) are stained by victoriablue 4 R after preoxidation of sections. Some macrophages (M) in the red pulp also reacted. $\times 145$.

TABLE I

SPLEEN OF FEMALE DIABETIC LEW-1W RATS AFTER SYNGENEIC ISLET TRANSPLANTATION. HISTOCHEMICAL INVESTIGATIONS

Staining reaction	Transplanted islets beta cells		Non-lymphoid cells of white and red pulp	
	Without preoxidation	With	Without preoxidation	With
Victoria blue 4 R	—	+	—	+
Aldehyde fuchsin	—	+	—	+
Pseudoisocyanin	—	+	—	+
Colloidal iron	(+)	+	(+)	+
Insulin staining by immunofluorescence	+		—	
Alcian blue, pH 1,5	—		(+)	
Toluidine blue, pH 4,5	(+)		(+)	
PAS reactivity	—		(+)	
Methylene blue	—		—	

blue, but not by immunohistochemical insulin demonstration (Figs. 3A, B). The same cells reacted in the white pulp of spleen in control rats, but with considerably less quantity and intensity. The staining properties of cells obtained by histochemical reactions are compiled in Tables I and II.

TABLE II

STAINING PROPERTIES OF CELLS BY VICTORIA BLUE IN THE SPLEEN OF FEMALE DIABETIC LEW-1W RATS AFTER SYNGENEIC ISLET TRANSPLANTATION

	Pretreatment of sections		Reacting substance
	Without preoxidation	With	
Transplanted beta cells	—	+	Insulin
Interdigitating reticular cells (White pulp)	—	+	Non-known (Electron microscopically: A high synthetic and secretory capacity of glycoproteins)
Activated macrophages (red and white pulp)	—	+	Phagosomal inclusions of insulin beta granules from attacked beta cells (?)
Mast cells	+		Heparin

Fig. 4. Well preserved beta cell in an islet of Langerhans 200 days after syngeneic islet transplantation into the spleen (cf. Figs. 2A–D). I = numerous mature insulin granules with light halo. The arrow heads point at immature insulin granules. ER = rough endoplasmic reticulum with partly dilated cisternae. RB = ribosomes in the cytoplasmic ground substance. M = mitochondria. N = nucleus. C = islet capillary with endothelial lining (E) and basement membrane (BM). PZ = parts of a pericyte. $\times 24400$.

Fig. 5. Well preserved beta cell in an islet of Langerhans 200 days after syngeneic islet transplantation into the spleen. I = numerous mature insulin granules with light halo. The arrow heads point at immature insulin granules. ER = rough endoplasmic reticulum. GA = Golgi complex. M = mitochondria. N = nucleus. NC = nucleolus. PS = perinuclear space. $\times 24800$.

Fig. 6. Infiltrating lymphocyte (L) into the cytoplasm of a beta cell in the periphery of a Langerhans' islet 200 days after syngeneic islet transplantation into the spleen (cf. Fig. 2B). External beta cell membrane cannot be detected at the contact site between lymphocyte and cytoplasm of the beta cell. I = insulin granules. ER = endoplasmic reticulum. M = mitochondria. RB = ribosomes. N = nucleus. $\times 24800$.

Fig. 7. Cytoplasmic components of beta cells with insulin granules (I), endoplasmic reticulum (ER), mitochondria (M) and ground substance (dissolved cytoplasm of beta cells) in splenic tissue between processes of reticular cells (RC). 200 days after syngeneic islet transplantation into the spleen of a female diabetic LEW.1W rat. $\times 25\,600$.

By electron microscopical investigation well preserved beta cells with immature and mature insulin granules in implanted islets were observed (Figs. 4 and 5). Corresponding to the light microscopical findings (Fig. 2B) lymphocytes and plasma cells in close contact with beta cells were seen. In these regions there are rarely infiltrating lymphocytes within the cytoplasm of beta cells. At the contact site between lymphocytes and beta cell plasma the external beta cell membrane could not be detected (Fig. 6). Between lymphocytes and processes of splenic reticular cells cytoplasmic components of destroyed beta cells were found (Fig. 7).

Discussion

Culture of pancreatic islets in tissue culture medium 199 from Parker at 10 mmol/l glucose and 5.3 mmol/l Mg^{++} , which was found to be favorable for the maintenance of islet functions to neonatal and adult Wistar rat islets [13,14] also proved to be suitable for neonatal Lewis rat islets in a large-scale culture of about 1000 islets per disk.

The syngeneic transplantation (LEW.1W $\rightarrow$ LEW.1W or LEW.1A $\rightarrow$ LEW.1A) into the spleen resulted in permanent graft survival and in normoglycemia of streptozotocin diabetic rats of the two strains (Fig. 1). In concordance with these functional results 200 days after transplantation the mass of islet cells in the spleen was demonstrable to be morphologically intact by histological, histochemical and electron microscopical investigation. The histochemical findings indicate an activation of red pulp macrophages and of phagocytic resp. secretoric active non-lymphoid cells in the white pulp as a consequence of the islet transplantation. There is no definitive interpretation of our findings in relation to the close contact between lymphocytes, plasma cells and transplanted beta cells and the infiltration of beta cells by lymphocytes (emperipolesis). The latter findings could be the expression of a mild rejection reaction of host lymphocytes against the transplanted beta cells in the syngeneic system too. Schwizer et al. [10] have observed the destruction of cultured mouse islet cells (CBA/J mice) by syngeneic splenic macrophages in vitro. These authors suggest the possibility that similar destructive interactions between macrophages and glycemia-stressed beta cells occur in vivo. The observed remains of dissolved beta cells between splenic reticular cells could derive from such cytolytic reactions or could be indications for a persistent dissolution of few beta cells ('physiological' molting?).

Acknowledgements

This study is part of the research project HFR 22 (Diabetes mellitus) of the Ministry of Health of the German Democratic Republic.

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