Hyperglycemia-induced B Cell Toxicity

The Fate of Pancreatic Islets Transplanted into Diabetic Mice Is Dependent on Their Genetic Background

Olle Korsgren, Leif Jansson, Stellan Sandler, and Arne Andersson
Department of Medical Cell Biology, Uppsala University, Uppsala, S-751 23 Sweden

Abstract

The role of pancreatic B cell dysfunction in the phase preceding clinical onset of insulin-dependent and non-insulin-dependent diabetes mellitus has been much debated. In this investigation, the impact of a prolonged diabetic environment on pancreatic islet B cells transplanted syngeneically under the kidney capsule of C57BL/6 (B6) and C57BL/Ks (BKs) mice was studied. Alloxan-diabetic mice bearing a subcapsular islet graft insufficient to normalize the blood glucose level were rendered normoglycemic by a second intrasplenic islet graft after various periods of hyperglycemia to examine the reversibility of hyperglycemia-induced B cell dysfunction. Using a perfusion technique of the graft-bearing kidney, it was found that both strains of mice exhibited a diminished glucose-induced insulin secretion after 6 wk of hyperglycemia, when compared with normoglycemic mice carrying islet grafts. When normoglycemia was restituted by the splenic graft after 4 or 12 wk, there was a normalization of glucose-stimulated insulin secretion in the renal islet grafts in B6 mice, whereas insulin secretion from the grafted BKs islets remained impaired. Morphometric measurements of the islet grafts demonstrated a 50% reduction in the graft volume in diabetic BKs mice after 12 wk, compared with normoglycemic animals, whereas no such decrease was observed in B6 mice. Islet grafts removed from hyperglycemic mice of both strains exhibited diminished insulin mRNA contents, and in the BKs mice there was also a reduced glucose oxidation rate in the islet grafts in vitro. This metabolic dysfunction can only partly be explained by a reduced graft size. The present findings emphasize the genetic constitution as a decisive factor for the survival and function during a period of sustained stress on a limited B cell mass. (*J. Clin. Invest.* 1990. 86:2161–2168.) Key words: pancreatic islets • transplantation • hyperglycemia • insulin secretion

Introduction

It has been proposed that persistent hyperglycemia induces an impairment of glucose-stimulated insulin release (1, 2). Experimental data indicate that prolonged glucose infusions in vivo for as short a time period as 48 h may induce an inability of islet B cells to release insulin in response to a glucose stimulus (3). Subtotal pancreatectomy, sufficient to cause elevated basal glucose concentrations, also seems to induce an impaired B cell function (4). However, it has been difficult to demonstrate these glucose effects in tissue culture where it seems as if B cells adapt more readily to high glucose concentrations (5–7).

We recently described a technique in which islets are transplanted beneath the renal capsule of mice, followed by perfusion of the transplant-bearing kidney and measurements of hormone contents in the effluent medium (8, 9). When a small number of islets were transplanted into alloxan-diabetic mice and thereby kept in a diabetic environment in vivo for various lengths of time, it was found that exposure to hyperglycemia for 4 wk severely impaired both the insulin release in response to glucose or arginine and also glucose-stimulated (pro)insulin biosynthesis in the grafts (9).

The aim of this study was to investigate, in a diabetes-susceptible and in a more resistant inbred strain of mice (10), whether the impairment in islet graft function observed in hyperglycemic recipients was reversible if normoglycemia was restored, and if the length of the exposure time to hyperglycemia had any importance in this context. Furthermore, a more detailed metabolic characterization of the islet grafts was performed to obtain a better insight into the mechanisms underlying impaired B cell function. Also, total islet graft volume was estimated by morphometric techniques to establish whether or not the long-term hyperglycemic stress led to a change of the graft volume.

Methods

Animals. Male and female inbred C57BL/6 (B6)[1] and C57BL/Ks (BKs) mice bred at the Biomedical Centre (Uppsala, Sweden), originally obtained from the Jackson Laboratory, Bar Harbor, ME, aged 3–5 mo, were used as islet donors. Syngeneic males of the same age served as recipients. Some of the animals had been made diabetic by a single intravenous injection of alloxan (75 mg/kg body wt; Sigma Chemical Co., St. Louis, MO) 6–8 d before the transplantation. All animals had free access to tap water and pelleted food (type R3, Ewos, Anticimex, Södertälje, Sweden) throughout the experimental period.

Islet isolation and transplantation. Pancreatic islets were prepared by a collagenase (Boehringer Mannheim, FRG) digestion method. Groups of ~ 150 isolated islets were cultured free-floating for 1–2 d in medium RPMI 1640 (Flow Laboratories, Ltd., Irvine, UK) supplemented with 10% (vol/vol) calf serum (Statens Bakteriologiska Laboratorium, Stockholm, Sweden) as previously described in detail (11).

After culture, syngeneic islets were implanted through an incision in the left renal capsule of ether-anesthetized normoglycemic or alloxan-diabetic mice. The islets were injected by means of a glass braking pipette, and the capsulotomy was left unsutured. At different times thereafter some diabetic animals were given a second intrasplenic islet graft, sufficiently large to normalize their blood sugar concentrations, via a microinfusion set (Butterfly-25, Abbot Ireland Ltd., Sligo, Eire).

Address reprint requests to Dr. Korsgren, Department of Medical Cell Biology, Biomedical Center, P.O. Box 571, S-751 23 Uppsala, Sweden.

Received for publication 28 February 1990 and in revised form 6 June 1990.

J. Clin. Invest.

0021-9738/90/12/2161/08 $2.00
Volume 86, December 1990, 2161–2168

1. *Abbreviations used in this paper:* B6, C57BL/6 (mice); BKs, C57BL/Ks (mice).

Nine different recipient animals groups were designated as follows: (A) normoglycemic B6 mice transplanted with 150 islets and otherwise untreated; (B) alloxan-diabetic, hyperglycemic B6 mice transplanted with 150 islets; (C and D) alloxan-diabetic, hyperglycemic B6 mice transplanted first with 150 islets and then, after (C) 4 or (D) 12 wk, with 500 islets into the spleen; this latter graft normalized their serum glucose concentrations for the remaining 2 wk; (E) normoglycemic BKs mice transplanted with 250 islets and otherwise untreated; (F) alloxan-diabetic, hyperglycemic BKs mice transplanted with 250 islets; (G and H) alloxan-diabetic, hyperglycemic BKs mice transplanted with 250 islets and then, after (G) 4 or (H) 12 wk, with a second intrasplenic transplant of 750 islets, which normalized their serum glucose concentration for the remaining 2 wk; (I) alloxan-diabetic, hyperglycemic BKs mice transplanted with 250 islets and then after 1 wk transplanted with a second intrasplenic graft of 750 islets, which normalized their serum glucose concentrations for the remaining 5 wk. Mice in groups A, B, E, and F were all killed after 6 wk. The outline of the different experimental groups is also given in Table I. Some animals belonging to group D became normoglycemic within 12 wk and were excluded from the perfusion experiments (see below). A nephrectomy was performed in these animals after 14 wk to examine the role of the islet graft in the maintenance of normoglycemia.

Blood samples for glucose determinations were collected, by puncture of the retro-orbital venous plexus, immediately before islet transplantation (diabetic recipients) and at the end of the observation period. The glucose concentrations of the samples were determined with an automated glucose oxidase method (Glucose Analyzer, model 2, Beckman Instruments, Inc., Fullerton, CA).

Perfusion of graft-bearing kidneys. This technique has recently been described in detail (9). Briefly, the kidney was removed together with part of the aorta and inferior vena cava. Both the ureter and the renal vein were cut, whereas the aorta was cannulated and infused with a continuously gassed (O_2/CO_2 = 95:5) Krebs-Ringer bicarbonate buffer supplemented with 2.0% (wt/vol) each of bovine serum albumin (fraction V, Miles Laboratories, Ltd., Slough, UK) and dextran T70 (Pharmacia Fine Chemicals, Uppsala, Sweden) and 2.8 or 16.7 mM D-glucose. In some cases 5 mM theophylline was also added. The medium was administered at a rate of 1.0 ml/min without recycling for 85 min with a perfusion pressure of ~ 40 mm Hg.

Perfusion experiments started with a 15-min period using a medium containing 2.8 mM glucose, followed by 20 min with 16.7 mM glucose, 15 min with 2.8 mM glucose, 20 min with 16.7 mM glucose + 5 mM theophylline, and finally 15 min with 2.8 mM glucose. A 1.0-ml sample of the effluent medium was collected every fifth minute, except for the first 10 min of perfusion with the high glucose concentrations when samples were taken after 1–5, 7, and 10 min. The insulin concentrations of these samples were measured by radioimmunoassay (12). The rate of insulin secretion was calculated by multiplying the insulin concentration in the sample by the flow rate, giving values expressed as nanograms of insulin per minute. The total stimulated insulin response to either glucose or glucose plus theophylline of the grafts was obtained by planimetry of the areas for each individual perfusion curve. Insulin release during perfusion with low glucose (2.8 mM) in each individual experiment did not differ between the experimental groups and was therefore designated as zero when the areas were measured. During perfusion with 16.7 mM glucose, areas were measured separately during the first 5 min and during the next 15 min to evaluate the first and second phases of insulin secretion separately. When the grafts were stimulated with both 16.7 mM glucose and 5 mM theophylline, the area corresponding to the total insulin secretion during the 20-min period was also calculated.

Insulin contents of the islet grafts. After perfusion the graft was removed from the kidney as previously described (9) and homogenized in 100 μl of redistilled water, and the vial was then washed three times, each with 100 μl of redistilled water. The whole aqueous homogenate (400 μl) was sonicated to disrupt any remaining intact cells. A sample of 50 μl was removed from the homogenate and transferred to tubes containing 125 μl acid-ethanol (0.18 M HCl in 95% [vol/vol] ethanol). The samples were extracted overnight at 4°C, followed by radioimmunological assay of insulin (12).

Glucose oxidation of islet grafts. For these experiments animals that had been either normoglycemic or hyperglycemic for 6 wk after transplantation were used (groups A, B, E, and F). After removing the graft (9), it was incubated for 90 min in 100 μl of a Krebs-Ringer bicarbonate buffer containing both D-[U-^{14}C]glucose (Amersham International, Amersham, UK) and nonradioactive glucose giving a final glucose concentration of 2.8 or 16.7 mM and a specific radioactivity of 0.79 or 0.13 mCi/mM. Glucose oxidation was then assessed by measurements of the amount of radioactive CO_2 released (5). In a separate series of experiments (groups E, F, and G) the dry weight of the incubated grafts was estimated. It was assumed that the nonendocrine elements of the graft (e.g., connective tissue, blood vessels, and the kidney capsule covering the graft) were present to the same extent in all experimental groups. This assumption is supported by the light micro-

Table I. Treatment of Different Groups of Mice and Serum Glucose Concentrations before and after Islet Transplantation

Group	Strain	Alloxan	*n*	1st renal islet graft	2nd splenic islet graft	Serum glucose: Before 1st graft	Serum glucose: Before 2nd graft	Serum glucose: At killing
				number of islets; length of time			*mM*	
A	B6	−	22	150; 6 wk	—	ND	—	8.6±0.3
B	B6	+	20	150; 6 wk	—	36.8±1.0	—	35.1±1.6
C	B6	+	5	150; 4 + 2 wk	500; 2 wk	35.4±2.5	28.0±1.7	9.8±0.7
D	B6	+	4	150; 12 + 2 wk	500; 2 wk	40.2±2.9	34.8±1.0	9.2±0.6
E	BKs	−	22	250; 6 wk	—	ND	—	9.3±0.2
F	BKs	+	17	250; 6 wk	—	43.7±1.3	—	37.5±1.4
G	BKs	+	5	250; 4 + 2 wk	750; 2 wk	29.2±1.6	33.2±1.3	9.4±0.6
H	BKs	+	5	250; 12 + 2 wk	750; 2 wk	45.9±2.6	37.5±1.1	11.1±0.4
I	BKs	+	5	250; 1 + 5 wk	750; 5 wk	45.7±1.0	27.0±0.9	9.1±0.8

Experimental group designation, mouse strain, treatment with alloxan, and number of animals (*n*) are indicated in columns 1–4. Number of syngeneic islets transplanted either beneath the kidney capsule or intrasplenically (columns 5 and 6) is given. Periods of time with only a renal islet graft with or without a second intrasplenic islet graft (columns 5 and 6) are given. Serum glucose concentrations before the first and second islet graft and death are shown in columns 7–9. Values for serum glucose concentrations are means±SEM. ND, not determined.

scopical appearance of the corresponding grafts used for volume measurements (see below). Furthermore, none of these nonendocrine tissues are likely to increase their rate of glucose oxidation when challenged high glucose (cf. 13, 14).

Insulin mRNA content of islet grafts. Transplanted mice which had been either normoglycemic or hyperglycemic for 6 wk (groups A, B, E, and F) were used for these experiments. The grafts were removed and immediately homogenized and sonicated in 400 μl 1% sodium dodecylsulphate buffer (SDS; BDH Chemicals, Poole, UK) and 400 μl phenol chloroform/isoamylalcohol solution (15). The sonicates were reextracted with phenol/chloroform/isomethylalcohol and nucleic acids precipitated with 0.07 M potassium acetate in ethanol overnight at −20°C. A dot-blot analysis was then performed as previously described after denaturation of RNA in glyoxal (15, 16). The probe for hybridization was pRI-7 (17). After hybridization at 42°C in 50% formamide the filters were washed three times for 30 min at 55°C in 15 mM NaCl, 1.5 mM Na-citrate + 0.1% SDS and exposed at −70°C to Hyperfilm-MP (Amersham International) with an intensifying screen. The absorbance of the spots obtained was determined by densitometry. The mean values recorded for the different control groups (*A* and *E*, respectively), were set to 100 arbitrary units. All individual values obtained were subsequently expressed in relation to this scale. The dot-blot analysis was run in one single assay for each mouse strain. The assay was linear over the range of concentrations of insulin mRNA tested, without any detectable background hybridization.

Volume measurements of islet grafts. In this separate series of experiments either alloxan-diabetic or normoglycemic mice of both mouse strains served as recipients of 150 syngeneic islets grafted under the kidney capsule as described above. The animals were killed at 2, 4, or 12 wk after the transplantation. The islet graft could easily be localized as a whitish spot, and before embedding it was cut out with a few millimeter-wide margin. It was then processed for light microscopy and the sections were stained with hematoxylin and eosin. Care was taken to use a uniform procedure for paraffin embedding of the tissue to avoid variations in shrinkage of the tissue during the histological processing. The area of the endocrine part of the graft, which could be clearly distinguished, was measured in every second of consecutive serial sections by using a computerized system for morphometry (MOP-Videoplan, Carl Zeiss, Svenska AB, Stockholm, Sweden). Total graft volume in each animal was then calculated as the total area two times the section thickness (7 μm) (18).

Statistical analysis. All values are given as means±SEM. Probabilities (*P*) of chance differences between the groups were calculated by Student's two-tailed *t* test. Glucose oxidation data were analyzed by two-factor analysis of variance, followed by multiple comparisons using Student's unpaired *t* test. *P* values were corrected for multiple comparisons using the Bonferroni method (19).

Results

Serum glucose concentrations (Table I). Alloxan induced a severe hyperglycemia before the animals received their islet grafts (Table I). The first renal islet graft, in general, slightly reduced the serum glucose concentrations, but the animals still remained markedly diabetic. When an additional 500 (B6 mice, groups C and D) or 750 (BKs mice, groups G, H, and I) islets were inserted into the spleen, the serum glucose concentrations were normalized in all animals and did not exceed 12 mM, 2 wk later. The serum glucose concentrations of mice belonging to group H were, however, slightly higher ($P < 0.05$) than those of control mice (group E). Of the diabetic animals implanted with 150 (B6 mice, group B) or 250 (BKs mice, group F) islets beneath the renal capsule, only those with serum glucose concentrations in excess of 22 mM at the end of the 6-wk observation period were included in the study. A total of 7 B6 mice (4 out of 8 animals originally belonging to group D, and 3 out of 23 belonging to group B) were excluded for this reason, since they all slowly reverted to normoglycemia. When the transplant-bearing kidney was removed 14 wk posttransplantation, all the "cured" animals of group D became diabetic again.

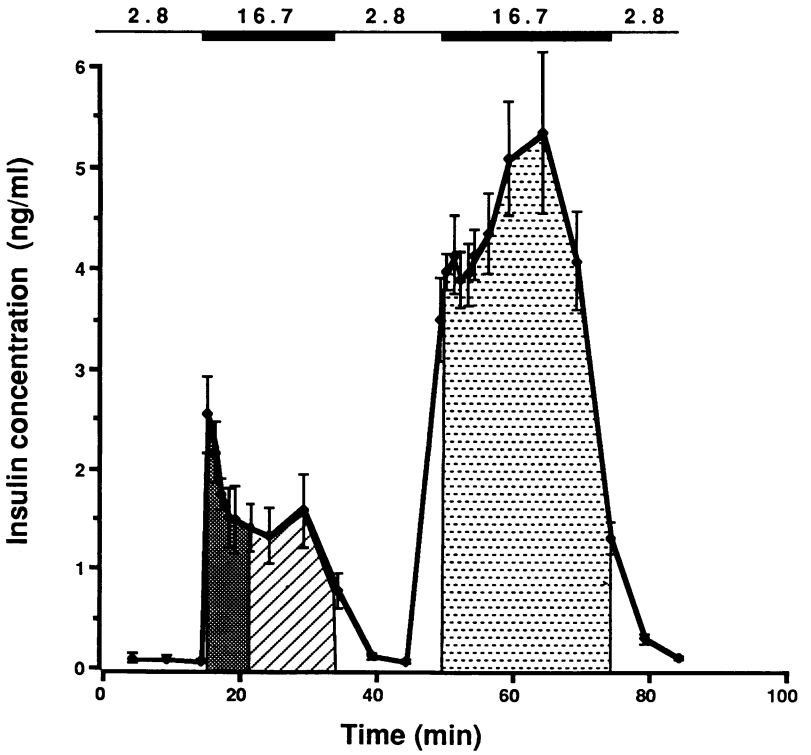

Figure 1. Insulin concentrations in the effluent medium collected from islet graft-bearing kidneys of BKs mice 6 wk after transplantation (group E). Kidneys were perfused with a medium containing either 2.8 or 16.7 mM glucose as indicated at the top. During the final stimulation with 16.7 mM glucose, 5 mM theophylline was also added. The gray, hatched, and dotted areas, respectively, correspond to the areas used to calculate the accumulated insulin secretion during the first (1–5 min) and second phases (5–20 min) of insulin secretion after stimulation with glucose, and during stimulation with both glucose and theophylline for 20 min.

Perfusion experiments (Figs. 1–3): A first-phase insulin release in response to stimulation with a high glucose concentration (min 16–20) and a sustained second phase (min 21–35 min) was observed in all animals being normoglycemic throughout the study (groups A and E). In contrast, only a minute insulin response was observed in the 6-wk hyperglycemic B6 mice (group B), and in the BKs mice (group F) there was no stimulation at all. The hyperglycemic B6 mice treated with a second curative intrasplenic graft (groups C and D) showed an almost complete restoration of glucose-induced insulin secretion irrespective of whether hyperglycemia had lasted for 4 or 12 wk. The corresponding BKs mice (groups G and H) were unable to restore glucose-induced insulin release under these experimental conditions. BKs islet grafts exposed to hyperglycemia for only 1 wk (group I) showed a first-phase glucose-stimulated insulin release which was almost identical to that of the normoglycemic controls (group E), but the second phase was slightly reduced.

When both a high glucose concentration and 5 mM theophylline were added to the perfusion medium, a marked insulin response was observed from all grafts in normoglycemic animals of both strains (groups A, C, D, E, G, and I), irrespective of the time period of hyperglycemia preceding the final 2 wk of normoglycemia. The only exception was the lower response of the grafts of the BKs mice (group H) that had been hyperglycemic for 12 wk before receiving the intrasplenic graft. Also in the grafts from the continuously hyperglycemic mice (groups B and F) the combined stimulation with glucose and theophylline exerted a stimulatory effect on the insulin release, although it was not as pronounced as in the normoglycemic animals. In all perfusion experiments insulin release returned to basal values, close to or below the detection limit of the radioimmunoassay for insulin, whenever the medium glucose concentration was lowered to 2.8 mM.

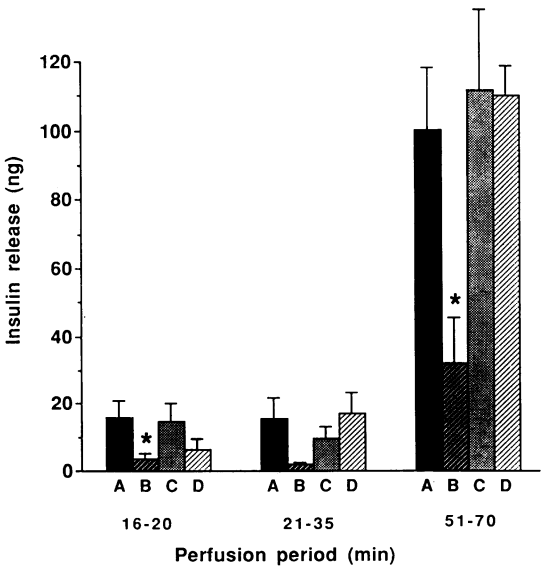

Figure 2. Insulin secretion in response to 16.7 mM glucose or 16.7 mM glucose plus 5 mM theophylline during perfusion of islet graft-bearing kidneys of B6 mice. The first group of bars represents the first phase of glucose-induced insulin secretion (gray area in Fig. 1), the second group the second phase (hatched area in Fig. 1), and the third group the total insulin secretion during stimulation with 16.7 mM glucose plus 5 mM theophylline (dotted area in Fig. 1). The bars represent means±SEM.* $P < 0.05$ vs. group A.

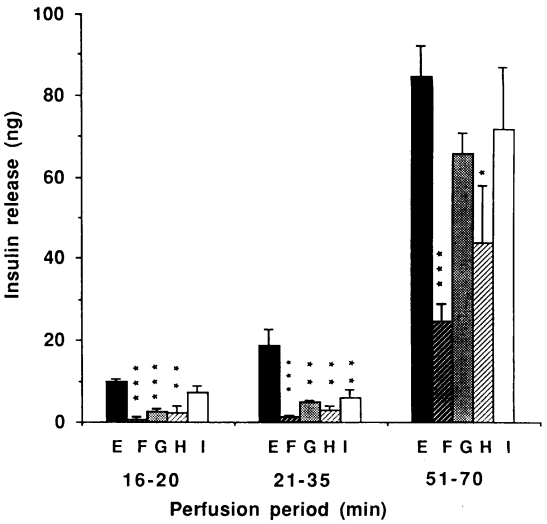

Figure 3. Insulin secretion in response to 16.7 mM glucose or 16.7 mM glucose plus 5 mM theophylline during perfusion of islet graft-bearing kidneys of BKs mice. Groups of bars as in Fig. 2. *, **, and *** $P < 0.05$, 0.01, and < 0.001 vs. group E.

Insulin contents of the islet grafts (Fig. 4). The insulin contents were significantly lower in the grafts removed from mice of both strains that had been continuously hyperglycemic for 6 wk (groups B and F). In the diabetic B6 mice, which had been cured after 4 or 12 wk of hyperglycemia (groups C and D), the insulin content of the graft was similar to that found in the normoglycemic mice (group A). In contrast, in the BKs mice that had been diabetic for 4 wk and then cured (group G), there was still a marked reduction of the insulin content of the graft. In the 12 wk diabetic BKs mice (group H) the decrease in insulin content did not, however, attain statistical significance. Somewhat surprisingly, the 1-wk diabetic BKs mice that subsequently were kept normoglycemic for 5 wk (group I), had islet grafts containing more insulin than the controls (group E).

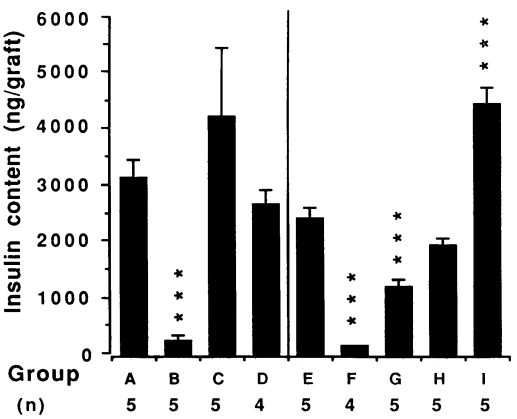

Figure 4. Graft insulin contents of syngeneic islets transplanted beneath the kidney capsule of either B6 (groups A–D) or BKs (groups E–I) mice. The bars represent means±SEM and the number of animals (n) is given below the bars. *** $P < 0.001$ compared with the corresponding values for the normoglycemic mice of the same strain.

Table II. Glucose Oxidation Rates and Insulin mRNA Contents of Pancreatic Islet Grafts Syngeneically Transplanted beneath the Renal Capsule of B6 and BKs Mice

Group	Strain	Glucose oxidation		Insulin mRNA
		2.8 mM glucose	16.7 mM glucose	
		pmol/graft × 90 min		*arbitrary units*
A	B6	923±51 (5)	3,903±463 (6)*	100±2 (6)
B	B6	1,117±98 (5)	4,308±865 (4)*	63±6 (6)§
E	BKs	937±126 (5)	4,407±554 (6)*‡	100±16 (6)
F	BKs	690±153 (4)	1,955±207 (4)*	21±4 (5)§

Glucose oxidation rates and insulin mRNA contents of the removed islet grafts were measured as described in the Methods section. Values are means±SEM for the number of animals given within parentheses. * $P < 0.05$ when comparing glucose oxidation rates at 2.8 and 16.7 mM glucose, of animals of the same group. ‡ $P < 0.05$ when comparing glucose oxidation rates at 16.7 mM glucose in animals of groups E and F. § $P < 0.001$ when comparing animals of the same strain.

Glucose oxidation and insulin mRNA contents of the islet grafts (Table II). Islet grafts removed from either normoglycemic or hyperglycemic B6 mice (groups A and B) both had higher glucose oxidation rates when incubated at a high glucose concentration (16.7 mM) than at low glucose (2.8 mM). The normoglycemic BKs mice (group E) had basal and stimulated glucose oxidation rates similar to those of the B6 mice. However, when determined at 16.7 mM glucose, the glucose oxidation rate of the islet grafts of the hyperglycemic BKs mice (group F) was only half of that observed in the normoglycemic mice (group E).

In a separate series of experiments incubated grafts of BKs mice (groups E, F, and G) were recovered for dry weight measurements after the glucose oxidation experiments. The oxidative rates (pmol glucose/90 min × μg graft) were 29.5±3.7 (group E; $n = 5$), 16.3±3.0 (group F; $n = 6$, $P < 0.05$ vs. group E) and 16.8±3.7 (group G; $n = 5$, $P < 0.05$ vs. group E).

Islet grafts of mice that had been hyperglycemic for 6 wk (groups B and F) had significantly lower insulin mRNA contents, when compared with normoglycemic animals (groups A and E). The reduction was more pronounced in the diabetic BKs mice.

Islet graft volumes (Table III). In the B6 mice the volumes of islet grafts transplanted into hyperglycemic and in normoglycemic mice were similar in all experimental groups irrespective of the length of time the animals had been transplanted. In the BKs mice the islet graft volume, however, decreased with time in both the normo- and hyperglycemic mice. This decrease was most marked in the diabetic animals, so that at 4 and 12 wk after transplantation the islet graft volumes were diminished to only two-thirds and one-half, respectively, of that in the corresponding normoglycemic mice.

Discussion

The possibility that a glucose-induced B-cell dysfunction, especially when imposed upon an already reduced B cell mass, can explain the insulin secretory defects in non–insulin-dependent diabetes and early phases of insulin-dependent diabetes has been suggested previously (1, 2). Experimental support for this notion is based mainly on three animal models, namely, neonatal streptozotocin injections in rats (20, 21), surgical reduction of the B cell mass (4, 22), or continuous intravenous glucose infusions (3, 23). In the present study, islets in a number insufficient to cure immediately a diabetic recipient were transplanted into either hyper- or normoglycemic mice. This technique offers unique possibilities for studies of long-term effects of hyperglycemia, i.e., several weeks or months, on B cell function without exposure of the B cells themselves or their progenitors to B cytotoxic agents. The localization of the grafted islets under the kidney capsule also makes it easy to remove the graft; i.e., it gives access to an islet population well suited for subsequent biochemical investigations.

To elucidate to what extent the genetic trait of the recipients influenced the alleged glucose toxicity two genetically related mouse strains, B6 and BKs, were used. It is known that when the autosomal diabetes (db) mutation is placed on the BKs background, the animals develop an overt diabetes, ultimately leading to premature death (24). In contrast, on the B6 background the diabetes evolved is milder and compensated for by islet hypertrophy. Moreover, male mice of the BKs strain are particularly sensitive to diabetes induced by multiple low-dose streptozotocin administration, whereas B6 mice are more resistant to this mode of treatment (25). The two strains

Table III. Serum Glucose Concentrations and Islet Graft Volumes of Various Groups of Mice at Different Time Points after Transplantation

Group	Serum glucose			Islet graft volume		
	2 wk	4 wk	12 wk	2 wk	4 wk	12 wk
		mM			*nl*	
Normoglycemic B6 mice	9.2±0.6 (7)	7.7±0.5 (8)	8.6±0.3 (11)	168±15	143±12	146±12
Alloxan-diabetic B6 mice	33.5±1.7 (8)*	34.6±2.5 (5)*	39.7±2.0 (5)*	181±24	146±13	168±34
Normoglycemic BKs mice	9.7±0.3 (8)	9.8±0.4 (7)	8.6±0.4 (6)	181±9	155±7	122±16
Alloxan-diabetic BKs mice	32.6±5.1 (7)*	41.0±1.8 (5)*	42.4±1.4 (8)*	194±28	92±10*	61±6*

Groups of animals given in the first column were transplanted beneath the kidney capsule with 150 syngeneic islets. Serum glucose concentrations at death are given in columns 2–4. Islet graft volumes (columns 5–7) were estimated in histological sections, using a computerized morphometric procedure. Values are means±SEM for the number of mice given within parentheses. * $P < 0.001$ compared with normoglycemic mice of the same strain at corresponding weeks after transplantation.

also exhibit different expressions of endogenous retroviral genes in response to high glucose (26), and BKs mice but not B6 mice spontaneously produce islet autoantibodies (27). Previous experiments with transplanted islets have also demonstrated that islet cells have a higher regeneratory capacity in B6 mice than in BKs mice (28). Taking all these findings into account, the BKs mice would represent animals more sensitive than B6 mice to various assaults on their islet cells. This notion was supported by the current volume measurements of the islet grafts, showing a marked reduction in the volume of endocrine tissue in grafts of hyperglycemic BKs mice, whereas such a reduction did not occur in the corresponding B6 mice. These data are in line with those of two previous studies dealing with intrasplenically transplanted islets (29, 30). Furthermore, some of the diabetic B6 mice transplanted with 150 islets became normoglycemic after 2–3 mo. In contrast, all BKs mice remained hyperglycemic despite being transplanted with 250 islets. This suggests that in those B6 mice, the B cells, probably by hypertrophy and hyperplasia, could overcome putative destructive processes to finally compensate the glycemic stress. The prompt return of hyperglycemia upon removal of the graft-bearing kidney rules out the possibility that a recovery of endogenous islets in the pancreas was the reason for normalization of the serum glucose.

In those BKs mice which were hyperglycemic for 4 or 12 wk after transplantation and then rendered normoglycemic for 2 wk (groups G and H), both the first and second phases of insulin response to stimulation with 16.7 mM glucose were markedly reduced. These findings suggest a permanent functional impairment of the islet grafts in these mice. The increased insulin content and the almost normal insulin secretion from the renal islet grafts exposed to hyperglycemia for only 1 wk (group I) show that the impaired B cell function depends on the length of exposure to high glucose. In addition, this finding suggests that the engraftment process is not influenced to any major degree by the diabetic state; i.e., the reported findings are not due to an immediate destruction of B cells. Nevertheless, the present estimates of islet graft volume indicate in the BKs mice that some endocrine cells had degenerated during the period 2–12 wk after implantation. On the other hand, the corresponding B6 mice had an almost completely restored glucose-stimulated insulin release after 2 wk of normoglycemia following the initial 4–12 wk of hyperglycemia. This may be taken to indicate that, in diabetic mice of this strain (group B), the low rate of insulin secretion from perfused grafts in response to glucose was due to a marked degranulation of the B cells. This presumably reflects a physiologic adaptation to the hyperglycemic conditions, rather than a permanent functional impairment or a reduction in B cell mass, and can therefore be viewed as a form of glucose desensitization (31, 32).

The obvious question arising is how a prolonged exposure to a hyperglycemic environment mediates inhibitory and perhaps cytotoxic effects to the B cells. The ability to increase glucose oxidation when stimulated with high glucose is a characteristic feature of the pancreatic B cell (33), and may also be a prerequisite for nutrient induced insulin secretion (34). The hyperglycemic BKs mice displayed a significantly decreased islet graft oxidation rate at 16.7 mM glucose, when compared with normoglycemic mice of the same strain. Although part of the decreased oxidative rate reflects a reduced graft volume, there are still reasons to believe that the diabetic condition induced a disturbed glucose metabolism in the remaining B cells. Furthermore, BKs mice diabetic for 4 or 12 wk (groups G and H) showed a markedly impaired glucose-induced insulin release despite their fairly large insulin contents. The almost identical decrease in glucose oxidation in both the chronically diabetic (group F) and the cured (group G) mice indicate an irreversible loss of B cell function during the diabetic period. It may be speculated that in these cells a disturbed metabolism of glucose is a primary defect attained in a chain of events finally leading to B cell dysfunction. Such a defect would directly lead to an impaired glucose-stimulated insulin release (34). Furthermore, a reduction in glucose oxidation may cause a lowered generation of certain metabolites, which may regulate proinsulin gene expression (35–37), eventually leading to lower levels of the messenger. This is substantiated by a correlation between the metabolic state of the B cell and the proinsulin mRNA content (38). The present findings of low insulin mRNA contents, and the previously observed marked reduction in the rate of glucose-stimulated (pro)insulin biosynthesis (9) in islet grafts in long-term hyperglycemic mice, are thus in good accord with the suggested chain of events. It also relates to two other models of diabetes in rodents, namely partially pancreatectomized rats and db/db mice, in which there is a short period of compensatory increase of pancreatic proinsulin mRNA, at the early stage of glucose intolerance followed by a marked impairment, when diabetes becomes manifest (39, 40). It should also be noted, that during a number of experimental conditions there is a disturbed glucose-induced insulin release accompanied with a deficient glucose metabolism: e.g., islets cultured after previous exposure to streptozotocin (41, 42), islets isolated from neonatally streptozotocin injected rats (43), islets cultured in the presence of interleukin 1 (44), and in islets isolated from nonobese diabetic mice before the development of hyperglycemia (14). Incidentally, in addition to the present findings (cf. Fig. 3, groups G and H) phosphodiesterase inhibitors like theophylline can partially or totally overcome the inhibition in insulin secretion in many of these conditions of impaired insulin secretion (42, 45, 46). Whether or not the events proposed above for causing impaired B cell function also lead to cell death remains, however, unknown.

It cannot be ruled out that factors other than the hyperglycemia per se in the diabetic state contribute to the development of impaired B cell function. Hence, it has been difficult to demonstrate a disturbed B cell function in rodent pancreatic islets maintained in vitro for prolonged periods in the presence of high glucose concentrations (5–7). The present data, however, lend support to the concept of the existence in vivo of a glucose-induced impairment of B cell function ("glucotoxicity") in the B cells of BKs mice, which ultimately lead to B cell death and an islet volume decrease. In contrast, transplanted islets from the B6 mice showed a reversible impairment of the insulin release ("desensitization") after prolonged hyperglycemia and, accordingly, there was no islet volume decrease. Thus, the present findings emphasize the importance of the genetic constitution as one decisive factor for function and survival of B cells after a sustained excessive glucose load, i.e., conditions that may prevail in the period preceding the development of both insulin-dependent and non–insulin-dependent diabetes mellitus.

Acknowledgments

The skilled technical assistance of Birgitta Bodin, Margareta Engkvist, Eva Forsbeck, Astrid Nordin, and Eva Törnelius is gratefully acknowledged. We thank Dr. Michael Welsh for helping us with the insulin mRNA measurements.

This study was supported by grants from the Swedish Medical Research Council (12X-109, 12X-8273, 12P-8435, 19P-8982, 12P-9287), the Swedish Diabetes Association, the Juvenile Diabetes Foundation International, the Nordic Insulin Fund, the Aage Louis-Hansens Mindefond, the Torsten and Ragnar Söderberg Foundation, the Hoechst Diabetes Foundation, Magnus Bergvalls Stiftelse, the Åke Wiberg Foundation, O.E. and Edla Johansson Foundation, the Ernfors Family Fund, the Swedish Society for Medical Research, and the Swedish Society for Medicine.

References

1. Unger, R. H., and S. Grundy. 1985. Hyperglycemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes. *Diabetologia.* 28:119–121.

2. Weir, G. C., J. L. Leahy, and S. Bonner-Weir. 1986. Experimental reduction of B-cell mass: implications for the pathogenesis of diabetes. *Diabetes Metab. Rev.* 2:125–161.

3. Leahy, J. L., and G. C. Weir. 1988. Evolution of abnormal insulin secretory responses during 48-h in vivo hyperglycemia. *Diabetes.* 37:217–222.

4. Bonner-Weir, S., D. F. Trent, and G. C. Weir. 1983. Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J. Clin. Invest.* 71:1544–1554.

5. Andersson, A. 1974. Long-term effects of glucose on insulin release and glucose oxidation by mouse pancreatic islets maintained in tissue culture. *Biochem. J.* 140:377–382.

6. Malaisse-Lagae, F., A. Sener, and W. J. Malaisse. 1986. Can desensitization of the β-cell to glucose be simulated in cultured pancreatic islets? *Acta Diabetol. Lat.* 24:17–25.

7. Eizirik, D. L., and S. Sandler. 1989. Sustained exposure of toxically damaged mouse pancreatic islets to high glucose does not increase β-cell dysfunction. *J. Endocrinol.* 123:47–51.

8. Korsgren, O., S. Sandler, A. Schnell Landström, L. Jansson, and A. Andersson. 1988. Large-scale production of fetal porcine pancreatic isletlike cell clusters. *Transplantation (Baltimore).* 45:509–514.

9. Korsgren, O., L. Jansson, and A. Andersson. 1989. Effects of hyperglycemia on the function of isolated mouse pancreatic islets transplanted under the kidney capsule. *Diabetes.* 38:510–515.

10. Leiter, E. H. 1989. The genetics of diabetes susceptibility in mice. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 3:2231–2241.

11. Andersson, A. 1978. Isolated mouse pancreatic islets in culture: effects of serum and different culture media on the insulin production of the islets. *Diabetologia.* 14:397–404.

12. Heding, L. G. 1972. Determination of total serum insulin (IRI) in insulin-treated diabetic patients. *Diabetologia.* 8:260–266.

13. Sener, A., J. Rasschaert, D. Zähner, and W. J. Malaisse. 1988. Hexose metabolism in pancreatic islets: stimulation by D-glucose of [2-^{3}H]glycerol detritiation. *Int. J. Biochem.* 20:595–598.

14. Strandell, E., D. L. Eizirik, and S. Sandler. 1990. Reversal of β-cell suppression in vitro in pancreatic islets isolated from nonobese diabetic mice during the phase preceding insulin-dependent diabetes mellitus. *J. Clin. Invest.* 85:1944–1950.

15. Brunstedt, J., and S. J. Chan. 1982. Direct effect of glucose on the preproinsulin mRNA level in isolated pancreatic islets. *Biochem. Biophys. Res. Commun.* 106:1383–1389.

16. Thomas, P. S. 1980. Hybridization of denaturated RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA.* 77:5201–5205.

17. Chan, S. J., B. E. Noyes, K. L. Agarwal, and D. F. Steiner. 1979. Construction and selection of recombinant plasmids containing full-length complementary DNAs corresponding to rat insulins I and II. *Proc. Natl. Acad. Sci. USA* 76:5036–5040.

18. Sandler, S., and L. Jansson. 1987. Blood flow measurements in autotransplanted pancreatic islets of the rat: impairment of the blood perfusion of the graft during hyperglycemia. *J. Clin. Invest.* 80:17–21.

19. Wallenstein, S., C. L. Zucker, and J. L. Fleiss. 1980. Some statistical methods useful in circulation research. *Circ. Res.* 47:1–9.

20. Portha, B., C. Levacher, L. Picon, and G. Rosselin. 1974. Diabetogenic effect of streptozotocin in the rat during the perinatal period. *Diabetes.* 23:889–895.

21. Weir, G. C., E. T. Clore, C. J. Zmachinski, and S. Bonner-Weir. 1981. Islet secretion in a new experimental model for non-insulin dependent diabetes. *Diabetes.* 30:590–595.

22. Imamura, T., M. Koffler, J. H. Helderman, D. Prince, R. Thirlby, L. Inman, and R. H. Unger. 1988. Severe diabetes induced in subtotally depancreatized dogs by sustained hyperglycemia. *Diabetes.* 37:600–609.

23. Leahy, J. L., H. E. Cooper, and G. C. Weir. 1987. Impaired insulin secretion associated with near normoglycemia: a study in normal rats with 96-h in vivo glucose infusions. *Diabetes.* 36:459–464.

24. Coleman, D. L. 1978. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia.* 14:141–148.

25. Rossini, A. A., M. C. Appel, R. M. Williams, and A. A. Like. 1977. Genetic influence of the streptozotocin-induced insulitis and hyperglycemia. *Diabetes.* 26:916–920.

26. Leiter, E. H., J. W. Fewell, and E. L. Kuff. 1986. Glucose induces intracisternal type A retroviral gene transcription and translation in pancreatic beta cells. *J. Exp. Med.* 163:87–93.

27. Serreze, D. V., E. H. Leiter, E. L. Kuff, P. Jardieu, and K. Ishizaka. 1988. Molecular mimicry between insulin and retroviral antigen p73: development of cross-reactive autoantibodies in sera of NOD and C57BL/KsJ *db/db* mice. *Diabetes.* 37:351–358.

28. Swenne, I., and A. Andersson. 1984. Effect of genetic background on the capacity for islet cell replication in mice. *Diabetologia.* 27:464–467.

29. Andersson, A. 1983. The influence of hyperglycemia, hyperinsulinemia and genetic background on the fate of implanted mouse islets. *Diabetologia.* 25:269–272.

30. Leiter, E. H. 1987. Analysis of differential survival of syngeneic islets transplanted into hyperglycemic C57BL/6J versus C57BL/KsJ mice. *Transplantation (Baltimore).* 44:401–405.

31. Grodsky, G. M. 1989. A new phase of insulin secretion: how will it contribute to our understanding of β-cell function. *Diabetes.* 38:673–678.

32. Robertson, R. P. 1989. Type II diabetes, glucose "non-sense," and islet desensitization. *Diabetes.* 38:1501–1505.

33. Malaisse, W. J., and A. Sener. 1988. Hexose metabolism in pancreatic islets. Feedback control of D-glucose oxidation by functional events. *Biochim. Biophys. Acta.* 971:246–254.

34. Malaisse, W. J. 1983. Insulin release: the fuel concept. *Diabète & Metab.* 9:313–320.

35. Nielsen, D. A., M. Welsh, M. J. Casadaban, and D. F. Steiner. 1985. Control of insulin gene expression in pancreatic β-cells and in an insulin producing cell line, RIN-5F cells. I. Effects of glucose and cyclic AMP on the transcription of insulin mRNA. *J. Biol. Chem.* 260:13585–13589.

36. Welsh, M., D. A. Nielsen, A. J. MacKrell, and D. F. Steiner. 1985. Control of insulin gene expression in pancreatic β-cells and in an insulin producing cell line, RIN-5F cells. II. Regulation of insulin mRNA stability. *J. Biol. Chem.* 260:13590–13594.

37. Welsh, M. 1990. Glucose regulation of insulin gene expression. *Diabète & Metab.* 15:367–371.

38. Welsh, M., J. Brunstedt, and C. Hellerström. 1986. Effects of

D-glucose, L-leucine, and 2-ketoisocaproate on insulin mRNA levels in mouse pancreatic islets. *Diabetes.* 35:228–231.

39. Orland, M. J., R. Chyn, and A. Permutt. 1985. Modulation of proinsulin messenger RNA after partial pancreatectomy in rats. Relationships to glucose homeostasis. *J. Clin. Invest.* 75:2047–2055.

40. Orland, M. J., and A. Permutt. 1987. Quantitative analysis of pancreatic proinsulin mRNA in genetically diabetic (db/db) mice. *Diabetes.* 36:341–347.

41. Strandell, E., D. L. Eizirik, O. Korsgren and S. Sandler. 1988. Functional characteristics of cultured mouse pancreatic islets following exposure to different streptozotocin concentrations. *Mol. Cell. Endocrinol.* 59:83–91.

42. Eizirik, D. L., S. Sandler, N. Welsh, and C. Hellerström. 1988. Preferential reduction of insulin production in mouse pancreatic islets maintained in culture after streptozotocin exposure. *Endocrinology.* 122:1242–1249.

43. Portha, B., M.-H. Giroix, P. Serradas, N. Welsh, C. Hellerström, A. Sener, and W. J. Malaisse. 1988. Insulin production and glucose metabolism in isolated pancreatic islets of rats with NIDDM. *Diabetes.* 37:1226–1233.

44. Sandler, S., A. Andersson, and C. Hellerström. 1987. Inhibitory effects of interleukin 1 on insulin secretion, insulin biosynthesis and oxidative metabolism of isolated rat pancreatic islets. *Endocrinology.* 121:1424–1431.

45. Eizirik, D. L., E. Strandell, K. Bendtzen, and S. Sandler. 1988. Functional characteristics of rat pancreatic islets maintained in culture following exposure to human interleukin 1. *Diabetes.* 37:916–919.

46. Rabinovitch, A., C. Pukel, and H. Baquerizo. 1988. Interleukin-1 inhibits glucose-modulated insulin and glucagon secretion in rat islet monolayers. *Endocrinology.* 122:2393–2398.