Length of metabolic normalization after rat islet cell transplantation depends on endocrine cell composition of graft and on donor age

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Summary In vitro studies have demonstrated that beta-cell functions are negatively influenced by age and positively by the presence of glucagon producing alpha cells. This study examines whether the function of beta-cell grafts varies with the age of the donor and with the presence of other endocrine islet cells. Islet beta and endocrine non-beta-cells were purified from 10- to 30-week-old Lewis rats, and reaggregated into pure beta and mixed endocrine cell aggregates. Grafts consisted of 1.2 to 1.7 million beta cells with or without 0.6–0.7 million alpha cells. Their intraportal transplantation in 10-week-old streptozotocin-diabetic rats corrected hyperglycaemia in all experimental groups, with normal glucose tolerance curves at post-transplantation week (PT wk) 4. Recipients of mixed endocrine cell grafts from 10-week-old donors maintained a glucose tolerant state until PT wk 20, but turned glucose intolerant thereafter; only 1 of 12 animals was overtly diabetic at PT wk 64. Recipients

of pure beta-cell grafts from 10-week-old donors became glucose-intolerant from PT wk 4 on, with 5 of 11 cases developing overt diabetes before PT wk 64. When grafts were prepared from 30-week-old donors, metabolic deterioration started earlier, again with a more rapid loss for pure beta-cell grafts; at PT wk 64, virtually all recipients were overtly diabetic. It is concluded that delayed graft failure can be the consequence of an insufficient number of islet endocrine non-beta-cells as well as of a higher donor age. This observation can explain late failures in animal and human islet transplantation using marginally low beta-cell numbers. The interpretation of long-term studies on islet cell transplantation can benefit from the use of standardized grafts with well defined cellular composition. [Diabetologia (1997) 40: 1152–1158]

Keywords Islet, beta cell, transplantation, diabetes, insulin.

Experimental diabetes mellitus in rodents can be corrected by different types of islet cell grafts which have been placed in different locations [1]. While numerous studies have demonstrated short-term benefits of islet transplantation, relatively few investigations have examined the conditions for long-term metabolic normalization. The latter is however a requisite for a preventive effect on chronic complications, the main objective for developing islet transplantation as a cure of diabetes. Several reports have described conditions under which metabolic

control by islet transplants was progressively lost during a mid- or long-term follow-up [1–8]. It was often not clear whether this delayed failure was due to an insufficient graft, an inadequate implantation site or an immune reactivity [1–8]. We recently observed that the composition of the donor tissue can be responsible for a late loss of metabolic control in streptozotocin-diabetic rats which had been successfully treated by islet cell transplantation [9]. Number of beta cells, proportion of other endocrine and non-endocrine cells and particle size were found to be potentially influential variables. The present study was designed to assess whether the duration of metabolic normalization is reduced by a higher age of the beta-cell donor and by the absence of islet endocrine non-beta-cells in the implant. The rationale for investigating these two variables comes from in vitro experiments in which both factors were found to

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Abbreviations: PT wk, Post-transplantation week.

Table 1. Characteristics of purified islet cell grafts at time of implantation

Preparation	DNA (μg)	Hormone content		Cellular composition		Cell number	
		Insulin (μg)	Glucagon (μg)	Insulin-pos. (%)	Glucagon-pos. (%)	Insulin-pos. (10^6)	Glucagon-pos. (10^6)
<i>Donor age 10 weeks</i>							
Beta cells	9 ± 4^a	52 ± 28	1.0 ± 0.5^a	89 ± 9^b	4 ± 3^b	1.2 ± 0.5	0.07 ± 0.08^b
Endocrine cells	15 ± 3	43 ± 18	11.3 ± 3.3	66 ± 9	29 ± 9	1.4 ± 0.3	0.61 ± 0.21
<i>Donor age 30 weeks</i>							
Beta cells	11 ± 3^b	44 ± 23	0.9 ± 0.3^b	87 ± 9^b	4 ± 2^b	1.2 ± 0.3	0.05 ± 0.02^b
Endocrine cells	20 ± 5	37 ± 11	10.6 ± 3.3	62 ± 11	26 ± 7	1.7 ± 0.6	0.69 ± 0.23

Values represent means $\pm$ SD of 5 to 9 measurements. Grafts consist of aggregated beta cells or mixtures of beta cells and islet non-beta cells (endocrine cells). Statistical significance is calculated for differences between beta-cell grafts and mixed endocrine cell grafts prepared from donors of same age:

^a $p < 0.05$ and ^b $p < 0.005$. No significant differences were measured between beta-cell grafts of 10- and 30-week-old donors and between mixed endocrine cell grafts of 10- and 30-week-old donors

exert major influences on the function of islet beta cells [10–14].

Materials and methods

Recipients. Two weeks before implantation, male inbred Lewis rats (age 8 weeks; Charles River, Sulzfeld, Germany) were rendered diabetic by intravenous injection of 60 mg/kg streptozotocin (Sigma, St. Louis, USA, freshly dissolved in citrate buffer [pH 4.5]). One week after injection, their diabetic state was confirmed by glycaemia (2-h fast) above 20 mmol/l, daily urine volumes above 100 ml and weight loss.

Graft preparation and transplantation. Donor islet cell aggregates were prepared from 10- or 30-week-old male Lewis rats as previously described [15]. Briefly, isolated islets were digested according to a modified collagenase method, selected by hand-picking and then enzymatically dissociated. The dispersed islet cells were purified by autofluorescence-activated cell sorting (FACS, Becton Dickinson, Sunnyvale, Calif., USA) to obtain suspensions of single beta cells and single endocrine non-beta cells. The non-beta-cell preparation consisted of at least 75 % alpha cells and 15 % other endocrine cells. The purified beta cells were reaggregated in the absence or presence of islet endocrine non-beta-cells in a cell number ratio of 1:0.6. Aggregates were formed during orbital shaking in bacteriologic petri dishes (9 cm diameter, Nunc, Roskilde, Denmark) filled with 9 ml HAM's F10 (Flow, Irvine, UK) containing 10 mmol/l glucose, 2 mmol/l L-glutamine, 0.5 % bovine serum albumin (Sigma), 2 % heat-inactivated fetal calf serum (Flow), 50 $\mu\text{mol/l}$ isobutylmethylxanthine (IBMX), 0.075 mg/ml benzyl penicillin and 0.1 mg/ml streptomycin sulfate. The rotatory incubation was stopped after 20 h, and culture continued for 4 days under static conditions in the same medium without fetal calf serum and IBMX. At the end of culture, the preparations were gently removed from the dishes and samples were taken for insulin, glucagon and DNA assays, and for electron microscopy and immunocytochemistry [16]. The data were used to calculate the number of beta- and alpha-cells in the graft at the time of implantation; the method used for this calculation has been previously described [9, 17]. After sampling, the islet cell preparations were injected into the portal vein of diabetic recipients [18].

Metabolic follow-up. Recipients were followed in metabolic cages. At post-transplantation weeks 4, 12, 20, 40 and 64, blood

samples were taken from a tail incision in non-anaesthetized animals, after a 2-h fast in the morning. Blood glucose was measured with a glucose oxidase method (Glucopath and Glucoscot II; Menarini Diagnostics Benelux, Brussels, Belgium). Animals with a basal glycaemia exceeding 7.8 mmol/l and daily urine volume of more than 20 ml/24 h (i.e. $>$ mean + 3 SD of normal controls) were considered to be overtly diabetic and were not submitted to a glucose tolerance test. The other animals received an intragastric bolus of a 30 % glucose solution (2 g/kg) as a single bolus. Glycaemia was measured after 15, 30, 60, 90 and 120 min. When two or more measurements between 60 and 120 minutes exceeded the mean + 3 SD of age-matched normal controls, recipients were considered to be glucose intolerant. At weeks 8, 16, 30 and 64 post-transplantation, tail blood was collected for measuring serum fructosamine levels [19]. At PT wk 64, all animals were decapitated and their liver and pancreas extracted for hormone assay or processed for histology [7].

Statistical analysis. Data are expressed as means $\pm$ SD. Statistical significance of difference between experimental groups was calculated by Macintosh Statview 512 + one-way analysis of variance. For any overall group effect in ANOVA, group values were compared using the post-hoc Fisher PTLTD test.

Results

Composition of islet cell grafts. The cellular composition of the grafts was determined at the time of implantation. By using electron microscopy all preparations were shown to consist of more than 95 % ultrastructurally intact endocrine cells. Immunocytochemistry confirmed the difference between pure beta-cell grafts (90 % insulin-positive cells) and mixed endocrine cell grafts (60–70 % insulin-positive and 25–30 % glucagon-positive cells) (Table 1). The four types of graft contained a similar number of beta cells (1.2 to 1.7 million) and a similar insulin content (Table 1). Mixed endocrine cell grafts contained, in addition, 0.6 to 0.7 million alpha cells and a corresponding glucagon store (Table 1). The composition of the grafts was comparable for 10- and 30-week-old donors.

Table 2. Metabolic follow-up during 64 weeks post-transplantation

Experimental group post-transplant week		Death		Overt diabetes				Glucose intolerance				Normal glucose tolerance			
		0–30	30–64	4	20	40	64	4	20	40	64	4	20	40	64
	(n)														
Normal controls	(22)	0	0	0/22	0/22	0/22	0/22	0/22	0/22	0/22	0/22	22/22	22/22	22/22	22/22
Diabetic controls	(31)	7	13	31/31	25/25	11/11	6/6	–	–	–	–	0/31	0/25	0/11	0/6
Intraportal transplant															
Donor age 10 weeks															
Beta cells	(12)	0	0	0/12	1/12	1/12	5/11	1/12	3/12	11/12	5/11	11/12	8/12	0/12	0/11
Endocrine cells	(7)	0	0	0/7	0/6 ^a	0/6	1/6	0/7	0/6	4/6	5/6	7/7	6/6	2/6	0/6
Donor age 30 weeks															
Beta cells	(8)	0	0	0/8	2/8	7/8	7/8	2/8	5/8	1/8	1/8	6/8	1/8	0/8	0/8
Endocrine cells	(7)	0	1 ^b	0/7	1/7	4/7	5/6 ^b	0/7	3/7	3/7	1/6	7/7	3/7	0/7	0/6

Number of animals at time of implantation (*n*), number that died spontaneously (Death), and number with overt diabetes, glucose intolerance and normal glucose tolerance until 64 weeks post-transplantation. Five diabetic control animals

died during blood sampling and two recipients^a of grafts from 10-week-old donors during glucose tolerance testing; one recipient^b of a mixed endocrine cell graft died in normoglycaemia

Table 3. Basal glycaemia after islet cell implantation

Experimental groups post-transplant week	Glycaemia (mmol/l)					
	4	12	20	30	40	64
Normal controls	5.2 ± 0.8 (22)	4.9 ± 0.6 (22)	4.8 ± 0.6 (22)	5.1 ± 0.7 (22)	5.4 ± 0.6 (22)	4.9 ± 0.7 (22)
Diabetic controls	28.7 ± 2.9 (31)	26.6 ± 4.2 (28)	25.5 ± 3.8 (25)	22.8 ± 5.8 (19)	24.4 ± 4.5 (11)	24.4 ± 4.3 (6)
Intraportal transplants						
Donor age 10 weeks						
Beta cells	5.6 ± 1.1 (12)	5.8 ± 0.7 (12)	6.5 ± 1.4 (12)	7.6 ± 3.3 (12) ^d	8.0 ± 1.5 (12) ^{a,d}	10.9 ± 5.4 (11) ^b
Endocrine cells	5.3 ± 1.1 (7)	5.7 ± 1.1 (7)	5.7 ± 0.9 (6)	5.8 ± 1.1 (6)	7.5 ± 0.8 (6) ^e	10.1 ± 4.9 (6) ^b
Donor age 30 weeks						
Beta cells	5.5 ± 0.6 (8)	5.9 ± 0.9 (8)	8.6 ± 5.8 (8) ^b	13.4 ± 8.4 (8) ^{b,c}	16.4 ± 7.1 (8) ^{b,c}	11.8 ± 4.3 (8) ^b
Endocrine cells	5.7 ± 1.1 (7)	6.0 ± 0.9 (7)	6.9 ± 0.8 (7)	7.9 ± 2.2 (7)	12.1 ± 3.4 (7) ^b	15.3 ± 5.2 (6) ^b

Values represent means ± SD from (*n*) animals, tested at different times following islet cell transplantation. Statistical significance was determined for differences between implants and normal controls (^a *p* < 0.05 and ^b *p* < 0.005), between pure beta-cell grafts and mixed endocrine cell grafts of 30-week-

old donors (^c *p* < 0.05), between pure beta-cell grafts of 10- and 30-week-old donors (^d *p* < 0.005), and between mixed endocrine cell grafts of 10- and 30-week-old donors (^e *p* < 0.05). Values in diabetic controls were always higher (*p* < 0.005) than those in the other groups

Short-term effects of islet cell transplantation. In each of the four transplant groups, 2 h fasting (basal) glycaemia and daily urine volumes normalized within 4 weeks (Tables 2 and 3). Correction of polyuria was slightly faster in recipients of 10-week-old beta-cell grafts (median 6 days, range 2–9 days) than of 30-week-old beta-cell grafts (median 10 days, range 2–25 days, not significant). Recipients of mixed endocrine cell grafts of 10- and 30-week-old donors normalized 2 to 3 days more rapidly than those of beta-cell grafts prepared from the same donors.

Four weeks after transplantation, all recipients of endocrine cell grafts exhibited a normal glucose tolerance (Table 2). After a gastric glucose load, the 15 and 30 min glycaemia was higher than in normal controls, as was also the case in previous islet transplant studies [17]; the 60 to 120 min values were however not different from control levels (Fig. 1). For recipients of beta-cell grafts from 10 or 30-week-old donors, blood glucose was at all time points higher than that in control animals (Fig. 1); most but not all animals fulfilled the criteria of glucose tolerance (Table 2).

Comparison of long-term metabolic effects by pure beta-cell grafts and mixed endocrine cell grafts prepared from 10-week-old donors. Only 1 of 34 transplanted animals died spontaneously within the 64-week study period (Table 2), which contrasts with the high mortality in diabetic controls (over 60% died). Up to PT wk 40, their weight gain paralleled that in normal controls (Fig. 2). The degree to which metabolic control was maintained differed with the composition of the islet cell graft.

All recipients of mixed endocrine cell grafts maintained normal 2-h fasting glycaemia, daily urine volumes and glucose tolerance curves over 20 weeks (Tables 2 and 3, Fig. 3). Their serum fructosamine levels were significantly lower than in diabetic controls and not different from control values (Table 4). However, all animals developed a glucose intolerance between PT wk 20 and 60 (Table 2, Fig. 3). At PT wk 64, only one animal had become overtly diabetic (Table 2) with diabetic fructosamine levels, causing a significant increase in the mean glucose (Table 3) and fructosamine levels (Table 4) at this time point.

Fig. 1. Intra-gastric glucose tolerance test at post-transplantation weeks 4 and 20 in normal controls (○---○) and in recipients of islet cells from 10- (□, ■) or 30-week-old (△, ▲) donors. Grafts consisted of pure beta cells (□, △ left panel) or endocrine cells (■, ▲ right panel). Data are means $\pm$ SEM. Statistical difference is determined vs normal controls (* p < 0.05, ** p < 0.005) and between recipient groups of 10- and 30-week-old donors († p < 0.05, †† p < 0.005)

In recipients of pure beta-cell grafts, metabolic control was lost more rapidly and more severely. Glucose tolerance curves were disturbed at all time points from PT wk 4 on and deteriorated progressively (Fig. 3), with 60 to 120 min glucose levels that, up to PT wk 40, were higher than those in recipients of mixed endocrine cell grafts. From PT wk 30 on, the mean 2-h fasting blood glucose levels (Table 3) and fructosamine levels (Table 4) were significantly higher than those in controls. Before PT wk

64, 5 out of 11 animals became overtly diabetic (Table 2).

Effect of donor age on long-term metabolic normalization. Islet cell grafts prepared from 30-week-old donors also prevented the high mortality seen in diabetic controls (Table 2). Weight gain of recipients paralleled that of normal controls up to PT wk 20 for pure beta-cell grafts and PT wk 30 for mixed endocrine cell grafts (Fig. 2). Beyond these time points, body weights stabilized at levels which remained markedly lower than the progressively increasing values in age-matched controls; they were significantly lower for recipients of pure beta-cell grafts than for those of mixed endocrine cell grafts (Fig. 2). As for implants prepared from 10-week-old donors, grafts of pure beta cells lost metabolic control more rapidly than those of mixed endocrine beta cells. This is expressed by the earlier elevation of 2-h fasting glycaemia (from PT wk 20 on) and of serum fructosamine (from PT week 8 on), the earlier occurrence of glucose intolerance (from PT wk 4 on) and the earlier transition to overt diabetes (7 out of 8 recipients at PT wk 40).

Implants from 30-week-old donors maintained metabolic control less efficiently than those with similar composition prepared from 10-week-old donors. Recipients of pure beta-cell grafts from older donors exhibited higher post-challenge glycaemia from PT wk 20 on (Fig. 1) and higher 2-h fasting glycaemia and serum fructosamine from PT wk 30 on (Tables 3 and 4); they also developed overt diabetes in more animals and at an earlier time point (Table 2). Recipients of mixed endocrine cell implants were similar with respect to the difference in donor age: those receiving grafts from the older donors (30 weeks) progressively developed glucose intolerance from PT wk 20 onwards (Fig. 1) and overt diabetes from PT wk 40 (Table 2), whereas those with grafts from the younger donors (10 weeks) became glucose intolerant at a later time (PT wk 40) (Figs. 1 and 3) and had

Table 4. Serum fructosamine levels after islet cell implantation

Experimental groups post-transplantation week	Fructosamine (μ mol/l)			
	8	16	30	64
Normal controls	128 $\pm$ 28 (22)	136 $\pm$ 22 (22)	146 $\pm$ 17 (22)	139 $\pm$ 17 (22)
Diabetic controls	309 $\pm$ 26 (31)	295 $\pm$ 42 (28)	292 $\pm$ 45 (19)	N.D.
Intraportal transplants				
Donor age 10 weeks				
Beta cells	143 $\pm$ 21 (12)	158 $\pm$ 18 (12)	177 $\pm$ 25 (12) ^{a d}	180 $\pm$ 43 (11) ^a
Endocrine cells	144 $\pm$ 11 (7)	144 $\pm$ 32 (6)	167 $\pm$ 32 (6)	187 $\pm$ 66 (6) ^a
Donor age 30 weeks				
Beta cells	153 $\pm$ 34 (8) ^a	172 $\pm$ 34 (8) ^a	238 $\pm$ 67 (8) ^{b c}	204 $\pm$ 57 (8) ^b
Endocrine cells	148 $\pm$ 23 (7)	167 $\pm$ 27 (7) ^a	182 $\pm$ 42 (7) ^a	202 $\pm$ 45 (6) ^b

Values represent means $\pm$ SD from (n) animals, tested at different times following islet cell transplantation. Statistical significance was calculated for differences between implants and normal controls (^a p < 0.05, ^b p < 0.005), between pure beta-cell grafts and mixed endocrine cell grafts of 30-week-old

donors (^c p < 0.05), between pure beta-cell grafts of 10- and 30-week-old donors (^d p < 0.005). Values in diabetic controls (PT week 8–30) were always higher (p < 0.005) than those in the other groups

Fig. 2. Body weight in normal (○---○) and diabetic (●---●) controls, in recipients of pure beta cells (□, △) or mixed endocrine cells (■, ▲) from 10- (□, ■) or 30-week-old (△, ▲) donors. Statistical difference is determined vs normal controls (* $p < 0.05$, ** $p < 0.005$), among recipient groups of beta cells from 10- or 30-week-old donors († $p < 0.05$, †† $p < 0.005$) and among recipient groups of endocrine cells from 10- or 30-week-old donors (‡ $p < 0.05$). All measurements in diabetic controls are lower ($p < 0.005$) than those in other groups from PT week 4 on

a low chance of becoming overtly diabetic (only 1 of 6 beyond PT wk 40) (Table 2).

Hormone stores in islet cell recipients. At PT wk 64, pancreatic insulin stores of islet cell recipients and diabetic controls represented less than 5% of the values measured in normal controls. Islet cell recipients that maintained normal 2-h fasting glycaemia during the whole study, contained significantly higher hepatic insulin reserves than recipients that had reverted to diabetes (Table 5). Their hepatic insulin content represented 50% of the implanted store and corresponded to 16% of the pancreatic insulin content in age-matched normal controls. It was comparable for normoglycaemic recipients of beta-cell grafts and endocrine cell grafts, irrespective of the age of the donors (Table 5). In both donor age categories, recipients of endocrine cell grafts contained a higher hepatic glucagon reserve than recipients of beta-cell grafts (Table 5), but its size was less than 15% of that in the initial implant.

Discussion

The present study confirms that intraportal injection of islet cell aggregates containing 1 to 2 million beta cells corrects overt diabetes in streptozotocin-diabetic

Fig. 3. Intra gastric glucose tolerance test at post-transplantation weeks 4, 20, 40 and 60 in normal controls (○---○) and in recipients of intraportally injected beta cells (△) or endocrine cells (■) of 10-week-old donors. Statistical difference is determined vs normal controls (* $p < 0.05$, ** $p < 0.005$)

rats with a body weight of 200 g [9, 15, 17]. This effect is achieved with beta cells isolated from young (10 weeks) as well as from middle-aged (30 weeks) rats. It did not require the presence of other endocrine or non-endocrine islet cell types, as was already shown in previous experiments [7, 15]. The duration of metabolic normalization varied however with the age of the donor as well as with the presence or absence of other endocrine islet cells in the beta-cell aggregates. This notion was demonstrated by comparing implants with selected composition as prepared

Table 5. Hepatic and pancreatic hormone content in islet cell graft recipients 64 weeks after implantation

Experimental groups	Metabolic status	(n)	Liver ($\mu\text{g}/\text{organ}$)		Pancreas ($\mu\text{g}/\text{organ}$)	
			Insulin	Glucagon	Insulin	Glucagon
Normal controls	N	(22)	< 0.1	< 0.1	155 $\pm$ 51	2.7 $\pm$ 1.3
Diabetic controls	D	(4)	< 0.1	< 0.1	5 $\pm$ 2	1.8 $\pm$ 0.2
Intraportal implants						
Donor age 10 weeks						
Beta cells	N	(5)	25 $\pm$ 14	0.4 $\pm$ 0.4	8 $\pm$ 4	2.3 $\pm$ 0.8
	D	(4)	4 $\pm$ 2	0.1 $\pm$ 0.1	4 $\pm$ 1	5.8 $\pm$ 5.2
Endocrine cells	N	(4)	20 $\pm$ 14	1.0 $\pm$ 0.4	5 $\pm$ 4	1.6 $\pm$ 1.4
Donor age 30 weeks						
Beta cells	D	(4)	6 $\pm$ 3	0.3 $\pm$ 0.3	6 $\pm$ 5	3.4 $\pm$ 0.7
Endocrine cells	D	(4)	6 $\pm$ 2	1.3 $\pm$ 0.6	4 $\pm$ 1	2.2 $\pm$ 1.4

Values represent means $\pm$ SD of (n) measurements. Recipient groups are divided according to metabolic states: basal normoglycaemia (N) or overt diabetes (D). Hepatic hormone stores in recipients are always higher ($p < 0.005$) than those in normal and diabetic controls. Hepatic insulin contents in normoglycaemic recipients are always higher ($p < 0.005$) than those in recipients with overt diabetes. No significant differences in

hormone stores were measured between normoglycaemic recipients of pure beta-cells grafts and mixed endocrine grafts of 10-week-old donors, between diabetic recipients of pure beta-cell grafts and mixed endocrine grafts of 30-week-old donors, and between diabetic recipients of pure beta-cell grafts of 10- and 30-week-old donors

from islet beta and islet endocrine non-beta cells that were purified from 10- to 30-week-old rats. At the time of implantation, grafts were composed of ultra-structurally intact cells, containing 1.2 to 1.7 million insulin-positive beta cells either without or with 0.6 to 0.7 million glucagon-containing alpha cells.

Implants which only consisted of beta cells were less capable of maintaining long-term metabolic control than those with a supplement of islet endocrine non-beta cells. Recipients of pure beta cells progressively developed glucose intolerance, a rise in basal glycaemia and fructosamine levels, and a return to an overt diabetic state. The number of animals exhibiting one or more of these metabolic deteriorations, was higher for grafts prepared from 30- than from 10-week-old donors. This loss of metabolic control also occurred at an earlier time point post-transplantation. The negative effect of older donor age was clearly observed in recipients of mixed islet endocrine cell aggregates: while overt diabetes was only a rare and late outcome of implants from 10-week-old rats, it developed earlier and affected virtually all recipients of grafts from 30-week-old animals.

It is not directly evident why mixed islet endocrine cell aggregates achieve a longer metabolic control than pure beta-cell aggregates. The vicinity of glucagon-producing alpha cells could improve the survival of beta cells during culture or during the first post-transplantation days, thus leading to engraftment of a larger functional beta-cell mass than when the same number of beta cells is aggregated without alpha cells. The latter implants may – as a consequence – contain a marginally low beta-cell mass, and therefore become functionally inadequate at a later time, when insulin needs have increased as a result of the greater body weight and/or older age of the recipients [10, 11, 20]. It is also conceivable that the local release

of glucagon amplifies the beta cells' secretory response to glucose, thus enhancing their homeostatic control. Such a mechanism is certainly operative in isolated beta-cell preparations [13, 14], but its existence in the endocrine pancreas or in islet cell grafts has not yet been demonstrated. While the observations with the cultured endocrine cell aggregates are indicative for processes that operate in intact islets, it cannot be excluded that the metabolic outcome of freshly isolated islet grafts also involves other components and mechanisms than those presently studied.

Previous studies have shown that age reduces the islet secretory responses to glucose [10–12, 20]. Sellawry et al. [21] reported that islets from 42-week-old donor rats failed to normalize plasma glucose levels in streptozotocin-diabetic rats while an identical number of islets from 10-week-old animals restored normoglycaemia in the majority of recipients. Little information is however available on the cellular composition of the islet preparations used in these comparative studies. It is for example not known whether “old” and “young” islets contained similar numbers of viable alpha and beta cells. The present data indicate that the use of older donors may shorten the post-transplantation period during which recipients maintain normoglycaemia. Between post-transplantation weeks 20 and 40, 7 out of 8 recipients of beta-cell aggregates from 30-week-old donors became overtly diabetic, whereas this was only the case for 1 out of 12 recipients of grafts from 10-week-old donors. The observation that beta-cell implants from older donors achieve a therapeutic action of shorter duration suggests that age reduces the functional capacity of the beta cells. This reduced cellular potential could have been present at the time of implantation without necessarily interfering with the normalization process; the increasing body mass of the

recipient is expected to increase the insulin needs, eventually resulting in insulin shortage and metabolic decompensation. The latter phenomenon is also held responsible for the delayed failure of grafts with a marginally low beta cell number [9]. It is also conceivable that the "older" beta cells progressively deteriorate in their ectopic site, leading to a shorter lifespan and/or to a loss of their physiologic responses. Such process could be the consequence of a reduced cellular adaptation to the new environment in the liver. A third factor can be sought in the respective potential of "old" and "young" implants for beta-cell neof ormation from pre-existing beta cells. It is known that the beta-cell proliferative compartment decreases with age [22]. Implants from older donors may therefore be less efficient in maintaining the beta-cell mass in the implant. Islet transplantation studies in man have not yet identified an effect of donor age; as in rodents, such observation may require a comparative analysis using standardized beta-cell grafts.

The present data underline the need for long-term follow-up of beta-cell transplants. Most islet transplant studies are terminated before post-transplantation week 20. At that time, few differences were noticed between the experimental groups that we chose to investigate (the present study and [9]). Evidence is also presented that delayed graft failure is not necessarily a consequence of the recipient's immune reactivity but can also be a sign of an inadequate composition of the donor tissue. This notion should be considered in the interpretation of late failures in both animal and human islet transplantation. We previously observed that allogeneic grafts of mixed endocrine islet cells maintained a long-term normalization in virtually all non-immunosuppressed rat recipients while allografted pure beta-cell aggregates lost metabolic control in 50% of the recipients [7]. According to the present observations, the absence of endocrine non-beta-cells may explain – at least in part – failure of the beta cells in the absence of any alloreactivity.

While the number of living beta cells seems to be the major – if not the only – determinant for the immediate correction of hyperglycaemia, several additional graft characteristics appear to influence the duration of metabolic normalization. Long-term correction was found endangered if the graft contained marginally low numbers of beta cells [9], if they were prepared from old donors (present data), if they were not associated with other endocrine islet cells (present data) or if they were transplanted as impure tissue [9]. In more general terms, our results stress the importance of using standardized islet cell grafts for comparative studies. Quality control tests of the graft should provide information on such critical factors as beta-cell number and presence of other cell types.

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