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Transplantation of allogeneic fetal pancreases combined from MHC-different donor strains does not change rejection of the graft

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Abstract. A number of 17.5- to 18.5-day-old fetal pancreases were grafted under the kidney capsule of streptozotocin-diabetic rats. Eight syngeneically grafted glands were sufficient to reverse the diabetes of the recipients within 4 weeks when the recipient rats were treated with insulin for 18 days after transplantation. Eight allogeneic fetal pancreases obtained from one donor strain were rejected after transplantation and the recipients relapsed into hyperglycemia immediately after insulin withdrawal. Eight allogeneic fetal pancreases obtained from eight MHC-different donor strains were also rejected and the recipients relapsed into hyperglycemia after insulin withdrawal. Using fetal pancreases as tissue sources, the combination of the allogeneic graft from different donor strains was not sufficient to prolong the survival time of the grafted tissue.

Key words: Pancreas transplantation, fetal, rat – Fetal pancreas transplantation, rat – Graft rejection, pancreas, fetal – Rejection, pancreas, fetal

The amount of insulin-producing tissue available for transplantation of type I diabetic patients can be increased by using fetal tissue. Syngeneic fetal pancreases grafted into streptozotocin-diabetic rats have been able to reverse diabetes in rats [1, 2]. However, the major drawback of fetal pancreas transplantation is the likelihood of rejection following allogeneic transplantation.

Recently, Gotoh et al. [4, 5] observed permanent allograft survival when a reduced number of Langerhans islets from four MHC-different donor strains of mice was grafted into streptozotocin-diabetic recipients. We decided to investigate this possibility by using a pool of eight MHC-different fetal pancreases for transplantation into streptozotocin-diabetic rats.

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Materials and methods

Animals and transplantation

Either adult female LEW.1A MaxK rats (haplotype $RT1^a$, body weight 170–230 g) or adult female LEW.1W MaxK rats (haplotype $RT1^u$, body weight 170–230 g) served as recipients. The experimental induction of diabetes was done intravenously with 50 mg streptozotocin per kilogram body weight (streptozotocin dissolved in citrate buffer, pH 4.5). Only animals with a plasma glucose level above 20.0 mmol/l (determined at least three times) were used. The LEW.1A rats were used as recipients for the syngeneic transplants and the LEW.1W rats as recipients for the allogeneic transplants.

Four to six adult female rats were mated with one male for 40 h. Some 17.5–18.5 days later, the pregnant rats were anesthetized (hexobarbitale, 100 mg/kg body weight, i.p.), the abdominal cavity was opened, and the fetuses were prepared and used for pancreas preparation by means of a stereo microscope. The following experimental transplantations were performed:

(1) syngeneic transplantation without subsequent insulin treatment

(2) syngeneic transplantation with subsequent insulin treatment

(3) allogeneic transplantation using one donor strain

(4) allogeneic transplantation using eight MHC-different donor strains

Eight fetal pancreases were grafted under the kidney capsule of one diabetic rat. Fetal pancreases from LEW.1A rats were used for syngeneic transplantations and for allogeneic transplantations using one donor strain.

For allogeneic transplantations of MHC-different fetal pancreases, the following donor strains [7] were used:

(1) LEW Max K (haplotype $RT1^l$)

(2) LEW.1A Max K (haplotype $RT1^a$)

(3) LEW.1W Max K (haplotype $RT1^u$)

(4) DA Ph K (haplotype $RT1^{av1}$)

(5) LEW.1N Ph K (haplotype $RT1^n$)

(6) LEW.1WR1 Ph K (haplotype $RT1^{r4}$)

(7) WOK a (haplotype $RT1^a$)

(8) WOK u (haplotype $RT1^u$)

Table 1. Pancreas and graft insulin content

Days after transplantation	0		30		60		120	
	Pancreas insulin (pmol/mg w. wt.)	Graft insulin (pmol)	Pancreas insulin (pmol/mg w. wt.)	Graft insulin (pmol)	Pancreas insulin (pmol/mg w. wt.)	Graft insulin (pmol)	Pancreas insulin (pmol/mg w. wt.)	Graft insulin (pmol)
Syngeneic transplantation								
– without insulin treatment	0.4 ± 0.2 (6)	108 ± 17 (21)	–	–	0.3 ± 0.1 (6)	353 ± 122 (4)		
– with insulin treatment	0.5 ± 0.1 (18)	108 ± 17 (21)	–	3294 ± 694* (5)	0.8 ± 0.1 (19)	3102 ± 830 (4)	0.9 ± 0.1 (13)	4220 ± 773 (9)
Allogeneic transplantation (fetal pancreases of LEW.1A rats)	0.2 ± 0.1 (8)	108 ± 17 (21)	0.5 ± 0.1 (8)	61 ± 21 (5)				
Allogeneic transplantation (pooled fetal pancreases)	0.8 ± 0.2 (9)	105 ± 19 (8)	0.5 ± 0.1 (9)	84 ± 40 (6)				

* $P < 0.01$ in comparison to the former time investigated

Eight glands from at least six different strains were combined for one transplantation. The remaining fetal pancreases were weighed and quickly frozen in liquid nitrogen. The insulin content was estimated radioimmunologically after acid-alcohol extraction [17].

To measure the pancreatic insulin content of the recipients, a pancreatic biopsy was taken surgically [10] and used for insulin determination [17]. The grafted animals were treated with insulin (2.0–3.5 IU/day) for 18 days. Body weight and postprandial plasma glucose (Beckman glucose analyzer, Fullerton, Calif., USA) were measured three times weekly for 4 weeks and thereafter weekly up until termination of the experiments. At that time the recipients' pancreases and the grafts were prepared and used either for insulin determination [14] or graft morphology. For morphology, the grafts were fixed in Bouin's solution. The fixed grafts were cut into 6-µm sections and were stained either with hematoxylin-eosin or using the indirect immunofluorescence technique [9].

Sixty and 120 days after successful syngeneic transplantation, a glucose tolerance test (2 g glucose/kg body weight) was carried out. At 0, 10, 30, 60, and 120 min after i.p. glucose injection, blood samples were taken from the tail vein and plasma glucose was measured. The glucose tolerance of the grafted rats was compared with that of healthy adult female LEW.1A rats (body weight 170–230 g).

Statistics

Results are given as mean ± SEM of n different animals. Statistical evaluation was checked by the paired or unpaired Student's t-test.

Results

The graft recipients were characterized by a markedly reduced pancreatic insulin content (Table 1) and a marked hyperglycemia (Fig. 1). Those syngeneically transplanted without insulin treatment did not develop normoglycemia within 56 days after transplantation (Fig. 1). Their body weight did not increase during the observation period, nor did the graft insulin content of these animals increase significantly (Tables 1, 2).

The other group of isograft recipients was treated with daily injections of insulin to keep them nearly normoglycemic. When the insulin requirement decreased to less than 0.5 units per day, the insulin was withdrawn. The rats were treated with insulin for 15 ± 1 days ($n = 23$). After 18 days of insulin treatment, the insulin requirement of nearly all syngeneically grafted rats had decreased to less than 0.5 units per day. For this reason, allogeneically grafted animals were treated with insulin for 18 days after transplantation.

The isograft recipients developed normoglycemia within 4 weeks after transplantation (Fig. 1) and maintained it for at least 120 days. Their body weight increased not only under insulin therapy but also after the development of normoglycemia (Table 2). The graft insulin content of these animals showed a marked increase, which could already be observed 30 days after transplantation (Table 1). The graft insulin content at 60 and 120 days after transplantation did not increase further to a significant degree in comparison with day 30 after transplantation. The recipients' pancreatic insulin content did not change up to 120 days after transplantation (Table 1).

The indirect immunofluorescence technique revealed islets in the graft that typically resembled adult islets, containing many insulin-positive β-cells (Fig. 2). In addition, groups of insulin-positive cells could be seen within the graft, surrounded by connective tissue, fat cells, duct

Table 2. Body weight gain

Days after transplantation	0	18	30
	Body weight (g)	Body weight gain (g)	
Syngeneic transplantation – without insulin treatment	174 ± 7 (6)	+ 3 ± 4 (6)	+ 5 ± 5 (6)
Syngeneic transplantation – with insulin treatment	199 ± 3 (20)	+ 23 ± 2 (20)	+ 31* ± 3 (20)
Allogeneic transplantation (fetal pancreases from LEW.1A)	172 ± 5 (9)	+ 35 ± 4 (8)	+ 25 ± 4 (8)
Allogeneic transplantation (pooled fetal pancreases)	175 ± 5 (9)	+ 28 ± 4 (9)	+ 15* ± 3 (9)

* $P < 0.05$ in comparison to the former time investigated

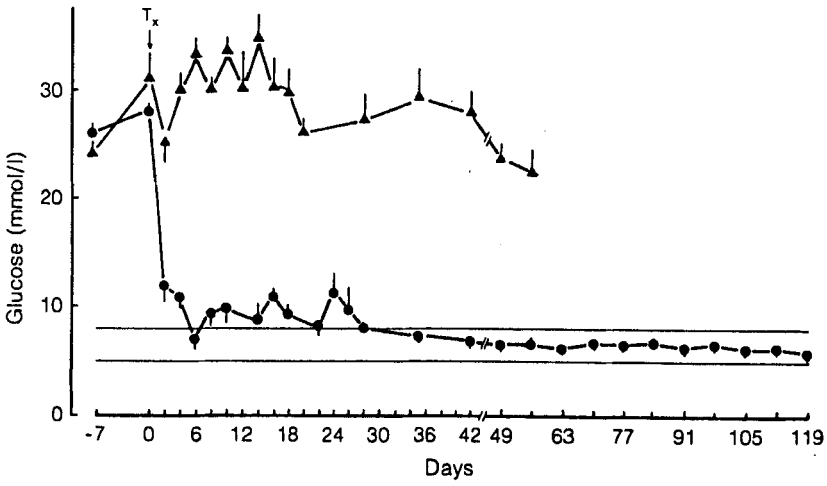


Fig. 1. Plasma glucose of syngeneically grafted rats without insulin treatment after transplantation (▲, $n = 6$) and of syngeneically grafted rats with insulin treatment for 18 days after transplantation (●, $n = 20$)

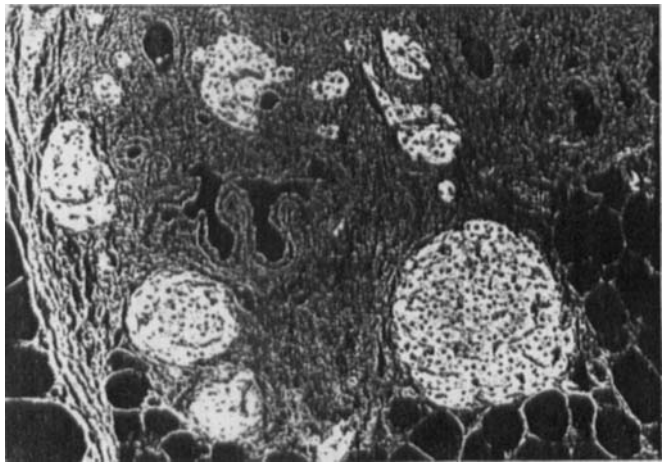


Fig. 2. Immunohistochemical demonstration of insulin-containing β-cells in a syngeneic graft (×220)

cells, and vessels. Other islets were surrounded by well-developed exocrine tissue, also containing vessels and ducts (Fig. 5).

The transplantation that resulted in normoglycemia did not normalize the glucose tolerance of the grafted rats (Table 3). However, the glucose intolerance did not become further impaired within the observation period.

Immediately after insulin withdrawal, recipients of LEW.1A allografts relapsed into hyperglycemia (Fig. 3), which was accompanied by a loss in body weight (Table 2). The insulin requirement was significantly enhanced when compared with that of the syngeneically grafted rats (Fig. 4). The recipients' graft insulin content did not change within 30 days after transplantation (Table 1).

Recipients of MHC-different allogeneic fetal pancreases were also characterized by an immediate hyperglycemia and loss in body weight after insulin withdrawal (Fig. 3, Table 2). The insulin requirement was significantly higher than that of the syngeneically grafted rats (Fig. 4). Graft insulin content had not changed at day 30 after transplantation (Table 2).

Morphological examination of the allogeneically grafted fetal pancreases showed complete destruction of the grafted tissue. Only connective tissue with infiltrating lymphocytes could be observed. A hematoxylin/eosin-stained section of such a typical graft is shown in Fig. 6.

Discussion

The insulin content of the grafted fetal pancreases was much lower than that of an islet graft obtained from adult or neonatal donors. Eight fetal pancreases with a gestational age of 17.5–18.5 days contained less than 1 % of the pancreas insulin content of an adult rat. In contrast to that, an islet graft consisting of 2000 isolated pancreatic islets of newborn rats was found to contain about 60 % of the pancreas insulin content of an adult rat [6]. Hence, growth and maturation of the fetal graft presumably account for its ability to reverse diabetes.

After syngeneic transplantation, recipients treated with daily insulin injections became normoglycemic within 4 weeks. In contrast, recipients not treated with insulin stayed hyperglycemic. We and others [12] conclude that a permanent hyperglycemia interferes with growth and maturation of fetal pancreases. Exogenous insulin is a necessary prerequisite for the metabolic compensation of the recipient's diabetes to induce or maintain growth and maturation of fetal pancreases. In an earlier experiment, we found an increase in the number of β cells in the native pancreas of diabetic rats after islet transplantation [6] (i. e., under conditions of a compensated glucose metabolism). The graft insulin content of syngeneically grafted insulin-treated rats increased within 30 days after transplantation up to a level of 20 %–25 % of the pancreas insulin

Table 3. Glucose tolerance after syngeneic transplantation (calculated as glucose area 0–120 min)

	Normal LEW.1A (min · mmol/l)	60 days after transplantation (min · mmol/l)	120 days after transplantation (min · mmol/l)
0–120 min reactive	353 ± 22 (22)	633 ± 77* (19)	628 ± 68* (14)

* $P < 0.01$ in comparison to normal LEW.1A rats

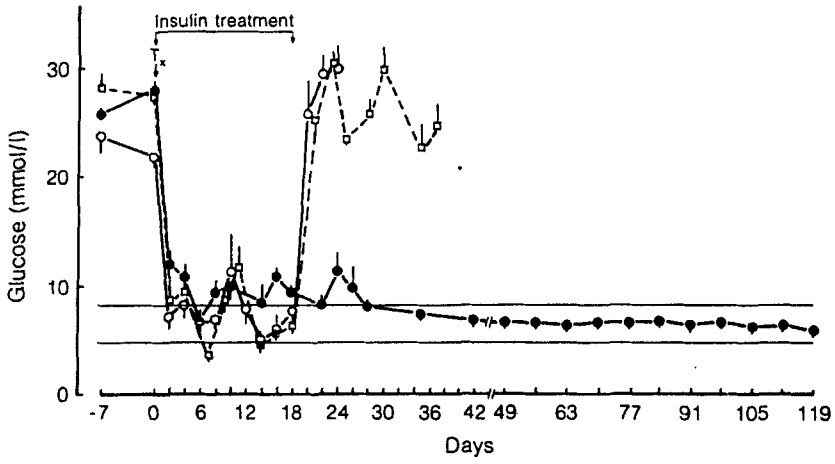


Fig. 3. Plasma glucose of syngeneically grafted rats (●, $n = 20$), of allogeneically grafted rats, donor strain LEW.1A (○, $n = 8$), and of allogeneically grafted rats, eight MHC-different donor strains (□, $n = 9$)

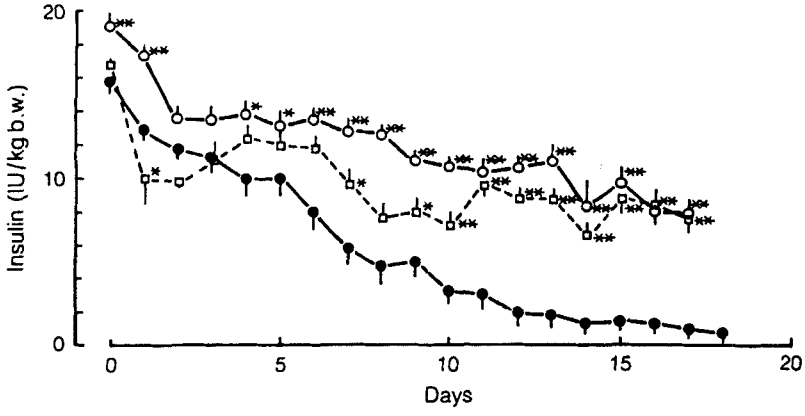


Fig. 4. Insulin requirement of syngeneically grafted rats (●, $n = 20$), of allogeneically grafted rats, fetal pancreases of LEW.1A, (○, $n = 8$), and of allogeneically grafted rats, MHC-different fetal pancreases (□, $n = 9$). ** $P < 0.01$, * $P < 0.05$: insulin requirement of allogeneically grafted versus insulin requirement of syngeneically grafted rats

content of an adult rat, but it did not increase further. This amount of insulin was sufficient to reverse diabetes and to maintain a permanent normoglycemia, but it was not sufficient to normalize the glucose tolerance of the recipients.

Our results confirm the findings of others that a fetal pancreas transplantation under the conditions described is able to reverse diabetes [2]. The major drawback of this kind of transplantation is the likelihood of rejection after allogeneic transplantation. Allogeneically grafted fetal pancreases from LEW.1A rats were acutely rejected in the LEW.1W-recipients. The insulin requirement of these rats was significantly enhanced in comparison with that of syngeneically grafted rats. The allogeneically grafted rats relapsed into hyperglycemia immediately after insulin withdrawal. The insulin content and the morphological appearance of the graft demonstrated its destruction.

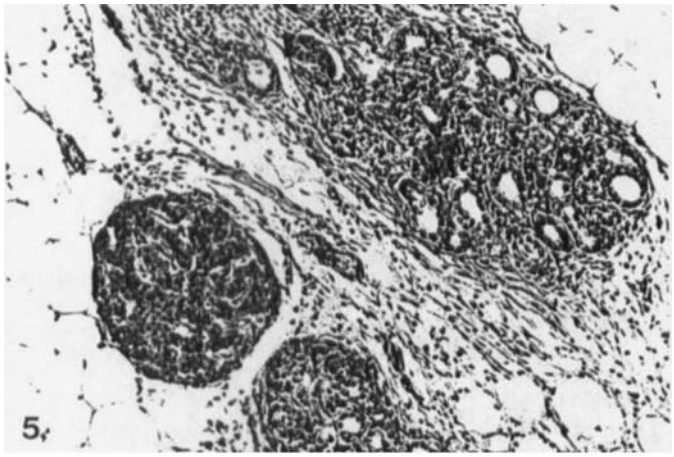
One possible way to prevent or delay the rejection of allogeneic grafts is to reduce the immunogenicity of the graft. This can be achieved by cultivation of the fetal pancreases under high-oxygen pressure or low-temperature conditions [3, 11, 13]. Another possibility, proposed by Gotoh et al. [4, 5], is to combine and transplant pancreatic islets from MHC-different donor strains. As the authors demonstrated, transplantation of a reduced number of pancreatic islets from four MHC-different donor strains resulted in permanent allograft survival and permanent normoglycemia of the grafted streptozotocin-diabetic mice. The authors hypothesized that the immune response elicited by suboptimal numbers of islets was too weak to cause rejection of the graft [5].

The combination of an allogeneic fetal pancreatic graft from eight MHC-different donor strains did not change the rejection of the graft.

The insulin requirement of the recipients was similar to that observed in rats receiving LEW.1A fetal pancreases. The recipients of combined allografts relapsed into hyperglycemia immediately after insulin withdrawal. The insulin content and the morphological appearance of the grafts did not differ from allografts consisting of LEW.1A fetal pancreases. Both recipients of LEW.1A and of combined allografts were characterized by a loss in body weight after insulin withdrawal.

The results demonstrated that the combination of an allograft from MHC-different fetal pancreases did not improve the survival of the grafted tissue on the condition that high-responder recipients [8] were used.

Simeonovic et al. [13–16] found that there is a difference between the immunogenicity of fetal pancreases and isolated adult islets or proislets. The numbers of macrophages and MHC class II-positive leukocytes counted in fetal pancreases of mice was significantly higher than those counted in proislets [14]. The mean survival time of allogeneically grafted proislets was markedly higher than that of whole fetal pancreases from the same donor [16]. The higher immunogenicity of fetal pancreases in com-



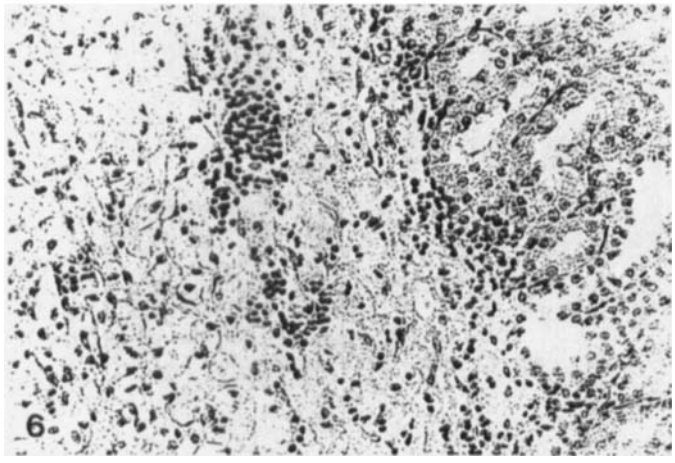


Fig. 5. Hematoxylin/eosin staining of an isograft (× 220)

Fig. 6. Hematoxylin/eosin staining of an allograft (× 430)

parison with isolated islets may be the reason for the difference between our results and those of Gotoh et al.

In sum, the combination of allogeneic fetal pancreases from MHC-different donors was not sufficient to improve the transplantation outcome and must, therefore, be combined with other methods that reduce the immunogenicity of fetal pancreases.

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