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Effect of genetic background on the capacity for islet cell replication in mice

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Summary. Proliferation of islet cells may compensate for both an increased peripheral insulin resistance and islet cell destruction but the capacity for regeneration may be genetically determined. For the latter reason, glucose-stimulated islet cell replication was estimated in both inbred C57BL/6J (BL/6) and C57BL/KsJ (BL/Ks) mice. Islets isolated from both strains were exposed to high concentrations of glucose in vitro or in vivo for a prolonged time period. This was achieved either by culturing the islets free-floating in a high glucose concentration medium for 3 days or implanting the islets intrasplenically in insufficient numbers to cure alloxan-diabetic syngeneic recipients. In both strains high glucose concentration culture was found to increase the autoradiographic labelling index of the islets but the replicatory activity decreased with age. The proliferative rate of the islet cells of the BL/6 mice was about twice as high as that of the BL/Ks mice irrespective of age and glucose concentration. Likewise, the labelling index of intrasplenic BL/6 islets implanted into alloxan-diabetic mice was twice as high as that of the islets implanted into alloxan-diabetic BL/Ks mice. The replicatory activity of the latter islets did not differ statistically from that of islets implanted into non-diabetic control BL/Ks mice. No differences in the rates of proinsulin and total protein biosynthetic rates were observed between high glucose concentration-cultured islets of the two mouse strains. The present results indicate that the proliferative response of pancreatic islets to a prolonged glucose stimulation may be genetically determined. This may play a significant role in the development of different diabetic syndromes both in laboratory animals and man.

Key words: Inbred mouse strains, alloxan diabetes, islet culture, islet implantation, islet cell replication, autoradiographic labelling index, proinsulin biosynthesis.

We have reported that intrasplenic transplantation of syngeneic pancreatic islets isolated from lean mice failed to cure obese hyperglycaemic mice, despite a considerable growth of the grafted islets [1]. In an attempt to elucidate the influence of hyperglycaemia on the growth of these islet grafts, syngeneic islets in numbers insufficient to cure diabetes (approximately 150) were implanted into alloxan-diabetic mice of two different inbred strains, C57BL/6J (BL/6) and C57BL/KsJ (BL/Ks) [2]. The volume of the islets implanted intrasplenically into alloxan-diabetic BL/Ks mice decreased markedly, whereas that of islets implanted into BL/6 mice was usually unaffected. Some mice of the latter strain, however, became normoglycaemic and indeed, in these particular mice an increase of the volume of the implanted islets was found.

The present study was carried out in order to elucidate to what extent a difference in islet cell replicatory activity of these two mouse strains contributes to the fate of intrasplenically implanted islets. For this purpose islets either were exposed to a high glucose environment in vitro by use of a tissue culture system [3] or in vivo were intrasplenically implanted using insufficient numbers of islets. Replicating cells were labelled with ^{3}H-thymidine and identified by an autoradiographic technique.

Materials and methods

Animals

Male and female inbred BL/6 and BL/Ks mice, aged 1, 3 or 9 months (belonging to strains originally obtained from the Jackson Laboratory, Bar Harbor, Maine, USA and then bred by continuous sister-brother mating), were used for preparation of islets either for culture or transplantation experiments. Three-to-5-month old, syngeneic males of both strains, injected intravenously with alloxan (75 mg/kg body weight) or non-injected controls, served as islet recipients. During the experimental period, animals were allowed free access to tap water and laboratory chow (Ewos-Anticimex, Type R3: Ewos, Södertälje, Sweden).

Table 1. Autoradiographic labelling indices of cultured mouse islets

Mouse strain and glucose concentration during culture and labelling	Age					
	1 month		3 months		9 months	
C57BL/6J mice						
2.7 mmol/l	0.405 ± 0.049	(8)	0.302 ± 0.015	(6)	0.147 ± 0.018	(6)
16.7 mmol/l	0.988 ± 0.064[a]	(8)	0.570 ± 0.044[a]	(6)	0.360 ± 0.033[a,c]	(7)
C57BL/KsJ mice						
2.7 mmol/l	0.273 ± 0.031	(9)	0.206 ± 0.017	(7)	< 0.1	(5)
16.7 mmol/l	0.503 ± 0.049[b]	(8)	0.372 ± 0.032[b]	(8)	0.151 ± 0.027[c]	(6)

Results expressed as mean ± SEM with the number of experiments in parentheses.
[a] $p < 0.001$; [b] $p < 0.01$, high versus low glucose culture within each strain; [c] $p < 0.001$, islets isolated from 9-month versus 1-month-old mice within each strain

Chemicals

Alloxan monohydrate was supplied by Sigma Chemicals, St. Louis, Missouri, USA. Collagenase was obtained from Worthington Biochemicals, Freehold, New Jersey, USA; tissue culture medium RPMI 1640 and Hanks' balanced salt solution (HBSS) from Statens Veterinärmedicinska Anstalt, Uppsala, Sweden; heat-inactivated fetal calf serum from Flow Laboratories, Irvine, Scotland; (L-(2,4,6^{-3}H)phenyl-alanine (sp. act. 82 Ci/mmol) and (methyl-^{3}H)thymidine (sp. act. 5 Ci/mmol) from Amersham International, Amersham, Bucks, UK; and antiserum against bovine insulin from Miles Laboratories, Kankakee, Illinois USA.

Islet isolation and culture

Pancreatic islets were prepared by a collagenase digestion method [4] from BL/6 or BL/Ks mice starved overnight. Groups of approximately 150 islets were cultured free-floating for 3 days as described previously [3] in RPMI 1640 medium with 2.7 or 16.7 mmol/l glucose and supplemented with 10% heat-inactivated fetal calf serum, 20 mmol/l Hepes and antibiotics. The medium was changed after 2 days of culture. In experiments intended for determinations of autoradiographic labelling indices, the medium was supplemented with 10 μCi/ml ^{3}H-thymidine during the last 60 min of the culture period. These islets were then rinsed in HBSS and fixed in Bouin's solution before further processing for autoradiography.

Estimation of proinsulin biosynthesis

Duplicate batches of 15 islets cultured in 16.7 mmol/l glucose as described above were transferred to multiwell plates containing, in each well, 50 μl of bicarbonate-buffered medium [5] supplemented with L(2,4,6-^{3}H)phenylalanine (5 μCi/ml), albumin (2 mg/ml) and glucose (16.7 mmol/l). The islets were incubated at 37 °C, in a humidified atmosphere of 5% CO_2 in air, for 2 h and subsequently sonicated in distilled water. The incorporation of ^{3}H-phenylalanine into proinsulin and trichloroacetic acid-precipitable proteins was determined using an immune binding technique [6]. Duplicate samples of the homogenates were removed for estimation of the DNA content [7, 8].

Islet implantation

Collagenase-isolated, syngeneic islets, prepared from 3–5 month old-male and female mice were cultured overnight in RPMI 1640 medium supplemented with 10% calf serum. The islets were suspended in HBSS and injected intrasplenically, in groups of 150, into lightly anaesthetized (ether) alloxan-diabetic or normal mice. Blood samples for glucose determination were collected by retroorbital puncture before islet implantation and at death. Only alloxan-diabetic mice with serum glucose concentrations > 25 mmol/l were used as recipients.

Serum glucose concentrations were determined with a glucose oxidase method using an automated analyser (Beckman Instruments, Fullerton, California, USA). At the end of the one week observation period, each mouse was injected intraperitoneally twice with 2 μCi ^{3}H-thymidine/g body weight, 120 and 90 min before killing. The pancreas and spleen were then rapidly removed and fixed in Bouin's solution.

Determination and autoradiographic labelling indices

The fixed tissues were embedded in paraffin and sectioned at 7 μm. After removal of paraffin, the sections were dipped in Kodak NTB-2 emulsion and exposed for 1 week (sections of cultured islets) or 4–5 weeks (sections of pancreas and spleen) at 4 °C. The autoradiographs were developed for 6 min in Kodak D-19 and fixed for 10 min in Kodak F-24. The emulsion base was allowed to dry overnight and the sections were lightly counterstained with haematoxylin and mounted. To determine the labelling index, radioactively labelled nuclei were counted at a magnification of × 1000 and expressed as a percentage of the total number of nuclei scored. In each preparation a minimum of 1000 nuclei were counted in at least 15 islet sections. In sections of cultured islets labelled nuclei were easily recognized due to the immense number of grains covering them. In intrasplenic and pancreatic islet cells 10 grains over the nucleus were required in order to constitute a labelled cell.

Statistical analyses

Results are expressed as mean ± SEM. Probabilities (p) of chance differences between groups were estimated by Student's two-tailed t-test.

Results

Labelling indices of cultured islets

High glucose concentration culture induced a two- to threefold increase of the autoradiographic labelling index when compared to culture in low glucose concentration (Table 1). In the oldest BL/Ks mice the labelling index after culture in low glucose concentrations was very low, thus making an accurate estimation of the labelling index impossible for this particular group. In both strains there was a considerable decrease of the labelling index with increasing age. Thus, islets of the 9-month-old mice had a labelling index approximately one-third that of 1-month-old mice. A comparison be-

tween the two strains showed that the labelling indices of high glucose concentration-cultured BL/6-islets were approximately twice as high as those of corresponding BL/Ks-islets ($p<0.001$ for 1- and 9-month-old mice and $p<0.01$ for 3-month-old mice).

Proinsulin biosynthesis of cultured islets

No differences in rates of islet proinsulin or total protein biosynthesis were observed between the two mouse strains (Table 2). In both strains there was a tendency towards increased proinsulin biosynthesis with increasing age, although a statistically significant difference was obtained only for the BL/Ks mice. In the BL/6 mice this increase, however, was not accompanied by an increase of the total protein biosynthetic rate. The percentage factor, expressing the ratio between the rates of proinsulin and total protein biosynthesis, therefore was increased significantly for the oldest BL/6 mice.

Islet implantation

Intrasplenic implantation of 150 syngeneic islets into alloxan-diabetic recipients did not restore the hyperglycaemia to normal levels in either strain within the observation period of 1 week. In normal animals of both strains the labelling index was similar in the pancreatic islets and in the islets implanted into the spleen (Table 3). At both locations, however, the islet labelling indices of BL/6 mice were two- to threefold higher than those of BL/Ks mice. The labelling indices of islets implanted intrasplenically into diabetic BL/6 mice were twice that of islets implanted into non-diabetic recipients. In the BL/Ks mice, however, there was no statistically significant difference in the mean labelling index of islets implanted into normal or diabetic mice. It is worthy of note that in four out of nine diabetic and in one out of five normal BL/Ks mice the implanted islets could not be recovered or were too small to be utilized for determination of the labelling index, despite serial sections of the entire spleens. In BL/6 mice implanted islets were recovered always in both the normal and diabetic animals. No attempts were made to localize islets possibly lost during transplantation.

Table 2. Proinsulin and total protein biosynthesis of high glucose concentration-cultured mouse islets

Biosynthesis (cpm/μg DNA)	Age		
	1 month	3 months	9 months
C57BL/6J mice			
Proinsulin	29.7± 3.9 (13)	34.7± 5.3 (14)	37.2± 7.8 (8)
Total protein	236 ±42	260 ±41	200 ±36
%	14.1± 1.1[a]	13.8± 0.6[b]	18.3± 1.2
C57BL/KsJ mice			
Proinsulin	27.3± 2.8[a] (17)	25.8± 2.8[a] (13)	38.5± 5.0 (7)
Total protein	171 ±18[a]	192 ±15[a]	277 ±33
%	16.8± 0.9	13.4± 0.8[c]	15.4± 2.8

Results expressed as mean ± SEM with the number of observations in parentheses. [a] $p<0.05$, 1 or 3 months versus 9 months; [b] $p<0.01$, 3 versus 9 months; [c] $p<0.05$, 3 months versus 1 month. % denotes the percentage radioactivity recovered in proinsulin per total protein biosynthesis

Discussion

In mice the severity and course of the diabetic syndrome produced by the two single gene mutations obese (ob) and diabetes (db) depends on their interaction with the genetic background [9]. Thus, when placed on one inbred background (BL/6) a mild and well-compensated diabetes develops, resembling human Type 2 (non-insulin-dependent) diabetes and compatible with a near-normal life span. On another background (BL/Ks) the syndrome, however, presents as an overt diabetes with islet cell degeneration leading to marked insulinopenia and hyperglycaemia and a much shortened lifespan. As yet, the primary defect of these two mouse mutations remains uncertain. There is an insulin resistance associated with a loss of receptor sites, which causes an excessive insulin demand and which in turn leads to a compensatory increase in size and number of the islets. In the diabetic BL/Ks mice there is, however, a decline of this β-cell mitotic activity after a few months as demonstrated with autoradiographic techniques [10, 11]. An imbalance between β-cell regen-

Table 3. Serum glucose and islet autoradiographic labelling indices of islet-implanted mice

Mouse strain	Serum glucose (mmol/l)		Autoradiographic labelling index	
	Before implantation	At death	Islets in pancreas	Islets in spleen
C57BL/6J mice				
Control	8.7±0.3 (4)	–	0.541±0.100 (4)	0.556±0.059 (6)
Alloxan-injected	33.2±2.1 (8)	33.6±2.0 (8)	–	1.057±0.076[a] (8)
C57BL/KsJ mice				
Control	8.6±0.3 (5)	–	0.198±0.023[b] (5)	0.279±0.054[b] (4)
Alloxan-injected	40.3±2.7[d] (9)	34.8±2.1 (9)	–	0.409±0.084[c] (5)

Results expressed as mean ± SEM with the number of observations in parentheses.
[a] $p<0.001$, control mice versus alloxan-injected C57BL/6J mice; [b] $p<0.05$; [c] $p<0.01$, C57BL/KsJ versus C57BL/6J mice; [d] NS, alloxan-injected C57BL/6J mice versus alloxan-injected C57BL/KsJ mice

eration and β-cell degeneration in the late stage of the syndrome thus probably favours the latter process, resulting in an overall reduction in the number of islet cells.

The present in vitro data indicate that there is a marked difference in the replicatory capacity of islets isolated from the two different inbred strains used in this study. Thus, in general there was a lower replicatory activity in the BL/Ks islets as compared to that of the BL/6 islets. Indeed, this discrepancy in replicatory capacity at the islet cell level may explain why the islets of the BL/Ks mice cannot keep up with the abnormal metabolic demand for insulin to control diabetes when the diabetes mutant gene is maintained on this particular inbred background.

In a previous study [12] we were unable to demonstrate a difference in the rate of DNA synthesis of cultured islets prepared from these two strains. The sensitivity of the present experimental design, however, is much higher due to the use of autoradiography, a shorter labelling period and the free-floating culture technique for the islets. Despite the use of a morphological method for quantitation of the replicatory activity in the present study, we did not examine whether different islet cell types were selectively affected by the experimental conditions. In general we observed no preferential location of the labelled cells in the islets, suggesting that the cell types were equally active in synthesizing DNA.

The difference between the two strains in islet cell replicatory activity was obvious also in the islet implantation experiments. Again, more labelled cells were found in the BL/6 islets irrespective of whether the islets were located in the spleen or the pancreas of the control mice. Naturally, the present finding of a lack of glucose effect on BL/Ks islet cell replication in vivo may explain why the size of syngeneic islets implanted in insufficient numbers into alloxan-diabetic BL/Ks recipients decreased so markedly [2]. It cannot, however, be ruled out that this islet atrophy and the poor islet recovery in some of the BL/Ks mice in this study could be explained by a putative hyperfunctional exhaustion and loss of β cells. It is worthy of note in this context that in the insulin biosynthesis experiments no indication of such an exhaustion was evident in either strain.

The present study forms an interesting parallel to the finding of a decreased islet volume in human Type 2 (non-insulin-dependent) diabetes [13, 14] and increased islet volume in obese non-diabetic subjects [15]. It may be implied, as proposed previously from these observations [16, 17], that Type 2 diabetes develops only in those individuals who have a diminished capacity for islet cell regeneration. This concept may add a new dimension to the debate of whether this type of diabetes is caused primarily by decreased peripheral sensitivity to insulin or by an insufficient insulin secretion.

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