

Role of Blood Glucose in Cytokine Gene Expression in Early Syngeneic Islet Transplantation

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In islet transplantation, local production of cytokines at the grafted site may contribute to the initial nonspecific inflammation response. We have determined whether the metabolic condition of the recipient modulates the cytokine expression in islet grafts in the initial days after transplantation. Normoglycemic and hyperglycemic streptozotocin-diabetic Lewis rats were transplanted with 500 syngeneic islets, an insufficient beta cell mass to restore normoglycemia in hyperglycemic recipients. The expression of IL-1 β , TNF- α , IFN- γ , IL-6, IL-10, and IL-4 genes was determined by real-time PCR in freshly isolated islets, in 24-h cultured islets and in islet grafts on days 1, 3, and 7 after transplantation. IL-1 β mRNA was strongly and similarly increased in normoglycemic and hyperglycemic groups on days 1, 3, and 7 after transplantation compared with freshly isolated and cultured islets. TNF- α mRNA was also strongly increased on day 1, and it remained increased on days 3 and 7. IL-6 and IL-10 were not detected in freshly isolated islets, but their expression was clearly enhanced in 24-h cultured islets and islet grafts. IL-6 was further increased in hyperglycemic grafts. IL-10 expression was increased in both normoglycemic and hyperglycemic grafts on day 1 after transplantation, and remained increased in hyperglycemic grafts compared to 24-h cultured islets. IFN- γ mRNA was barely detected in a few grafts, and IL-4 mRNA was never detected. Thus, the inflammatory response in islet grafts was maximal on day 1 after transplantation, it was sustained, although at lower levels, on days 3 and 7, and it was partly enhanced by hyperglycemia.

Key words: Islet transplantation; Cytokine; Real-time PCR; Hyperglycemia

INTRODUCTION

The promising results achieved by islet transplantation in the treatment of type 1 diabetes (35) are attenuated by the severe limitation imposed by the scarcity of islet tissue available for transplantation and the high number of islets required for successful transplantation (35). The enthusiasm generated by the Edmonton protocol has been recently tempered by the demonstration of high recurrence of diabetes (34). The vulnerability of islets in the initial days after transplantation, when more than 60% of the islet tissue is lost (4), clearly increases the number of islets required to achieve initial normoglycemia after transplantation, and also may play a role in the long-term failure of the graft (15). Reduction of this initial beta cell death has been shown to improve the metabolic outcome of the graft (25). The study of candidate genes to contribute to initial damage of transplanted islets could improve our understanding of the mechanisms leading to early damage of transplanted is-

lets and could be used to design strategies aimed to improve the survival of transplanted islets.

The mechanism of early graft failure is probably multifactorial, and although mediators of this initial beta cell death are not well known, it is generally accepted that after transplantation an inflammatory response takes place in the context of a “transplant environment activation” comprising ischemia/reperfusion damage and production of proinflammatory mediators (32). This inflammatory response occurs before immunological rejection or recurrence of autoimmunity take place, and is a general phenomenon afflicting syngeneic, allogeneic, and xenogeneic islet transplantation (32). Although inflammatory cytokines have been pointed out as important players in this nonspecific inflammation (32), their expression has been rarely determined in islet grafts in this context.

It is clearly established that the metabolic condition of the recipient modifies the outcome of islet transplantation. Islet grafts exposed to sustained hyperglycemia

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show impaired beta cell function (14,16,43), limited replicative capacity, increased beta cell apoptosis (4), and reduced beta cell mass (11,24). Moreover, we have reported that insulin-induced normoglycemia has a beneficial effect on the outcome of pancreatic islets transplanted to diabetic recipients in rodents (23). It is not known, however, whether the deleterious effects of hyperglycemia on transplanted islets enhance the nonspecific inflammation characteristic of early islet transplantation. Thus, the aim of the study was to determine the expression of pro- and anti-inflammatory cytokine genes in the initial days after syngeneic islet transplantation and to study whether blood glucose modulates their expression in islet grafts.

MATERIALS AND METHODS

Animals

Male Lewis rats (Harlan, Horst, The Netherlands) aged 7–10 weeks, were used as donors and recipients of transplantation. Hyperglycemic recipients were made diabetic by a single IP injection of streptozotocin (STZ) (Sigma Immunochemicals, St Louis, MO, USA) 65 mg/kg body wt, freshly dissolved in citrate buffer (pH 4.5). Only those rats with a blood glucose higher than 20 mmol/L on two consecutive measurements were transplanted. Blood glucose was determined between 0900 and 1100 h in nonfasting conditions. Blood was obtained from the snipped tail and glucose was measured with a portable glucose meter (Glucocard, A. Menarini Diagnostics, Barcelona, Spain). Animals were kept under conventional conditions in climatized rooms with free access to tap water and standard pelleted food.

Experimental Design

Normoglycemic, non-STZ-injected, and hyperglycemic, STZ-injected, Lewis rats were transplanted with 500 freshly isolated syngeneic islets. Because 500 syngeneic islets is an insufficient beta cell mass to consistently restore normoglycemia in this model (40), the diabetic animals were expected to remain hyperglycemic after transplantation. Grafts from normoglycemic and hyperglycemic rats were harvested on days 1, 3, or 7 after transplantation (six groups, $n = 6$ for each experimental group).

Six groups of 500 freshly isolated islets were used to determine cytokine expression in islets before transplantation. In addition, six groups of 500 islets were cultured for 24 h in RPMI-1640 medium supplemented with 11.1 mM glucose and 10% fetal calf serum (FCS) (Biological Industries, Beit Haemek, Israel) at 37°C and 5% CO₂ to determine cytokine expression after culture.

Islet Isolation, Transplantation, and Graft Harvesting

Islets were isolated by collagenase (Collagenase P, Boehringer Mannheim Biochemicals, Germany) digestion of the pancreas as previously described (27). All isolations were performed with the same batch of collagenase to avoid differences in endotoxin activity among isolations (41). Isolated islets were hand-picked under a stereomicroscope two or three times until a population of pure islets was obtained. Islets 75–250 μm in diameter were counted into groups of 500 islets and transplanted into the recipient on the same day of the isolation. Animals were anesthetized with a mixture of ketamine (100 mg/kg), diazepam (7.5 mg/kg), and atropine (0.05 mg/kg) and the left kidney was exposed through a lumbar incision. A capsulotomy was performed and islets were injected under the kidney capsule. After transplantation, the capsulotomy was cauterized with a disposable low-temperature cautery pen (AB Medica, S.A, Barcelona, Spain), and the lumbar incision was sutured. To harvest the grafts, the kidney was exposed, and the kidney capsule surrounding the graft was incised and removed with the graft. After removal from the kidney, the graft was immediately immersed in lysis buffer and total RNA was extracted.

RNA Extraction and Complementary DNA (cDNA) Synthesis

Total RNA was extracted from freshly isolated islets, 24-h cultured islets, and islet grafts using RNeasy Mini Kit (Qiagen, Crawley, UK), according to the manufacturer's protocol. Islets and grafts were immersed in lysis buffer containing 350 μl guanidine isothiocyanate-containing buffer plus 3.5 μl β -mercaptoethanol. Samples were homogenized and lysed by mechanical disruption with repeated passing through a Pasteur pipette. The lysate was centrifuged and the nonlysed kidney capsule was discarded. Total RNA was eluted in RNase-free water, quantified at 260 nm using DU® 640 Spectrophotometer (Beckman, Fullerton, CA, USA) and its integrity visualized in 1% agarose gel.

A constant amount of 1 μg of total RNA was treated with 1 U RQ1 RNase-free DNase (Promega, Madison, WI, USA) at 37°C for 30 min and then predenatured at 65°C for 10 min with 500 ng random hexamer primers (Promega,) and 10 mM of dNTP mix (10 mM each dATP, dGTP, dCTP, and dTTP) (Invitrogen, Carlsbad, CA, USA). cDNA synthesis was carried out in a final volume of 40 μl mixture containing 5' first strand buffer (Invitrogen), 40 U RNasin (Promega), 0.1 M DTT and 400 U SuperScript™ II RNase H⁻ reverse transcriptase (Invitrogen). Reactions were performed in a GeneAmp PCR System 9600 Perkin-Elmer Thermocycler (Applied Biosystems, Cheshire, UK) at the following conditions:

25°C for 10 min, 42°C for 50 min, and stopped at 70°C for 15 min. Resulting cDNA was stored at -20°C until use.

Relative Quantification Gene Expression Using Real-Time PCR

cDNA of IL-1 β , TNF- α , IFN- γ , IL-6, IL-10, and IL-4 were amplified and quantified by real-time PCR (ABI Prism® 7700, Applied Biosystems). Cytokine expression levels were normalized using eukaryotic rRNA 18S expression as internal control (33). Gene expression signals (C_T , or threshold cycle) were used to calculate $\Delta C_T = C_{T, \text{target gene}} - C_{T, 18S}$ (47). Because ΔC_T can be used when the amplifying efficiencies of both amplicons (target gene and internal control) are similar, we established the similarity of PCR amplification efficiencies of target gene and internal control for every pair of amplicons. With the exception of IL-4, which could not be detected, we confirmed that the absolute value of the slope of log input amount versus the difference between C_T for target gene and C_T for reference gene was closer to zero for all target genes (User Bulletin #2, ABI Prism 7700 Sequence Detection System, Applied Biosystems).

The target and the internal control genes were amplified in separated wells. For every PCR reaction, 1 μ l of each cDNA sample (corresponding to 25 ng of starting RNA for target gene, and 25 pg for the internal control 18S) were mixed with 2 \times TaqMan® Universal PCR Master Mix (12.5 μ l), 20 \times target or internal control primers and probe (PDAR, TaqMan® Pre-Developed Assay Reagents, Applied Biosystems) (1.25 μ l) in a total volume of 25 μ l. Amplification was performed following the universal amplification program: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min. Data were analyzed using the SDS software (Applied Biosystems).

CD68 Immunohistochemistry

To identify the presence of macrophages, a potential source of proinflammatory cytokines in islet grafts, we stained CD68-expressing cells (monocyte/macrophage lineage marker) in freshly isolated islets and in islet grafts. Rat spleen sections were used as a positive control. Endogenous peroxidases were blocked with 1% hydrogen peroxidase solution and antigen retrieval was performed by incubation in citrate buffer. Then sections were blocked with 5% horse serum (Biological Industries, Beit Haemek, Israel), and incubated overnight at 4°C with mouse anti-rat CD68 antibody (final dilution 1:100) (Serotec, Oxford, UK). Immunostaining was performed using an Immuno Pure® ABC Peroxidase Mouse IgG Staining Kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Peroxidase

reaction was visualized with 3,3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide. Bright-field microscope was used to visualize the CD68-stained sections.

Statistical Analysis

Results were expressed as mean \pm SEM. Differences among groups were evaluated using the one-way analysis of variance (ANOVA) or the Kruskal-Wallis H test, and when determined as significant (values of $p < 0.05$) the Fisher's PLSD (protected least significant difference) method, or the Mann-Whitney U test was used to determine specific differences between groups. Subsequently, the Hochberg's Sequential Method was used as post hoc test to adjust the type I error (α) for multiple testing. However, the use of adjustments for multiple tests has been seriously questioned (29), and it increases the likelihood of type II errors. Thus, the result of the Mann-Whitney U test is presented when it is statistically significant and it is followed by the result after the adjustment with the Hochberg's Sequential Method. The statistical software SPSS 12.0 and the Multiplicity Program from the Department of Biomathematics of the University of Texas M.D. Anderson Cancer Center (Houston, TX) were used.

RESULTS

Metabolic Evolution

Blood glucose and body weight of experimental groups are summarized in Table 1. On transplantation day, blood glucose level was 28.3 ± 1.1 mmol/L in the hyperglycemic group and 6.1 ± 0.2 mmol/L in the normoglycemic group. STZ-diabetic rats remained hyperglycemic during the study, confirming that 500 islets is an insufficient mass to restore normoglycemia.

Cytokine mRNA Expression

The total amount of RNA obtained from 500 isolated islets was 9.9 ± 1.0 μ g, and 24-h cultured islets had a similar total RNA content (11.0 ± 0.8 μ g). The RNA recovered from the 500-islet grafts was reduced to $\sim 40\%$ of the RNA obtained from 500 freshly isolated islets or from 500 cultured islets. This reduction is in agreement with the previously reported reduction in beta cell mass in the initial days after islet transplantation (4). There were no differences in recovered total RNA among days after transplantation or between normoglycemic and hyperglycemic groups (Fig. 1).

In freshly isolated islets IL-1 β and TNF- α mRNA were detected at low levels, whereas IL-6 and IL-10 transcripts were barely detectable, and IFN- γ and IL-4 mRNA were not detected (Fig. 2). The expression of IL-6 and IL-10 genes became clearly detectable in 24-h

Table 1. Characteristics of Experimental Groups

Group	Day of Graft Removal	n	Transplantation		Graft Removal	
			Blood Glucose (mmol/L)	Body Weight (g)	Blood Glucose (mmol/L)	Body Weight (g)
Normoglycemia	1	6	6.4 ± 0.5	214 ± 9	5.5 ± 0.1	203 ± 8
	3	6	5.7 ± 0.2	235 ± 11	5.5 ± 0.2	233 ± 11
	7	6	6.1 ± 0.2	220 ± 5	5.3 ± 0.1	242 ± 7
Hyperglycemia	1	6	25.8 ± 1.6	195 ± 7	25.9 ± 1.2	195 ± 6
	3	6	30.1 ± 1.3	202 ± 6	22.7 ± 1.9	203 ± 5
	7	6	29.0 ± 2.0	195 ± 6	23.2 ± 2.1	208 ± 3

Values are means ± SEM.

cultured islets. The expression of IL-1 β , TNF- α , IL-6, and IL-10 was increased in islet grafts compared to freshly isolated islets. In contrast, IFN- γ mRNA remained undetectable in cultured islets and it was barely detectable or undetectable in islet grafts, whereas IL-4 was never detected in cultured islets or islet grafts. Thus, the detailed analysis of the expression of cytokines was limited to those consistently detected, IL-1 β , TNF- α , IL-6, and IL-10.

IL-1 β . IL-1 β mRNA was detected in freshly isolated islets and in 24-h cultured islets. On day 1 after transplantation, IL-1 β expression increased strongly in both normoglycemic and hyperglycemic groups compared with freshly isolated ($p = 0.002$) and cultured ($p =$

0.002) islets. Although IL-1 β expression decreased on days 3 and 7 after transplantation compared to 1-day grafts, it remained higher than in freshly isolated and cultured islets even when adjusted for multiple testing. Hyperglycemia did not modify IL-1 β expression at any time after transplantation.

TNF- α . TNF- α mRNA was detected in freshly isolated islets and in 24-h cultured islets. TNF- α expression increased on day 1 after transplantation both in normoglycemic and hyperglycemic groups, and although it decreased on days 3 and 7, it remained higher than in freshly isolated ($p = 0.002$) and 24-h cultured islets ($p = 0.002$). On day 1 after transplantation TNF- α gene expression was higher in grafts from hyperglycemic recipients ($p = 0.026$), but the difference was not statistically significant when it was adjusted for multiple testing. On days 3 and 7 grafts transplanted to normoglycemic and hyperglycemic recipients showed comparable levels of TNF- α transcripts.

IL-6. IL-6 mRNA was barely detectable and could not be quantified in freshly isolated islets, but it was clearly detected in 24-h cultured islets, and was further increased on day 1 after transplantation in islet grafts exposed to hyperglycemia ($p = 0.009$), a difference that was in the limit of significance after adjusting for multiple testing ($p = 0.054$). On days 3 and 7 after transplantation, IL-6 gene expression in hyperglycemic groups remained increased to values similar to those of 24-h cultured islets. In contrast, in normoglycemic groups IL-6 was not increased on day 1 compared to 24-h cultured islets, and was even reduced on days 3 ($p = 0.015$) and 7 ($p = 0.015$), a difference that was of borderline significance after adjusting for multiple testing ($p = 0.060$).

IL-10. IL-10 mRNA was barely detectable and could not be quantified in freshly isolated islets, but it was clearly detected in 24-h cultured islets, and was further increased on day 1 after transplantation in both normog-

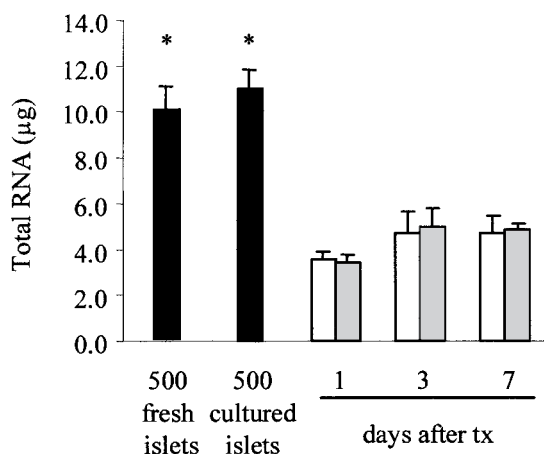


Figure 1. Total RNA obtained from freshly isolated islets ($n = 3$), 24-h cultured islets ($n = 6$), and 500-islet grafts transplanted to normoglycemic (open bars) and hyperglycemic (gray bars) rats and harvested on days 1, 3, and 7 after transplantation. Values are means ± SEM. ANOVA, $p < 0.001$; Fisher's PLSD, $*p < 0.001$ freshly isolated and 24-h cultured islets versus all other groups.

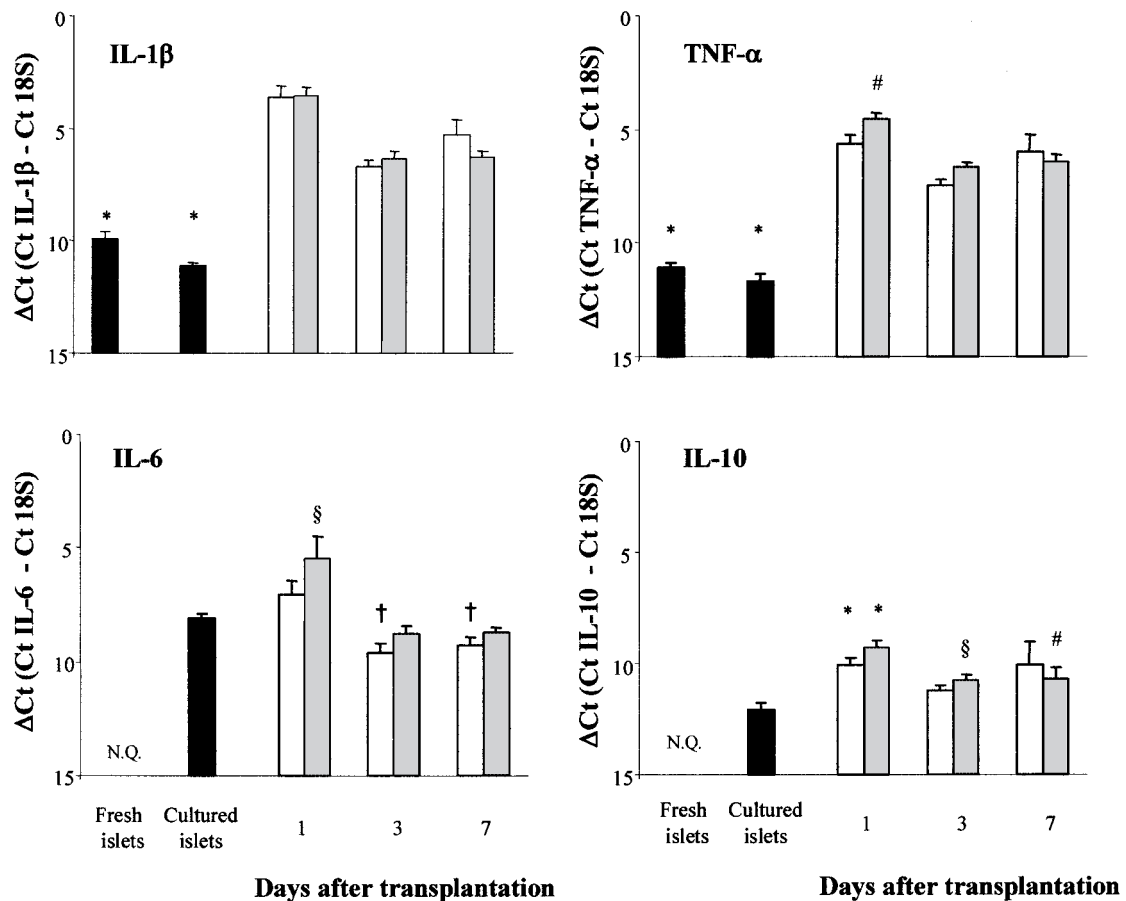


Figure 2. Real-time PCR results for expression levels of target genes on freshly isolated islets, 24-h cultured islets, and islet grafts on days 1, 3, and 7 after transplantation to normoglycemic (open bars) and hyperglycemic (gray bars) recipients. Gene expression was expressed as ΔC_t . Note that minimum value of target gene expression was 40 and the maximum 0. Values are means \pm SEM. N.Q.: not quantified due to the very low expression level. Kruskal-Wallis, $p < 0.001$ for each of the four cytokines; Mann-Whitney U test: * $p = 0.002$ versus all transplanted groups (IL-1 β and TNF- α) or versus cultured islets (IL-10), # $p = 0.026$ versus day 1 normoglycemic group (TNF- α) or versus cultured islets (IL-10), § $p = 0.009$ versus cultured islets (IL-6 and IL-10), † $p = 0.015$ versus cultured islets (IL-6).

lycemic ($p = 0.002$) and hyperglycemic ($p = 0.002$) grafts, and it remained increased in hyperglycemic grafts on day 3 ($p = 0.009$). These differences remained statistically significant when adjusted for multiple testing. Although IL-10 gene expression was also higher on day 7 in hyperglycemic grafts ($p = 0.026$), the difference was not statistically significant when it was adjusted for multiple testing. In contrast, in normoglycemic groups IL-10 gene expression was not increased on days 3 and 7 after transplantation compared to 24-h cultured islets.

CD68-Positive Cells in Islet Grafts. In islet grafts, macrophages were abundant in the periphery of the islet tissue and in the necrotic areas that are commonly found (4) in the initial days after transplantation (Fig. 3). A few CD68-positive cells were identified in freshly isolated islets, in agreement with previously published data (1).

DISCUSSION

We have identified the expression of proinflammatory cytokines IL-1 β , TNF- α , IL-6, and anti-inflammatory IL-10 in syngeneic islet grafts immediately after transplantation. In contrast, the expression of IFN- γ and IL-4 genes was barely detectable or undetectable. Cytokine gene expression in islet grafts was maximal on day 1 after transplantation, and it declined thereafter even though in most cases it remained higher than in freshly isolated islets or 24-h cultured islets. This inflammatory response was enhanced in islets transplanted to hyperglycemic recipients.

Previous studies have shown that the endotoxin activity present in collagenase preparations elicits an inflammatory response that may be responsible for the induction of several proinflammatory cytokine genes in islets during the isolation process (41). Thus, the cytokine ex-

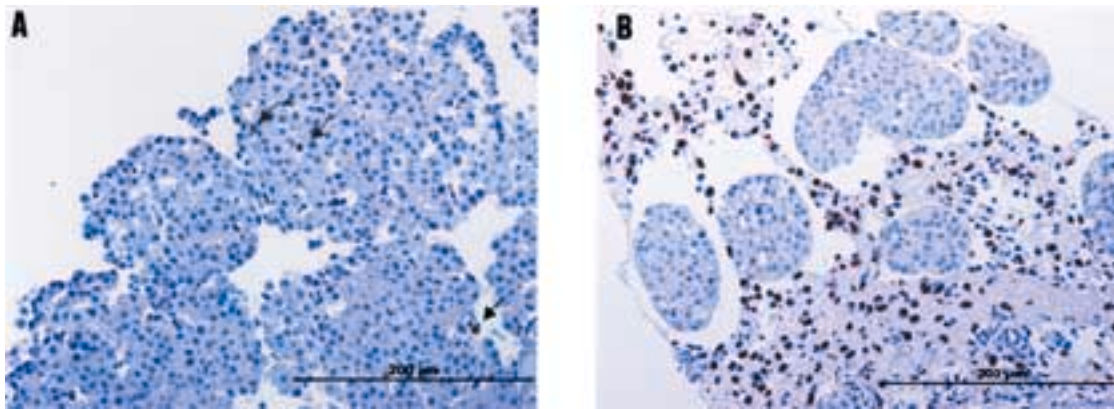


Figure 3. Macrophage detection in isolated islets and islet grafts. Sections were stained using an anti-CD68 antibody (monocyte/macrophage lineage marker), and visualized with 3,3'-diaminobenzidine tetrahydrochloride on bright-field microscope (brown staining). Freshly isolated islets (A) and a typical day 1 graft (B) are shown. Few macrophages were identified in freshly isolated islets (arrows), but abundant macrophages were found in the periphery of islet tissue and in necrotic areas in grafts.

pression found in the initial days after transplantation, and particularly on day 1, could reflect the inflammation induced by the isolation procedure. To minimize differences due to endotoxin-induced inflammation, all isolations were performed with the same batch of collagenase. Moreover, in order to ascertain the contribution of the isolation and transplantation procedures to cytokine gene expression in islet grafts, a group of freshly isolated islets and a group of 24-h cultured islets were included in the study. Cytokine gene expression was higher in islet grafts on day 1 after transplantation than in freshly isolated or in 24-h cultured islets, showing that the transplantation played a role inducing the inflammation at the grafted site. The effect of transplantation procedure was particularly important on IL-1 β and TNF- α expression, which was much higher on islet grafts than in isolated or cultured islets. In contrast, the impact of transplantation was milder on IL-6 and IL-10 expression, which was clearly increased 1 day after isolation in cultured islets, but only modestly enhanced in islet grafts.

The strong expression of IL-1 β on day 1 after transplantation, and its persistently increased expression, even though at lower levels, on days 3 and 7 is particularly relevant due to the well-established deleterious effects of IL-1 β on beta cells. IL-1 β impairs beta cell function, induces beta cell death, and suppresses beta cell replication (12,18,36,39), defects that have all been reported in recently transplanted islets. In addition, IL-1 β induction of TNF- α production by beta cells (45) could amplify the inflammatory response in islet grafts. Hyperglycemia, however, did not modify IL-1 β gene expression at any studied time after transplantation. Even though glucose induction of IL-1 β expression was reported in human islets (20), our results are in agreement

with previous data in rodent islets and with the recent report in human islets indicating that glucose does not induce IL-1 β production (13,44). However, hyperglycemia could modify beta cell sensitivity to the deleterious effects of IL-1 β (19,38).

TNF- α gene expression was detected in freshly isolated islets, in agreement with previous data in rodent and human islets (3,41), and was increased in transplanted islets. TNF- α expression was higher in day 1 hyperglycemic group, but because the difference was not confirmed after adjustment for multiple comparisons, the effect of hyperglycemia on TNF- α expression in islet grafts could not be definitively established. The increased expression of TNF- α in islet grafts had not been previously found (28), and could play a deleterious role on graft survival due to the direct deleterious effects of TNF- α on islet cells (6,17), particularly in combination with IL-1 β (19). In addition, TNF- α upregulation of IL-6 production by pancreatic islets (5) could enhance the inflammatory response in islet grafts. However, IL-6 actions depend on cell type, and in rat islets, both IL-6-induced damage (37,42) and protection (8) have been reported. IL-6 was detected in cultured islets but not in freshly isolated islets, and on day 1 after transplantation it was increased exclusively in the hyperglycemic group. The similar increment in IL-6 expression in 24-h cultured islets and day 1 normoglycemic grafts compared to the nondetectable levels in freshly isolated islets suggests that initial IL-6 expression in islet grafts was mostly related to pretransplantation processes, but we could not determine whether it was mostly related to the isolation procedure (41) or to factors taking place after islet isolation. A similar consideration is valid for IL-10 expression in islet grafts. On days 3 and 7, IL-6 expression remained as high as in 24-h cultured islets in hyper-

glycemic groups, but tended to decrease in normoglycemic groups. Overall, the results indicate that although transplantation did not modify per se the expression of IL-6 gene in islet grafts, hyperglycemia enhanced IL-6 gene expression in transplanted islets.

IFN- γ was just barely detected in a few islet grafts, and ambient blood glucose did not modify its expression. In agreement with our results, IFN- γ expression has been previously found in islet allotransplantation, but not or barely in syngeneic grafts (7,28). However, recent data have implicated IFN- γ in early graft loss in syngeneic islets transplanted to the liver (46), suggesting that the expression of IFN- γ may be dependent on the transplantation site.

Type 2 cytokines IL-4 and IL-10 may have a protective effect on islets exposed to proinflammatory cytokines (22,30), and predominant intragraft production of type 2 cytokines has been related to graft acceptance in organ transplantation (2). We did not detect IL-4 gene expression, in agreement with previous organ transplantation data showing IL-4 only in allogeneic transplantation (9). In contrast, IL-10 gene expression was significantly increased on day 1 after transplantation both in normoglycemic and hyperglycemic recipients. In normoglycemic groups IL-10 expression decreased subsequently to levels similar to those of 24-h cultured islets, but it remained increased in hyperglycemic groups. The data indicate a role for IL-10 against nonspecific inflammation in islet grafts, and we suggest that the higher expression in hyperglycemic animals may be in response to a more potent inflammation experienced by transplanted islets exposed to hyperglycemia.

This study has some potential limitations. First, although gene expression analysis provides useful information about the events that take place in islet grafts, posttranscriptional and posttranslational modifications may result in discrepancies between mRNA and protein expression. Second, the intensity and characteristics of inflammatory events may be different in other transplantation sites. In particular, inflammation may be higher in islets transplanted into the liver (46). It is remarkable, therefore, that we have found a clear induction of inflammatory cytokines using a relatively privileged transplant site and a syngeneic model. These results support the potential important role of early inflammation in islet transplantation. Third, the results found in murine models may not directly apply to human islets. For instance, differences in islet sensitivity to inflammatory cytokines among species have been recognized for long time (10). However, differences in glucose-induced expression of cytokines between murine and human islets (20) have not so far been confirmed (44). Finally, although we have identified that macrophages, a well-known source of proinflammatory cytokines, were abundant in

islet grafts we have not shown that they produced the cytokines. Moreover, transplanted islet preparations contain other cell types that may contribute to cytokine expression, as suggested by the reported production of IL-1 β , IL-6, and TNF- α by beta cells (5,20,45) and acinar cells (31,44), of IL-10 by acinar cells (31), of TNF- α by pancreatic ductal cells (26), or of IL-1 and IL-6 among others by endothelial cells (21).

In summary, we have characterized the nonspecific inflammation that takes place in the immediate days after islet transplantation. We have shown that the expression of proinflammatory cytokine genes in islet grafts is maximal on day 1 after transplantation, and it is partly modified by the ambient blood glucose. Even though the enhanced expression of cytokine genes in grafts exposed to high blood glucose could be relatively modest, the results suggest that the deleterious effects of hyperglycemia on islet grafts include the enhancement of nonspecific graft inflammation. Understanding the mechanisms of beta cell destruction in islet transplantation will contribute to develop strategies to protect islet grafts.

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