IL-1β and glycemia levels are inversely proportional in early period of allo- and xeno-transplantation of islets of Langerhans

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ABSTRACT

The aim of diabetes treatment must be to obtain normal glucose metabolism and prevent or reverse the late complications of the disease. Interest in islet transplantation for type 1 diabetes treatment is increasing, but immune mediated graft rejection is still a major concern at the time of transplantation. Previous findings have shown that interleukin 1-beta (IL-1β) is important in initiating β-cell dysfunction and cellular damage. In this study, IL-1β release and its relationship to glycemia levels following transplantation of allo- or xenogeneic islets of Langerhans into the liver of healthy and diabetic rats were investigated. IL-1β and glycemia levels were found to be inversely related in early post-transplant period. Monitoring cytokine profiles of recipients, especially in peri-transplant period, seems to be a promising measure of graft functioning.

KEY WORDS: Islet of Langerhans, transplantation, IL-1beta, glycemia, allograft, xenograft

INTRODUCTION

Autoreactive T cells mediated destruction of β-cells in the pancreatic islets of Langerhans leads to insulin deficiency with subsequent autoimmune type 1 diabetes (1-5). The aim of diabetes treatment must not only be to obtain normal glucose metabolism, but also to prevent or reverse the late complications of the disease, since the relationship between glycemic control and the risk of developing chronic complications was well determined (6, 7). Currently, pancreas and islet transplantation are considered as the most powerful therapies to obtain normoglycemia in diabetics (8). The endocrine component of the pancreas comprises <2% of the gland, and interest in islet transplantation is increasing as it is a simple and safer method than whole organ transplantation (9-11). However, immune mediated graft rejection at the time of transplantation is still a major concern. In a recent study, it has been demonstrated that intraportal administration of islet cells may lead to a clogging of the portal vessels and induce ischemia reperfusion injury to the surrounding liver tissue and islet cells (12). Local hypoxia and elevated pro-inflammatory cytokines levels further deteriored graft survival. Particularly interleukin 1-beta (IL-1β) is important in elucidating β-cell dysfunction and initiating β-cell damage within islets (13). Treatment of rodent islets with this cytokine results in a potent inhibition of insulin secretion followed by islet destruction (14).

As rodents are important models for the study of immunologic allo- and xeno-grafts rejection (9, 15), in this study, IL-1β release and its relationship to glycemia levels *in vivo* following transplantation of allo- and xeno-geneic islets of Langerhans in the liver of healthy and diabetic rats with/without immunosuppression were investigated.

MATERIALS AND METHODS

Donors

Outbred Wistar albino female rats (n=108) 220 to 250 g and aged 16 to 18 weeks obtained from the Institute of Experimental Medicine of Istanbul University, and heart-beating humans served as donors.

Recipients

Inbred Wistar albino male rats (n=36) similar to donor rats for weight and age were used as islet graft recipients. Two main groups (Allo-graft and Xeno-graft) were divided into 6 subgroups: non immunosuppressed diabetics (1), immunosuppressed diabetics (2) as allo-grafts, and non immunosuppressed non diabetics (3), immunosuppressed non diabetics (4), non immunosuppressed diabetics (5), immunosuppressed diabetics (6) as xeno-grafts.

Immunosuppression

The immunosuppressive regimen in the recipient rats consisted of Cyclosporine A (CyA, Novartis, Switzerland) dissolved at 6 mg/ml (w/v) concentration in 50% ethanol. CyA (9 mg/kg bw.) was injected into femoral muscle for immunosupression on days –1, 0, +1, and +2 of transplantation.

Diabetes induction

Experimental diabetes was induced in the fed recipients using a single intraperitoneal dose of 60 mg/kg of streptozotocin (STZ, Sigma, St. Louis, Mo, USA), dissolved at 40 μg/μl concentration in 0.9% NaCl solution. Diabetes was diagnosed based on blood glucose concentrations >200 mg/dl the day after STZ injection.

Islet isolation

Rats

Allogeneic Wistar albino rats weighing 220-250 g were used as pancreas donors for the allo-transplantation. Under ether anesthesia, the abdominal wall was opened by a midline incision, the liver was retracted cranially, and the distal end of the common bile duct was clamped. The proximal common duct is incised, cannulated with a 16-gauge polyethylene catheter, and slowly injected with 10 ml of Hank's solution to distend the pancreas. The pancreata were resected, disrupted into small pieces with scissors, and washed with cold Hank's balanced salt solution (HBSS). The tissue was digested with Collagenase P 4 mg/pancreas (Clostridium histolyticum; Boehringer Mannheim, Mannheim, Germany) for 10 min at 37^0C and simultaneously oxigenated with O_2 95% + CO_2 5% in a water bath. After 10 minutes the digested tissue was shaken vigorously by hand for 90 seconds in the water bath and washed with HBSS for 3 times. The islets were collected by hand-picking under a dissecting microscope. A mixture of approximately 850 islets were isolated from 3 rats.

Humans

The pancreata were harvested from three multiorgan cadaveric donors, 25-30 years of age, dead after a traumatic brain injury. The warm ischemia time and the preservation of the organs in University of Wisconsin (UW) solution each took about 30 minutes. Tissue pieces chopped in 2-3 g each were maintained in -20^0C. Tissues of 1.2 g were distended by injecting Hank's solution and disrupted. The tissues were digested in 37^0C water bath for 21 minutes by adding Collagenase P and simultaneously oxigenated. The digestion was halted with cold Hank's solution. The tissues were mixed and confirmed to be islet tissue by dithizone (DTZ) staining. Approximately 1500 islets were hand-picked under a dissecting microscope.

Islet transplantation

Islet transplantation was conducted at laparotomy under ether anesthesia. Approximately 850 and 1500 islets suspended in 1 ml HBSS per transplant were injected into portal vein for allo- and xeno-transplantation, respectively.

IL-1β and glycemia assessment

IL-1β in sera (fg/ml) and glycemia (mg/dl) were measured by ELISA kit [Endogen, Woburn (MA), USA] and vein blood samples obtained at post transplant days +1, +2, and +195.

Statistical analysis

All results are presented as mean values (± SEM) together with the number of individual observations (n). The statistical significance of differences between mean values was assessed by either ANOVA tests or Student's *t*-test. A probability below 0.05 was considered statistically significant.

RESULTS

Glycemia

At day 0, the glycemia was much higher ($p < 0.001$) in the diabetic rats (360 ± 12 mg/dl; n = 23) than in the non-diabetic animals (107 ± 6 mg/dl; n = 12). In this respect, there was no significant difference between either immunosuppressed and non-immunosuppressed rats or allografted and xenografted animals (Table 1). At day +1, the glycemia was significantly lower than at day 0, such a decrease being, as expected, less pronounced ($p < 0.02$ or less) in non-diabetic rats (26 ± 6 mg/dl; n = 11) than in either allografted diabetic animals (195 ± 21 mg/dl; n = 12) or xenografted diabetic animals (132 ± 39 mg/dl; n = 11). Once again, in this respect, there was no significant difference between immunosuppressed and non-immunosuppressed rats . The lesser decrease in glycemia recorded in the xenografted diabetic rats, as distinct from allografted diabetic animals, only achieve statistical significance ($p < 0.02$) after exclusion of one of the former rats which yielded a decrease in glycemia (250 mg/dl) well above ($p < 0.001$) the mean value recorded in the other 10 xenografted diabetic rats (102 ± 28 mg/dl; n = 10). As a matter of fact, even when taking into account the measurement made in the atypical xenografted diabetic rat, the mean glycemia recorded at day + 1 was significantly higher ($p < 0.005$) in the xenografted diabetic rats (248 ± 25 mg/dl; n = 12) than in the allografted diabetic rats (144 ± 20 mg/dl; n = 12). As judged from the paired difference in glycemia at day 0 and when the nadir value was reached, the results again followed the same hierarchy with mean values of 30 ± 5 mg/dl (n = 11) in non-diabetic rats, 152 ± 40 mg/dl (n = 11) in xenografted diabetic rats and 199 ± 21 mg/dl (n = 12) in allografted diabetic rats. Moreover, when considering the last available measurements of glycemia, highly significant differences were observed not only between non-diabetic rats (86 ± 5 mg/dl; n = 12) and allografted diabetic animals (154 ± 21 mg/dl; n = 12; $p < 0.005$), but also between the latter animals and xeonografted diabetic rats (268 ± 27 mg/dl; n = 12; $p < 0.005$).

IL-1ß

At day 0, the serum IL-1ß concentration was comparable in all 6 groups of rats with an overall mean value of 109 ± 2 fg/ml (n = 36). At day 1, the mean values recorded in each group of rats were always higher than those found at day 0. The mean values for the paired difference between these two measurements failed to differ significantly, however, in the 6 groups of rats, with an overall mean value (+ 16 ± 4 fg/ml; n = 33) higher ($p < 0.001$) than zero. Such paired differences also failed to differ significantly in immunosuppressed animals and non-immunosuppressed rats, the values recorded in the latter rats averaging 53.7 ± 20.5 % (n = 16; $p > 0.17$) of the corresponding mean values found in the former animals (100.0 ± 26.2 %; n = 16). In 20 out of 24 animals, the serum IL-1ß concentration was lower on day + 2 than on day + 1. However, such a difference only achieved statistical significance (paired decrease: - 11 ± 2 fg./ml; n = 23; $p < 0.001$) after exclusion of one xenografted non-diabetic animal yielding a mean increase between day + 1 and day + 2 of 150 fg/ml, well above the upper limit of the 95% confidence interval for the measurements made in the other 23 rats, i.e. + 14 fg/ml.

At the exclusion of the atypical xenografted non-diabetic animal just-mentioned, the values for IL-1ß reached on day + 2 averaged 106 ± 2 fg/ml (n = 9) in the allografted rats, as distinct ($p < 0.02$) from 118 ± 3 fg/ml (n = 16) in the xenografted rats. In this respect, there was no significant difference between either immunosuppressed and non-immunosuppressed rats or xenografted diabetic and non-diabetic animals. At the exclusion of the already mentioned atypical xenografted non-diabetic rat, the paired difference for the IL-1ß concentration between day + 1 and day + 2, when available, was comparable in the rats with a survival time after transplantation not exceeding 9 ± 1 days (- 11.8 ± 3.3 fg/ml; n = 10) and those animals with a survival time of 125 up to 780 days (- 11.5 ± 5.6 fg/ml; n = 8). Likewise, the absolute values for IL-1ß concentration reached on day 2 failed to differ significantly in these two sets of rats, with an overall mean value 113 ± 3 fg/ml (n = 18).

Table 1. Values of IL-1β, glycemia, and survival times of recipients.

Groups		IL-1β (fg/ml)				Glycemia (mg/dl)				Survival (Days)
		Pre-tx	Day 1.	Day 2.	Day 195.	Pre-tx	Day 1.	Day 2.	Day 195.	
Allograft	Non immunosuppressed diabetics (Subgroup 1)	*n=6* 110±2	*n=5* 115±4 [a]	*n=4* 105±3		*n=6* 348±17	*n=6* 161±40 [b, c]	*n=4* 141±25 [j]		*n=6* 22±17
	Immunosuppressed diabetics (Subgroup 2)	*n=6* 104±5	*n=5* 117±2	*n=5* 106±3	*n=2* 136±5	*n=6* 329±10	*n=6* 127±8 [d, e]	*n=5* 141±5 [k]	*n=2* 227±16	*n=4* 227±140
Xenograft	Non immunosuppressed non diabetics (Subgroup 3)	*n=6* 118±1	*n=6* 127±7	*n=5* 146±2	*n=3* 130±4	*n=6* 109±8	*n=6* 82±3 [f, g]	*n=5* 86±9 [l, m]	*n=2* 104±6	*n=5* 262±142
	Immunosuppressed non diabetics (Subgroup 4)	*n=6* 109±4	*n=5* 144±2	*n=4* 121±8	*n=1* 137	*n=6* 104±8	*n=6* 83±6 [h, i]	*n=4* 85±7 [n, o]	*n=1* 99	*n=6* 131±124
	Non immunosuppressed diabetics (Subgroup 5)	*n=5* 103±4	*n=5* 119±7	*n=3* 115±1	*n=1* 130	*n=6* 378±22	*n=6* 250±40	*n=4* 200±57 [p]	*n=1* 118	*n=5* 149±140
	Immunosuppressed diabetics (Subgroup 6)	*n=6* 108±4	*n=6* 130±2	*n=4* 113±5	*n=1* 133	*n=5* 388±43	*n=6* 246±33	*n=3* 365±45	*n=1* 405	*n=4* 52±47

[a] = p<0.05 differences between Subgroup 1 and Subgroup 4 in IL-1β level at day +1, [b]= p<0.05 differences between Subgroup 1 and Subgroup 5, [c]= p<0.05 differences between Subgroup 1 and Subgroup 6, [d]= p<0.05 differences between Subgroup 2 and Subgroup 5, [e]= p<0.05 differences between Subgroup 2 and Subgroup 6, [f]= p<0.001 differences between Subgroup 3 and Subgroup 5, [g]= p<0.001 differences between Subgroup 3 and Subgroup 6, [h]= p<0.001 differences between Subgroup 4 and Subgroup 5, [i]= p<0.001 differences between Subgroup 4 and Subgroup 6 in glycemia levels at day +1, [j]= p<0.05 differences between Subgroup 1 and Subgroup 6, [k]= p<0.05 differences between Subgroup 2 and Subgroup 6, [l]= p<0.001differences between Subgroup 3 and Subgroup 5, [m]= p<0.001 differences between Subgroup 3 and Subgroup 6, [n]= p<0.05 differences between Subgroup 4 and Subgroup 5, [o]= p<0.001 differences between Subgroup 4 and Subgroup 6, [p]= p<0.05 differences between Subgroup 5 and Subgroup 6 in glycemia levels at day +2.

DISCUSSION

The present measurements of glycemia document the validity of the experimental design used in this study, and this in several respects. First, the glycemia at day 0 was much higher in diabetic rats than in non-diabetic animals. Second, the glycemia was in all groups significantly lower at day + 1 than at day 0, illustrating the functional contribution of the transplanted islets. Third, such a decrease was, as expected, less pronounced in the non-diabetic rats than in the diabetic animals. Fourth, the decease in glycemia was, also as expected, less marked in the xenografted diabetic rats than in the allografted diabetic rats, whether considering the paired difference between the measurements made at day 0 and those recorded at day + 1 or the last available measurements of glycemia.

As judged from the changes in glycemia, no beneficial effect of immunosuppression was observed under the present experimental conditions. Likewise, the preavalence of rats with a long survival time (125 days or more), as distinct from animals with a survival time not exceeding 1 to 16 days, failed to be higher in the immunosuppressed rats than in the non-immunosuppressed animals, with respective overall values of 26.7 and 31.3 % among 15-16 rats and mean values in the 3 types of rats (xenografted non-diabetic and either allografted or xenografted diabetic rats) of 28.9 ± 10.6 % and 32.2 ± 13.9 % (n = 3 in both cases; p > 0.8).

The measurements of serum IL-1ß also provided selected pieces of information in fair agreement with theoretical expectation. For instance, the concentration of IL-1ß was higher, in all groups of rats, at day + 1 than at day 0. Second, the lower values recorded at day + 2, as distinct form day + 1, were significantly higher in the xenografted rats than in the allografted animals. As a matter of fact, this difference was the sole one with predictive value in terms of the beneficial effect of islet transplantation upon glcyemia. Indeed, no significant difference in IL-1ß concentration, whether considering the paired difference between day + 1 and day + 2 or the absolute nadir values reached on day 2, could be found when comparing animals with a short or prolonged survival time.

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