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Failure to prolong pancreatic islet allograft survival in rats with donor-specific blood transfusions and immunosuppression

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Abstract. The effects on pancreatic islet allograft survival of donor-specific blood transfusions (DST) in combination with pre- and posttransplant immunosuppression were studied. A total of 12 groups of rats ($n=105$) with chemically induced diabetes underwent islet allotransplantation. Multiple DST or third-party blood transfusions (TPT) were given prior to transplantation. Pretransplant immunosuppression consisted of azathioprine and prednisolone, and low-dose cyclosporin A was used for posttransplant immunosuppression. TPT, as well as separate or combined pre- and posttransplant immunosuppression without blood transfusions, did not prolong islet allograft survival. DST resulted in either primary nonfunction of the islet allografts or a markedly decreased islet allograft survival. These findings contrast with the beneficial effect of DST on whole-organ allograft survival in rats previously described by others.

Key words: Pancreatic islet transplantation - Donor-specific blood transfusions - Allotransplantation in rats.

The prolongation of renal and cardiac allograft survival in rats by means of combined donor-specific blood transfusions (DST) and immunosuppression has been demonstrated in several studies [3, 6, 7, 9, 10]. However, it was reported that the survival of nonvascularized pancreatic rat islet allografts following DST alone was shortened rather than prolonged, in contrast to the results of renal allotransplantation [2]. The present experiment was designed to investigate the effects of DST, third-party blood transfusions (TPT), and immunosuppression on the survival of nonvascularized pancreatic islet allografts in rats.

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Materials and methods

Animals

Lewis rats ($RT1^{L}$) (Simonson, Gilroy, Calif.) weighing 250-300 g were used as islet graft recipients. The animals were rendered diabetic (plasma glucose > 400 mg%) by the IV administration of 55 mg/kg streptozotocin (Sigma, St. Louis, Mo.) 1 week prior to transplantation. Fischer rats ($RT1^{Lvl}$) (Simonson, Gilroy, Calif.) weighing 200-250 g served as pancreas and blood donors. Blood for TPT was obtained from ACI rats ($RT1^{a}$) (Simonson, Gilroy, Calif.).

Islet transplantation

Pancreatic islets were isolated according to a previously described technique [14]. Briefly, donor animals were laparotomized under ether anesthesia and the common duct was cannulated. The pancreas was distended by the injection of 20 ml Hanks' solution containing 0.32 mg/ml type XI collagenase (Sigma, St. Louis, Mo.; 2200 IU/mg). A total pancreatectomy was carried out, maintaining the integrity of the pancreatic capsule. The pancreas was incubated with an additional 5 ml enzyme solution for 25 min at 37°C, and digestion was stopped by the addition of 50 ml cold Hanks' solution. The pancreatic tissue was gently dispersed and washed by centrifugation, then passed through a steel mesh (pore size, 800 μm), discarding the lymph nodes and ductal and vascular structures. The filtrate was centrifuged and suspended in a discontinuous gradient of dextran (Sigma, St. Louis, Mo.; mol. wt. 70,000, industrial grade) in Hanks' solution, containing three layers with a density of 1.094 g/ml, 1.081 g/ml, and 1.041 g/ml, respectively. The gradient was centrifuged at 500 g for 10 min and pure islets were harvested from the interface of the topmost layers with a pipet. Finally, the islets were washed twice by centrifugation in RPMI 1640 medium.

Diabetic recipient rats were laparotomized under ether anesthesia, and pancreatic islets from two donors (1700-2200 islets with a tissue volume of 0.05 ml dispersed in 2 ml RPMI 1640) were injected in a mesenteric vein with a 23-gauge butterfly needle. The animals were bled daily during the first 2 weeks after transplantation and twice per week thereafter for plasma glucose measurement. Allograft rejection was considered to have occurred on the first of 3 consecutive days at which plasma glucose levels of > 200 mg% were measured in previously normoglycemic recipients.

Transfusion and immunosuppression

Transfusions of 1 ml whole heparinized blood (either DST or TPT) were given IV at 5, 4, 3, 2, and 1 week prior to islet transplantation. Pretransplant immunosuppression, consisting of 24 mg/kg azathioprine and 20 mg/kg prednisolone (AZA/Pred), was given IV immediately following the third and fourth transfusions, and a double dose was injected following the fifth transfusion, as described by Zheng et al. [17]. Postoperatively, 1 mg/kg cyclosporin A (CyA) dissolved in intralipid (Kabi Baxter, Alameda, Calif.) was daily injected IP. The treatment protocols in 12 different groups of islet allograft recipients are shown in Table 1.

Statistical analysis

A two-tailed Student's *t*-test was used for the statistical analysis.

Results

Islet allograft survival is shown in Table 1. Islet transplantation without blood transfusions or immunosuppression (series I) resulted in a mean ($\pm$SE) allograft survival of 8.1 ± 0.9 days. Separate or combined pre- and posttransplant immunosuppression without blood transfusions (series II, III, IV) did not prolong allograft survival (P= NS). DST without immunosuppression resulted in primary nonfunction of all grafts (series V); the same result was obtained with DST in combination with pre- or posttransplant immunosuppression (series VI, VII). Pretransplant AZA/Pred with DST and posttransplant CyA resulted in a decreased allograft survival of 1.0 ± 0.5 days ($P<0.001$). TPT alone did not affect islet allograft survival (series IX), but combined with pretransplant immunosuppression alone (series X), it led to a decreased allograft survival of 2.5 ± 0.9 days ($P<0.001$). No prolongation of allograft survival was recorded for combinations of TPT and posttransplant immunosuppression (series XI, XII).

Discussion

The mechanisms by which DST exerts a beneficial effect on allograft survival is unknown. It has been suggested that this effect is due to suppressor cell stimulation, but others have provided evidence for the clonal deletion of immunoreactive cells by DST in combination with immunosuppression [5, 7, 10, 13, 15, 16]. According to the clonal deletion theory, the beneficial effect of DST is explained by the induction of immunoreactive cell clones that respond with an accelerated cell division on restimulation. If immunosuppressive drugs are given in conjunction with a secondary stimulus, such as an allograft, the susceptible clones of responding cells are selectively killed. Complete clonal deletion may not occur, but the effect is sufficient to lower significantly the dose of immunosuppression necessary to abrogate the rejection response [13]. Several authors have found support for the clonal deletion theory in rat heart allograft studies with DST pretreatment of recipients [10. 12, 17].

In the present study, the successful DST protocol of Zheng et al. [17] was adopted in a pancreatic islet allotransplant model, using the identical recipient strain. A major histocompatibility-complex-compatible, allogeneic, donor-recipient strain combination was tested as previously described in similar experiments [9]. In the present experiment, the pre- and posttransplant immunosuppressive regimen itself could not prolong islet allograft survival. DST alone resulted in primary allograft non-

Table 1. Treatment protocols and allograft survival (in days) in 12 groups of pancreatic islet transplant recipients. DST, Donor-specific blood transfusions; TPT, third-party blood transfusions; AZA/Pred, azathioprine and prednisolone; CyA, cyclosporin A; NS, not statistically significant

Series	Transfusion	Pretransplant immunosuppression	Posttransplant immunosuppression	*n*	Survival (mean $\pm$ SE)	*P* values vs series I
I	-	-	-	13	8.1 ± 0.9	-
II	-	AZA/Pred	-	7	9.6 ± 3.5	NS
III	-	-	CyA	7	9.9 ± 3.5	NS
IV	-	AZA/Pred	CyA	8	5.9 ± 0.6	NS
V	DST	-	-	8	0 ± 0	-
VI	DST	AZA/Pred	-	6	0 ± 0	-
VII	DST	-	CyA	6	0 ± 0	-
VIII	DST	AZA/Pred	CyA	11	1.0 ± 0.5	$P<0.001$
IX	TPT	-	-	12	9.1 ± 3.7	NS
X	TPT	AZA/Pred	-	10	2.5 ± 0.9	$P<0.001$
XI	TPT	-	CyA	9	3.9 ± 3.3	NS
XII	TPT	AZA/Pred	CyA	8	5.5 ± 3.7	NS

function, probably due to accelerated rejection, as concomitant controls were successful in all cases [4]. DST combined with immunosuppression resulted in significantly decreased allograft survival. Islet allograft survival was not prolonged by TPT. Instead, a decrease in graft survival was found following TPT, possibly due to the sharing of minor histocompatibility antigens of blood and islet donors and subsequent sensitization.

The results of this study show a harmful effect of the DST protocol on islet allograft survival, whereas a beneficial effect on whole-organ grafting has been observed. These findings cannot be explained by the clonal deletion theory. Gray et al. [2] have reported similar observations in a rat pancreatic islet and renal transplant experiment. These authors described a successful DST protocol for renal allografts, and the relative unresponsiveness obtained was explained by the production of suppressor cells. However, islet transplants conducted in a similar fashion resulted in primary nonfunction of all grafts due to accelerated rejection.

Perloff and Barker [9] found a prolongation of rat cardiac allograft survival following DST, but this effect was not observed in pancreatic or skin allografts. In contrast, Selawry et al. [11] have been successful in prolonging rat islet allograft survival with DST combined with posttransplant antilymphocytic serum (ALS) therapy. Donor-specific unresponsiveness was not obtained with this regimen, as skin grafts were separately rejected. It was suggested that enhancement by blocking antibody resulted in extended islet allograft survival. It has also previously been demonstrated that immunosuppressive agents, successfully used to prevent the rejection of vascularized whole-organ grafts, are ineffective in prolonging the survival of islet transplants [8]. The possibility should therefore be considered that other mechanisms play a dominant role in the rejection of nonvascularized grafts as opposed to whole-organ transplants.

The presence of cytotoxic lymphocytes in unrejected renal allografts following DST treatment in rats has been described by Ruiz et al. [10]. Bendtzen et al. [1] found interleukin-1, a product of activated mononuclear cells and localized immune reactions, to be cytotoxic for pancreatic islet cells, which could be an explanation for the different behavior of cardiac, renal, and islet allografts. Clearly, the rejection process in pancreatic islet allografts should be the subject of further investigation.

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References

1. Bendtzen K, Mandrup-Poulsen T, Nerup J, Nielsen JH, Dinarello CA, Svenson M (1986) Cytotoxicity of human pI7. Interleukin-1 for pancreatic islets of Langerhans. Science 232: 1545-1547
2. Gray DWR, Hibberd AD, McShane P, Morris PJ (1984) Islet allografts in rats made tolerant of renal allografts by whole blood transfusion compared to islet allografts after blood transfusion alone. Transplant Proc 16: 863-864
3. Johnson CP, Munda R, Alexander JW, Balakrishnan K, Blanton M (1984) The effect of donor-specific transfusions on rat heart allograft survival. Transplantation 38: 575-578
4. Kaufman DB, Rabe FL, Stock PG, Vliet JA van der, Platt J, Sutherland DER (1988) Cyclosporine suppresses immune-mediated pancreatic islet allograft primary non-function and enhances long-term functional survival. Transplant Proc [Suppl 3] 20: 426-430
5. Marquet RL, Heystek GA, Niessen GJCM, Jeekel J (1982) Induction of suppressor cells by a single blood transfusion in rats. Transplant Proc 14: 397-399
6. Martin DC, Dowdy SF, Hewitt CW, Black KS, Domingues D, Gonzalez GA (1985) Comparison of renal allograft survival in rats pretreated with multiple blood transfusions combined with either cyclosporine or azathioprine. Transplant Proc 17: 1087-1090
7. Martinelli GP, Chung-Loy R, Sher L, Racelis D, Miller CM, Schanzer H (1985) Long-term prolongation of cardiac allografts by subtherapeutic levels of cyclosporine in rats conditioned with pretransplant blood transfusions and cyclosporine. Transplantation 39: 1-5
8. Morris PJ, Finch DR, Garvey JF, Poole MD, Millard PR (1980) Suppression of rejection of allogeneic islet tissue in the rat. Diabetes [Suppl 1] 29: 107-112
9. Perloff LJ, Barker CF (1985) Variable response to donor-specific blood transfusions in the rat. Transplantation 38: 178-182
10. Ruiz P, Coffman TM, Howell DN, Straznickas J, Scroggs MW, Badlwin WM, Klotman PE, Sanfilippo F (1988) Evidence that pretransplant donor blood transfusion prevents rat renal allograft dysfunction but not the in situ cellular alloimmune or morphologic manifestations of rejection. Transplantation 45: 1-7
11. Selawry HP, Cohen HL, Mui MM (1981) The effect of donor strain blood and ALS therapy on pancreatic islet allograft survival in rats. Diabetes 30: 947-950
12. Takiff H, Novak M, Yin L, Iwaki Y, Terasaki PI (1987) Examination of the clonal deletion hypothesis following transfusions in rat cardiac transplants. Transplantation 43: 145-151
13. Terasaki PI (1984) The beneficial transfusion effect on kidney graft survival attributed to clonal deletion. Transplantation 37: 119-125
14. Vliet JA van der, Field MJ, Meloche RM, Chen D, Kaufman DB, Sutherland DER (1988) Pancreatic islet isolation in rats with ductal collagenase distention, stationary digestion and dextran separation. Transplantation 45: 493-495
15. Waymack JP, Munda R, Johnson C, Alexander JW (1987) Immunomodulation of donor-specific transfusions. Transplantation 43: 761-764
16. Yoshimura N, Kahan BD (1985) Nature of the suppressor cells mediating prolonged graft survival after administration of extracted histocompatibility antigens and cyclosporine. Transplantation 39: 162-168
17. Zheng T, Johnson CP, Sutherland DER (1987) Pretransplant cytotoxic conditioning produces effects consistent with clonal deletion mechanisms. J Surg Res 42: 498-502