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Primary nonfunction of islet xenografts: the role of macrophages

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Abstract Primary nonfunction (PNF) of hamster islets occurs when they are transplanted under the kidney capsule of diabetic Lewis rat recipients. PNF is absent if the islets are grafted into the liver. Previous results have indicated that macrophages might be involved in the phenomenon of PNF. To test this hypothesis, two procedures affecting macrophages were employed. First, recipients were pretreated with liposomes containing dichloromethylene disphosphonate (L-MDP), killing macrophages upon phagocytosis. Second, recipients were pretreated with the arginine analogue, L-NA, in order to inhibit the synthesis of nitric oxide (NO), a molecule believed to mediate beta cell dysfunction. Injection of L-MDP into the peritoneal cavity had no effect on PNF. However, co-transplantation of L-MDP with hamster islets under the kidney capsule reduced PNF significantly. Eradication of Kupffer cells with L-MDP did not prolong the survival of islets transplanted into the liver, indicating that acute xenogeneic rejection in this model is not mediated by macrophages. A single injection of L-NA, 3 h before transplantation resulted in complete annihilation of PNF, all recipients became normoglycemic within 1 day and remained so for 1.4 ± 0.5 days. These results confirmed the finding that macrophages and NO play a crucial role in PNF of islet grafts.

Key words Hamster islets
Xenograft · Liposomes · Nitric oxide

Introduction

Attempts at human islet allotransplantation have been hampered by a substantial rate of primary nonfunction (PNF). Also xenogeneic transplantation of islets from large animals or humans into rats is characterized by a high rate of PNF [10]. In contrast, murine recipients of islet xenografts are usually rendered normoglycemic prior to rejection of the graft. However, even in permissive species such as the mouse, contamination of islets by exocrine tissue may lead to PNF [5]. It has been hypothesized that PNF is the result of islet inactivation by specific and nonspecific immunologic and inflammatory reactions, in which macrophages and monocytes play a pivotal role [2]. Recently, it has been demonstrated that nitric oxide (NO), a ubiquitous substance released by activated macrophages, among other cells, is involved in the process of PNF [13].

We encountered the phenomenon of PNF quite unexpectedly in our hamster to rat model when islets where placed under the renal capsule of Lewis rat recipients. Surprisingly, in the same model, PNF did not occur if the

hamster islets were transplanted into the liver. Neither did PNF occur when hamster islets were transplanted under the kidney capsule of diabetic hamsters [7]. PNF in our model, therefore, is the result of a specific interaction between rat immunocompetent cells or cellular products and hamster islets, only occurring at a specific site. We have demonstrated that treatment with cyclosporin A (CsA) does not affect PNF; only total body irradiation substantially reduces PNF [7]. Supported by data from recent literature, we postulated that PNF was not likely to be caused by T cells, but rather by inflammatory cells, such as macrophages. In the present study, we further elaborated on the relationship between PNF and macrophages by eliminating the function of macrophages in two ways, by treating recipients with macrophage-depleting liposomes and second, by pretreatment with a L-arginine analogue in order to inhibit the synthesis of NO.

Materials and methods

Animals and transplantation

Golden Syrian hamsters of both sexes were used as donors, male inbred Lewis rats (RT1^1), as recipients. Rats were made diabetic by injection of streptozotocin (60 mg/kg) and were considered to be diabetic when consecutive blood glucose readings were greater than 20 mmol/l. Hamster islets were isolated by stationary collagenase digestion (type p; Boehringer, Mannheim, Germany) and hand-picking. About 1000 islets were either transplanted under the kidney capsule, via a cannula inserted through the kidney parenchyma up to the capsule, or into the liver by injection via the portal vein. PNF was defined as a glucose level persistently greater than 15 mmol/l, or more rigorously: not reaching normoglycemia (11 mmol/l). Rejection was diagnosed when blood glucose reverted to greater than 11 mmol/l.

Liposomes

Eradication of macrophages was done by treatment of recipients with liposome-encapsulated dichloromethylene diphosphonate (L-MDP). On injection, this substance (a gift from Boehringer, Mannheim, Germany) is rapidly phagocytized by macrophages leading to intracellular release of MDP which eventually kills the cell. L-MDP has been demonstrated to eliminate macrophages from spleen, liver, peritoneal cavity or lungs, depending on the route of administration [11]. We used L-MDP for the elimination of macrophages from the peritoneal cavity by giving 1 ml L-MDP i.p. on days-1 and 0. Elimination of macrophages from the renal capsule was performed by mixing 0.2 ml L-MDP with the islet mass to be transplanted under the capsule. Kupffer cels in the liver were eradicated by injecting 1 ml L-MDP i.v. 2 days before intraportal islet transplantation.

Inhibition of NO synthesis

The L-arginine analogue N omega L-nitro arginine (L-NA) was used to inhibit the synthesis of NO [8]. L-NA (Sigma) was given i.v. in a dose of 30 mg/kg, 3 h before islet transplantation.

Table 1 Effect of macrophage elimination and inhibition of nitric oxide synthesis on primary nonfunction (PNF) of hamster islets in diabetic rats. Survival time is given in days

Implantation site	Treatment	Survival	% PNF
Kidney	None	0, 0, 0, 0, 3	80
Liver	None	2, 2, 3, 4	0
Kidney	Liposomes i.p.	0, 0, 0, 0	100
Kidney	Liposomes s. caps.	0, 0, 0, 2, 4	60
Liver	Liposomes i.v.	3, 3, 3, 4, 5	0
Kidney	L-NA	1, 1, 1, 2, 2	0

Results

The results are summarized in Table 1. When hamster islets were placed under the kidney capsule of untreated recipients, PNF occurred in 80% of cases. PNF did not take place when the hamster islets were transplanted into the liver. Injection of L-MDP into the peritoneal cavity had no effect on PNF. Co-transplantation of L-MDP with islets under the kidney capsule reduced PNF to 60%. However, when the criterion for islet function was made less rigid and set at 15 mmol/l instead of 11 mmol/l, PNF was reduced on 0%, remaining 80% in the untreated group. Eradication of Kupffer cells by L-MDP had no effect on islet survival in the liver (3.6 ± 0.9 days vs. 2.8 ± 1 days in controls). A single injection of L-NA to inhibit NO synthesis, 3 h before transplantation led to complete annihilation of PNF under the kidney capsule. All recipients became normoglycemic within 1 day and remained so for 1.4 ± 0.5 days.

Discussion

PNF has been a major cause of failure in both clinical and experimental islet transplantation. Speculations about the possible etiology of PNF have included technical and immunopathological aspects [10]. It has recently been demonstrated in a pig to rat islet transplantation model that a combination of deoxyspergualin and antithymocyte serum is particularly effective in reducing PNF, which is highly suggestive of a crucial role for macrophages [3]. Others have found that modulation of macrophage function by silica administration completely abolishes PNF of islets transplanted under the kidney capsule [5]. Our results strongly supported the notion that macrophages are involved in PNF of islet grafts. In our particular model of PNF, depletion of macrophages using L-MDP at the site of implantation was effective in reducing PNF. If macrophages are such important

mediators of PNF it is difficult to explain why PNF did not occur in the liver where macrophages are so abundant. If we assume that only activated macrophages are involved in PNF, a possible explanation may be that transplantation of clean, handpicked islets into the liver via the portal vein is a relatively atraumatic operation, not leading to Kupffer cell activation. On the other hand, transplantation of islets under the kidney capsule through the kidney parenchyma is traumatic and may provoke inflammation and activation of tissue macrophages. It is conceivable that a more atraumatic technique at this site might reduce PNF considerably, whereas injection of a crude islet mass into the liver might provoke PNF.

Tumor necrosis factor α (TNF-α) and interleukin-1 beta (IL-beta), both products of activated macrophages, have recently been implicated in the autoimmune and posttransplant destruction of islets [12, 13]. The prime cytotoxic mediator in this destructive process is thought to be NO. Islets are extremely susceptible to lysis by NO [6]. NO is a product of the oxidation of L-arginine to L-citrulline by nitric oxide synthetase. Cytokines and endotoxin induce the expression of one isoform of nitric oxide synthetase, which generates a high level of NO that is cytotoxic to target cells. The other isoform is constitutive and the low levels of NO produced via this pathway function as a signaling molecule [1]. By using competitive inhibitors of nitric oxide synthetase, NO production can be inhibited [8]. Our present results with the NO inhibitor L-NA strongly suggested that PNF in our model was mediated by NO. They confirmed previous results by others indicating that NO may be the common denominator in PNF of islet grafts [4, 9]. It remains to be investigated whether protracted administration of L-NA may further prolong the survival of hamster islets in our model.

References

1. Corbett JA, Sweetland MA, Wang JL, Lancastar JR Jr, McDaniel ML (1993) Nitric oxide mediates cytokine-induced inhibition of insulin secretion by human islets of Langerhans. Proc Natl Acad Sci USA 90:1731–1735
2. Farney AC, Najarian JS, Nakhleh R, Field MJ, Morel P, Lloveras J et al (1992) Long-term function of islet autotransplants. Transplant Proc 24:969–971
3. Henretta J, Pittman K, McFadden T, Thomas J, Thomas F (1993) Deoxyspergualin and rabbit antithymocyte globulin markedly prolong discordant pig pancreatic islet xenografts. Transplant Proc 25:412–413
4. Jahr H, Nicolae M, Ketchum R, Koeberlein B, Barker CF, Brayman KL (1993) Islet xenografts in mice and rats II: mixed islet-spleen cell assay to analyze nitric oxide production and inhibition in xenogeneic species combinations (abstract). Presented at the Fourth International Congress on Pancreas and Islet Transplantation, Amsterdam, The Netherlands, June 27–30
5. Kaufman DB, Platt JL, Rabe FL, Dunn DL, Bach FH, Sutherland DER (1990) Differential roles of Mac-1$^+$ cells, and CD4$^+$ and CD8$^+$ T lymphocytes in primary nonfunction and classic rejection of islet allografts. J Exp Med 172:291–302
6. Kroncke KD, Rodriguez ML, Kolb H, Kolb-Bachofen V (1993) Cytotoxicity of activated rat macrophages against syngeneic islet cells is arginine-dependent, correlates with citrulline and nitrite concentrations, and is identical to lysis by nitric oxide donor nitroprussine. Diabetologica 36:17–24
7. Marquet RL, Bouwman E, Bonthuis F, Wolvekamp MCJ, Kouwenhoven E, van Rooijen N, Scheringa M, IJkermans JNM (1993) Local immunological factors determine the occurrence of primary nonfunction of islet xenografts. Transplant Proc (in press)
8. Moncada S, Palmer RMJ, Higgs EA (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 90:109–142
9. Nussler AK, Carroll PB, Di Silvio M, Rilo HLR, Simmons RL, Starzl TE, Ricordi C (1992) Hepatic nitric oxide generation as a putative mechanism for failure of intrahepatic islet cell grafts. Transplant Proc 24:2997
10. Pitman K, Thomas F, McFadden T, Haisch C, Thomas F (1993) Reduction of primary nonfunction of pancreas islet transplants with antithymocyte globulin agents, deoxyspergualin, and splenectomy. Transplant Proc 25:986–987
11. Van Rooijen N, Kors N, van de Ende M, Dijkstra CD (1990) Depletion of macrophages in spleen and liver after intravenous treatment with liposome-encapsulated dichloromethylene disphosphonate. Cell Tissue Res 260:215–222
12. Xenos ES, Farney AC, Widmer MB, Casanova D, Stevens RB, Blazar BR, Sutherland DER (1992) Effect of tumor necrosis factor α and of the soluble tumor necrosis factor receptor on insulin secretion of isolated islets of Langerhans. Transplant Proc 24:2863–2864
13. Xenos ES, Stevens RB, Gores PF, Casanova D, Farney AC, Sutherland DER, Platt JL (1993) IL-1 beta induced inhibition of beta cell function is mediated through nitric oxide. Transplant Proc 25:994