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The role of exocrine tissue in pancreatic islet transplantation

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Abstract. Isolated pancreatic islet transplantation has been proposed as a possible way of treating diabetes, but despite extensive experimental research, successful clinical transplantation remains elusive. A major problem has been the isolation of sufficient viable islet tissue for transplantation, especially from the human pancreas. It is possible to improve the yield of islet tissue by omitting purification steps, and unpurified dispersed pancreas has been successfully transplanted experimentally. However, attempts to apply the same technique clinically have been unsuccessful and have produced unacceptable complications. There is evidence that exocrine contamination may impair the implantation of islet tissue when transplanted to restricted sites, such as the kidney capsule. Yet, complete purification of islet tissue is probably not necessary for safe transplantation with adequate implantation of tissue in sites such as the spleen or liver. Exocrine tissue may be more immunogenic than islet tissue, and complete purification may have advantages for the prevention of rejection.

Key words: Pancreatic islet transplantation - Purification of islets - Role of exocrine tissue.

The development of a successful technique for clinical isolated islet transplantation remains an elusive goal, but hasty dismissal of the entire concept should be tempered by the realisation that the separation of islets for transplantation has required the development of a completely new field of biotechnology. Transplantation of single cells in the form of blood has been performed for many years, and more recently it has been possible to transplant tissue that has been dispersed to single cells for the purpose of bone marrow transplantation. Transplantation of vascularised organs such as the kidney has also been performed for many years. However, the isolation of islets for transplantation requires the separation of an intact organ (the islet) from within another organ prior to transplantation, and this has never been attempted in other fields.

The concept behind islet transplantation is that the implanted insulin-secreting cells may be able to cure diabetes mellitus by providing tight control of glucose metabolism and thus prevent the development of the complications of diabetes that are associated with long-term insulin injection therapy. It is not known exactly how perfect the control of glucose metabolism must be to achieve this aim, but it is certain that, as a minimum, patients should be rendered insulin-independent. Experimental and clinical evidence suggest that the mass of islet tissue required to allow insulin independence is at least 10% of the normal pancreatic islet tissue mass [5, 34].

The earliest successful techniques for islet isolation were applied to the rodent pancreas [2, 23, 27] and they measured the islet yield in terms of numbers of islets obtained. Whilst there are pitfalls to using islet numbers as a measure of islet mass [16], this way of expressing yield does have crude validity. The number of islets obtainable from a single rat pancreas using this early technique was approximately 200 [2]. The purity of the islets obtained was above 95% islet tissue and could easily be improved by hand-picking (personal observation). Two hundred islets represents approximately 5%-10% of the estimated total number of islets (approximately 3000) within a normal rat pancreas (unpublished observations), and for this reason it was never possible

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to reverse experimental diabetes of any severity by single donor transplants. Furthermore, despite encouraging early reports [2, 29, 32, 36], subsequent studies found that the yield of islets obtained from the more fibrous pancreases of larger mammals and from the human pancreas, using the same technique, was reduced still further [35], making the failure of early attempts at clinical islet transplantation unsurprising.

The poor yield of islets obtained after purification procedures led some investigators to abandon the islet purification stage and to transplant unpurified digested pancreatic tissue. This was first shown to be a reasonable approach by autotransplantation of dispersed pancreas in the totally pancreatectomised canine model [26], using the spleen as the implantation site. Many investigators confirmed this finding [7, 15, 21, 22], although it was noted that transplantation of unpurified tissue directly into the portal vein was associated with portal hypertension and death [24]. Similar problems sometimes occurred after intrasplenic injection [7].

Notwithstanding these cautionary findings, several clinical trials of transplantation of dispersed unpurified pancreatic tissue were undertaken, using either allografts of cadaveric pancreas [20, 37] or, more frequently, autografts of tissue prepared after total or subtotal pancreatectomy for pancreatic disease [4, 6, 29, 30, 41]. Although the canine experiments used the splenic site, all but one group [20] favoured the intraportal route for clinical transplantation. The reason, although never actually stated, was probably that the small size of the human spleen proved unsuitable for the large mass of tissue that was to be transplanted. Despite several claims [3, 29, 30, 41], there was little proof that these grafts provided sufficient insulin-secreting tissue to allow insulin independence, unless some intact pancreatic tissue had been left behind at operation. Distressingly, there were several instances of severe portal hypertension [6, 24] and even death due to the procedure [4]. The most severe complications followed autotransplantation procedures, and it was eventually realised that diseased pancreatic tissue might not behave the same as normal pancreas when dispersed and transplanted, a suspicion confirmed by canine experiments [25], sadly enough after the event. These reports produced a sense of disillusionment with the concept of islet transplantation and are a salutary reminder to those proposing further clinical trials.

The experience reported above has been accumulated with little attention to the basic questions that must surely underlie the role of exocrine contamination in the process of islet isolation and transplantation. Exocrine tissue contamination may potentially affect islet transplantation in several ways. The most obvious effect may be to induce harmful or frankly dangerous systemic effects, probably depending on the site that is used, some of which have been noted above. The implantation of islet tissue in the chosen transplantation site may be affected, most probably adversely. This effect would be expected to vary between different sites and would probably depend on the extent of exocrine contamination. A further possibility is that exocrine contamination may affect the immunogenicity of the tissue transplanted, either beneficially or adversely.

What are the harmful systemic effects of exocrine contamination?

The experience reported above indicates an absolute limit for exocrine contamination of islets for clinical transplantation: unpurified dispersed pancreatic tissue cannot be safely transplanted, at least not into the portal system. Other sites, such as the kidney capsule, may be much safer and may allow for the use of unpurified islet tissue. However, the implantation of islet tissue in such sites may be impaired by exocrine contamination. The mechanism underlying the harmful effects of exocrine contamination upon intraportal transplantation remains unknown. A simple mechanical effect of the large number of relatively large particles causing obstruction of the portal vein radicles is likely to contribute to the production of portal hypertension. This would be in line with the observation that the level of portal hypertension is directly related to the volume of tissue infused (unpublished observations). Yet, portal hypertension can also be produced by infusion of nonparticulate digestion supernatant alone [39]. Furthermore, exocrine tissue is highly thrombogenic, at least in vitro (personal observation), and superadded thrombosis may increase this effect. Experimental attempts to prevent thrombosis by the addition of heparin (in addition to aprotonin) to dispersed pancreatic grafts before intraportal transplantation was reported by one group to lessen the deleterious effect [24]; in another report, heparin alone had no effect [39]. Exocrine tissue may also affect the portal and systemic circulation by the release of enzymes or precursors either locally or into the circulation, a possibility that has been put forward to explain the severe shock with systemic hypotension seen in some animal models and clinically [39]. Furthermore, it has been suggested that these enzymes may trigger the release of a vasoactive substance such as kallikrein [24, 39];

however, no proof of this hypothesis has been provided. The addition of protease inhibitors such as aprotonin was found to be beneficial in experimental models [24, 39] but did not prove particularly effective in the clinical situation [4].

Although it is clear that completely unpurified pancreatic tissue is harmful, can we be sure that completely purified islets will not cause similar problems? Furthermore, if purified islets are safe, just how purified do the islets have to be in order to allow for safe transplantation? Totally purified islet transplants have only been possible in rodents, without apparent complications. Experimental islet transplantation in large animal models has provided some answers to these questions; however, there have been no systematic studies to specifically examine the question of the effect of exocrine contamination. Islet preparations derived from the animals' own pancreas have been transplanted to the spleen and portal vein of animals such as the dog [1, 31] and monkey [12]. These preparations have varied from 5% to 90% islets, as estimated by histological examination. Preparations that are 5%-10% pure have been autotransplanted regularly into the portal vein of monkeys without causing the systemic complications noted with entirely unpurified tissue [12]. They have, however, been associated with initially high - and almost unacceptable - rises in portal pressure. This pressure is reduced somewhat within a few minutes of transplantation and still further after a period of some weeks (unpublished observations).

Recently, the purity of islets obtainable for transplantation in experimental models has improved, and the rise in portal pressure caused by transplantation of this tissue has been reduced. For example, intraportal transplantation of islet tissue of greater than 80% purity into cynomolgus monkeys was found to cause virtually no rise in portal pressure; yet, sufficient islet tissue was implanted to allow long-term normoglycemia (R. Sutton, recent unpublished data). What conclusions can be drawn from these experiments? In terms of the safety of clinical transplantation, assuming that the intraportal route is chosen, it seems likely that tissue derived from a single pancreas could be transplanted if the purity was greater than 10% islet tissue and provided the portal pressure was monitored very carefully during infusion and the transplant discontinued if portal pressure rose significantly (above 20 cm water). Increasing the purity would tend to increase the safety of transplantation. Indeed, if the purity could be increased to 80% or more, there should be no problems directly related to the transplantation procedure.

What is the effect of exocrine contamination on islet implantation?

The first reported methods for separation of islets of Langerhans from the rodent pancreas produced a relatively low yield of islets [2]. However, the purity obtained, although rarely actually quoted, was quite good, as evidenced by more recent employment of the same technique (personal observations). Probably for this reason, little attention has been paid to the role of exocrine contamination in the rodent model. The failure of the rodent islet isolation method, when applied to the pancreas of large mammals, led to the subsequent use of unpurified islet transplants, with success in the canine model [26]. The success of these transplants showed that exocrine contamination was compatible with sufficient islet implantation to give reasonable graft function, at least when transplanted to a site such as the canine spleen.

Exocrine contamination may be even more important when transplantation is undertaken to other sites. One centre recently reported the use of unpurified mechanically dispersed pancreas implanted under the renal capsule [38]. The technique used to prepare the tissue was a modification of earlier similar techniques [18, 19] and was reported in the canine model. This report was remarkable not only because of the use of a mechanical tissue dispersion technique, but also because the tissue transplanted was allogeneic and because no attempt was made to prevent rejection by immunosuppression. These results have not been confirmed by any other centre. In a quantitative study in the rat, syngeneic unpurified pancreatic tissue prepared by an identical mechanical method and transplanted to the kidney capsule site showed that virtually no endocrine tissue survived the process [14]. Another group autotransplanted unpurified pancreatic tissue to the kidney capsule in the dog and failed to show any function [17], although the tissue was dispersed by a different technique, namely collagenase digestion. Interestingly, the same tissue functioned well when transplanted into the spleen.

The only systematic study of the effect of exocrine contamination on islet implantation that has thus far been done examined the insulin content of islet grafts contaminated with varying proportions of exocrine tissue and transplanted to the kidney capsule site in nondiabetic rats [14]. The islets were isolated by a collagenase digestion technique. This study showed that exocrine contamination of 50% or more produced a deleterious effect, as shown by histological examination, and reduced the implantation of islet tissue by approximately 50%. Histological

studies of exocrine contamination of human islets transplanted into nude rats and of monkey islets autografted beneath the kidney capsule demonstrated features compatible with a similar impairment of implantation [14].

The nature of the detrimental effect of exocrine contamination has not been examined by the above studies. However, it is tempting to speculate from the histological appearance that the exocrine tissue undergoes necrosis, presumably releasing its content of enzymes locally, which may damage the islet tissue if it is nearby. If this is the case, better dispersion of impure preparations may allow more islet tissue to implant away from exocrine tissue, something which may account for the success of transplants to such sites as the canine spleen.

What is the effect of exocrine contamination on graft immunogenicity?

It is perhaps surprising, given the large numbers of studies that have examined the rejection of islet grafts, that until recently little consideration had been given to the notion that exocrine contamination may affect the rejection process. The first clue that this might be the case came indirectly. Early studies of the effect of the removal of so-called passenger leucocytes from islet grafts were performed in the mouse [8, 9]. Apart from the effect of treatment of the islets to remove the putative dendritic cells, it was noted that, even without treatment, some 20%-30% of grafts went on to spontaneous survival. This was attributed at the time to the very "clean" islets transplanted, consequent on hand-picking using the newly developed "green light" technique [10]. This technique allowed for the removal of all contaminating lymph nodes, but also, coincidentally, of all the contaminating ductular and exocrine tissue (D. Faustman, personal communication). The class II bearing dendritic cells within islets have since been demonstrated to be central to the rejection process for islets. Yet, little notice was taken of the observation that the exocrine gland actually contains more class II bearing cells than islets [33] until studies of islet rejection in the rat demonstrated that contamination with lymphoid, ductular, or acinar tissue resulted in more rapid rejection, whilst completely pure islets could implant without rejection [11]. Purified endocrine cells were also found to be less immunogenic than isolated islets, and the effect of contaminating elements may have been more important than the loss of dendritic cells [40]. These findings, if demonstrated to be consistent, might mean that previous reports of the severe immunogenicity of islets must be re-examined, since it is likely that some degree of acinar, ductular, or even lymphoid tissue contamination was present in many of the grafts. It is also important that precautions are taken to document the purity of islet preparations used in allograft experiments in the future.

Finally, if the immunological advantages of complete purification can be shown to be applicable to humans, this may justify the use of additional, more complex purification steps to allow for the complete purification of human islets for clinical transplantation. Such procedures may include the removal of exocrine tissue by lectin-bound microspheres [28] or by fluorescence-activated sorting [13].

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References

1. Alejandro R, Cutfield RG, Shienvold FL, Polonsky KS, Noel J, Olson L, Dillberger J, Miller J, Mintz DH (1986) Natural history of intrahepatic canine islet cell autografts. J Clin Invest 78: 1339-1348
2. Ballinger WF, Lacy PE (1972) Transplantation of intact pancreatic islets in rats. Surgery 72: 175-186
3. Cameron JL, Mehigan DG, Harrington DP, Zuidema GD (1980) Metabolic studies following intrahepatic autotransplantation of pancreatic islet grafts. Surgery 87: 397-400
4. Cameron JL, Mehigan DG, Broe PJ, Zuidema GD (1981) Distal pancreatectomy and islet autotransplantation for chronic pancreatitis. Ann Surg 193: 312-317
5. Child CG, Frey CJ, Fry WJ (1969) A reappraisal of removal of ninety-five per cent of the distal portion of the pancreas. Surg Gynecol Obstet 129: 49-56
6. Dobroschke J, Schwemmle K, Langhoff G, Laube H, Bretzel RG, Federlin K (1978) Autotransplantation von Langerhans'schen Inseln nach totaler Duodenopancreatektomie bei einem Patienten mit chronischer Pankreatitis. Dtsch Med Wochenschr 103: 1905-1910
7. DuToit DF, Reece-Smith H, McShane P, Denton T, Morris PJ (1982) Effect of cyclosporin A on allotransplanted pancreatic fragments to the spleen of totally pancreatectomised dogs. Transplantation 33: 302-307
8. Faustman D, Hauptfeld V, Lacy P, Davie J (1981) Prolongation of murine islet allograft survival by pretreatment of islets with antibody directed to Ia determinants. Proc Natl Acad Sci USA 78: 5156-5159
9. Faustman DL, Steinman RM, Gebel HM, Hauptfeld V, Davie JM, Lacy PE (1984) Prevention of rejection of murine islet allografts by pretreatment with anti-dendritic cell antibody. Proc Natl Acad Sci USA 81: 3864-3868
10. Finke EH, Lacy PE, Ono J (1979) Use of reflected green light for specific identification of islets in vitro after collagenase isolation. Diabetes 28: 612-613
11. Gotoh M, Maki T, Porter J, Satomi S, Monaco AP (1986) Effect of contaminating lymph nodes, ductal and vascular tissue, and exocrine tissue on the survival of purified pancreatic islet allografts. Transplant Proc 18: 1848-1850

12. Gray DWR, Warnock GL, Sutton R, Peters M, McShane P, Morris PJ (1986) Successful autotransplantation of isolated islets of Langerhans in the cynomolgus monkey. Br J Surg 73: 850-853
13. Gray DW, Gohde W, Carter N, Heiden T, Morris PJ (1989) Separation of pancreatic islets by fluorescence activated sorting. Diabetes 38 [Suppl 1]: 133-135
14. Gray DW, Sutton R, McShane P, Peters M, Morris PJ (1988) Exocrine contamination impairs implantation of islets transplanted beneath the kidney capsule. J Surg Res 45: 432-442
15. Hanson SL, Sutherland DE, Field MJ, Rabe F, Najarian JS (1981) Comparison of techniques for pancreatic islet transplantation in dogs. Surg Forum 32: 383-385
16. Hellman B (1959) Actual distribution of the number and volume of the islets of Langerhans in different size classes in nondiabetic humans of varying ages. Nature 184: 1498-1499
17. Hesse UJ, Sutherland DE, Gores PF, Sitges-Serra A, Najarian JS (1986) Comparison of splenic and renal subcapsular islet autografting in dogs. Transplantation 41: 271-274
18. Hinshaw DB, Jolley WB, Knierim KH, Hinshaw DB (1981) New nonenzymatic method for the isolation of functional pancreatic islets. Surg Forum 32: 381-383
19. Jolley WB, Knierim K, Hinshaw DB, Ham JM, Hinshaw DB (1982) Successful allografts of pancreatic islets in diabetic rabbits after donor-specific blood transfusion and antilymphocyte serum treatment. Transplant Proc 14: 413-414
20. Kolb E, Largiader F (1980) Clinical islet transplantation. Transplant Proc 12 [Suppl 2]: 205-207
21. Kolb E, Ruckert R, Largiader F (1977) Intraportal and intrasplenic autotransplantation of pancreatic islets in the dog. Eur Surg Res 9: 419-426
22. Kretschmer GJ, Sutherland DE, Matas AJ, Cain TL, Najarian JS (1977) Autotransplantation of pancreatic islets without separation of exocrine and endocrine tissue in totally pancreatectomized dogs. Surgery 82: 74-81
23. Lacy PE, Kostianovsky M (1967) Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16: 35-39
24. Mehigan DG, Bell WR, Zuidema GD, Eggleston JC, Cameron JL (1980) Disseminated intravascular coagulation and portal hypertension following pancreatic islet autotransplantation. Ann Surg 191: 287-293
25. Mehigan DG, Zuidema GD, Eggleston JC, Cameron JL (1980) Pancreatic islet autotransplantation: results in dogs with chronic duct ligation. Am J Surg 139: 170-174
26. Mirkovitch V, Campiche M (1977) Absence of diabetes in dogs after total pancreatectomy and intrasplenic autotransplantation of pancreatic tissue. Transplant Proc 9: 321-323
27. Moskalewski S (1965) Isolation and culture of the islets of Langerhans of the guinea pig. Gen Comp Endocrinol 5: 342-353
28. Muller-Ruchholtz W, Leyhausen G, Petersen P, Schubert G, Ulrichs K (1987) A simple methodological principle for large scale extraction and purification of collagenase-digested islets. Transplant Proc 19: 911-915
29. Najarian JS, Sutherland DE, Matas AJ, Steffes MW, Simmons RL, Goetz FC (1977) Human islet transplantation: a preliminary report. Transplant Proc 9: 233-236
30. Najarian JS, Sutherland DE, Baumgartner D, Burke B, Rynasiewicz J, Matas AJ, Goetz FC (1980) Total or near total pancreatectomy and islet transplantation for treatment of chronic pancreatitis. Ann Surg 192: 526-542
31. Rajotte RV, Kneteman NM, Warnock GL, Procyshyn AW (1986) Long-term normoglycemia in pancreatectomized dogs transplanted with frozen/thawed pancreatic islets. Cryobiology 23: 214-221
32. Scharp DW, Murphy JJ, Newton WT, Ballinger WF, Lacy PE (1975) Transplantation of islets of Langerhans in diabetic rhesus monkeys. Surgery 77: 100-105
33. Steiniger B, Klempnauer J, Wonigeit K (1985) Altered distribution of class I and class II MHC antigens during acute pancreas allograft rejection in the rat. Transplantation 40: 234-239
34. Sun AM, Coddling JA, Haist RE (1974) A study of glucose tolerance and insulin response in partially depancreatectomised dogs. Diabetes 23: 424-432
35. Sutherland DE (1981) Pancreas and islet transplantation. II. Clinical trials. Diabetologia 20: 435-450
36. Sutherland DE, Steffes MW, Bauer GE, McManus D, Noe BD, Najarian JS (1974) Isolation of human and porcine islets of Langerhans and islet transplantation in pigs. J Surg Res 16: 102-111
37. Sutherland DE, Matas AJ, Goetz FC, Najarian JS (1980) Transplantation of dispersed pancreatic islet tissue in humans: autografts and allografts. Diabetes 29 [Suppl 1]: 31-44
38. Toledo-Pereyra LH, Bandlien KO, Gordon DA, Mackenzie GH, Reyman TA (1984) Renal subcapsular islet cell transplantation. Diabetes 33: 910-914
39. Traverso LW, Gomez RR (1982) Hemodynamic measurements after administration of aprotinin and/or heparin during pancreatic cell autotransplantation in the dog, pig and monkey. Ann Surg 195: 479-485
40. Tze WJ, Tai J (1984) Intracerebral allotransplantation of purified pancreatic endocrine cells and pancreatic islets in diabetic rats. Transplantation 38: 107-111
41. Valente U, Ferro M, Campisi C, Cataldi L, Traverso C, Fontana I, DeRosa E, Cama A, Tosatti E (1980) Report of clinical cases of islet autotransplantation. Transplant Proc 12 [Suppl 2]: 202-204