Upsala J Med Sci 86: 213–218, 1981

Transplantation of Pancreatic Islets

B. Petersson, A. Andersson, C. Hellerström, C.-G. Groth, R. Gunnarsson, S. Sandler, I. Swenne and J. Östman

From the Department of Medical Cell Biology, University of Uppsala and the Departments of Surgery and Medicine, Huddinge Hospital, Huddinge, Sweden

Despite a number of brilliant achievements in research on diabetes mellitus the problem of an efficient cure of the disease remains to be solved. Recent efforts to reach this goal have been directed along two main avenues, one dealing with the development of artificial devices for glucose-controlled, long-term delivery of insulin and the other with transplantation of either whole pancreas or isolated pancreatic islets. The present paper is a brief review of the latter approach.

1. Present state of the art

Systematic trials of human pancreas transplantation began about 15 years ago when Lillehei and his associates (11) grafted vascularized pancreatic glands into a series of diabetic patients. Striking improvements in glucose tolerance were noted in several of these patients in the immediate postoperative period and some were able to stay off insulin for several months. However, the rate of complications and the mortality were high and none of the grafts functioned for more than a year. Except for rejection of the organ, failures were related to sepsis, thrombosis, fistula and necrosis of the duodenal portion of the graft. Since these initial attempts many improvements of the operative procedure have been made leading to a significant drop in the rate of complications (8, 21). According to most recent statistics about 100 vascularized pancreatic transplantations have now been carried out, somewhat more than half of them having been reported in the literature. However, up till now only 8 patients have carried a functioning graft for more than one year and the maximum time achieved is reported as four years (6).

As an alternative to whole pancreatic transplantation Lacy (3) and others (5) demonstrated that diabetes in experimental animals could be cured by the intraportal or intrasplenic, transplantation of isolated islets of Langerhans. In addition, tissue culture procedures or even cryopreservation could be applied to store the islet specimens in a tissue bank. Indeed, animals with experimental diabetes have been cured permanently by grafts of syngeneic isolated islets

and only minimal deviation of their glucose tolerance has been noted after the transplantation (23). Moreover, regression of diabetic nephropathy has been recorded in animals treated with islet transplantation (20).

If these approaches were applicable to the clinical situation thrombosis, exocrine leakage and other complications would be eliminated and major surgery avoided. It was therefore disappointing to find that isolated islets could not be obtained from the human pancreas in quantities sufficient for transplantation (2, 18). This is due to the fibrous nature of the human pancreas and the variations between individual cadaveric pancreases, which precludes the application of standardized preparative procedures. In order to circumvent these difficulties pancreatic microfragments partially digested with collagenase, have been used. Several attempts at allotransplantation of such fragments have now been carried out in patients (22, 14) but only in one case has the procedure led to lasting alleviation of the diabetic state (14). An additional case which was transplanted with pancreatic material obtained from fetuses (see below) showed a transitient rise in C-peptide excretion (7,10).Transplantation of microfragments obtained from the patient's own pancreas following total pancreatectomy for chronic pancreatitis has, however, prevented from postoperative diabetes in several cases (15). This observation suggests that pancreatic microfragments are useful and that the main obstacle to successful transplantation in the diabetic recipients remains the problem of immune rejection.

Although in diabetic patients rejection of a transplanted pancreas may be prevented by immunosuppressive regimes, such a treatment often leads to an aggravation of the diabetic state. Theoretically this could be avoided if the immunogenicity of the donor tissue were abolished before transplantation. Recent evidence suggests that a considerable alleviation of the immunogenic properties may be achieved by maintaining the graft in tissue culture before transplantation (12,1,4). This may be explained by the elimination in culture of certain cells, presumably passenger leucocytes, implicated in the allograft response (13). However, fragments of adult pancreas do not lend themselves for tissue culture, since they survive for only short periods in these conditions. We therefore have studied the possibility of using the fetal human pancreas, which appears more suitable for maintenance in tissue culture. In addition, growth of the fetal B-cells would be sufficient to gradually compensate for the deficient insulin production of the recipient. Indeed, by this particular mechanism, transplantation of fetal pancreas has been found to reverse fully experimental diabetes in rodents (17).

2. Ongoing attempts to improve the methodology

In a recent report we presented data on the survival of human fetal pancreas maintained in organ culture for 6-14 days (24). We concluded that human fetal B-cells may retain their viability in culture for more than a week but there

was a conspicuous variability between different fetal preparations with regard to their ability to respond to an acute glucose challenge. Moreover, in a certain number of experiments the structural integrity of the explants was less satisfactory, which may have been due to a deficient oxygen supply to cells in the central parts of the explants.

In order to improve the culture conditions we have recently applied the culture technique of Mandel and Kennedy (16) in which small pieces of human fetal pancreas are placed in culture dishes on top of Millipore-filters (8 /um pore size), supported by gelfoam (Spongostan^{R}), so that the tissue is maintained at the air-liquid interphase. Preliminary trials, indeed, suggest that this approach leads not only to greatly improved yields of tissue but also to differentiation and growth of the pancreatic cells in vitro. Pancreatic sections immunochemically stained for the demonstration of insulin showed that both islets and scattered B-cells were present in the culture explants (Fig. 1). It was also found that such specimens respired linearly for several hours when incubated in Cartesian divers (9), although the respiratory rate was lower than that earlier observed for isolated adult human islets (2).

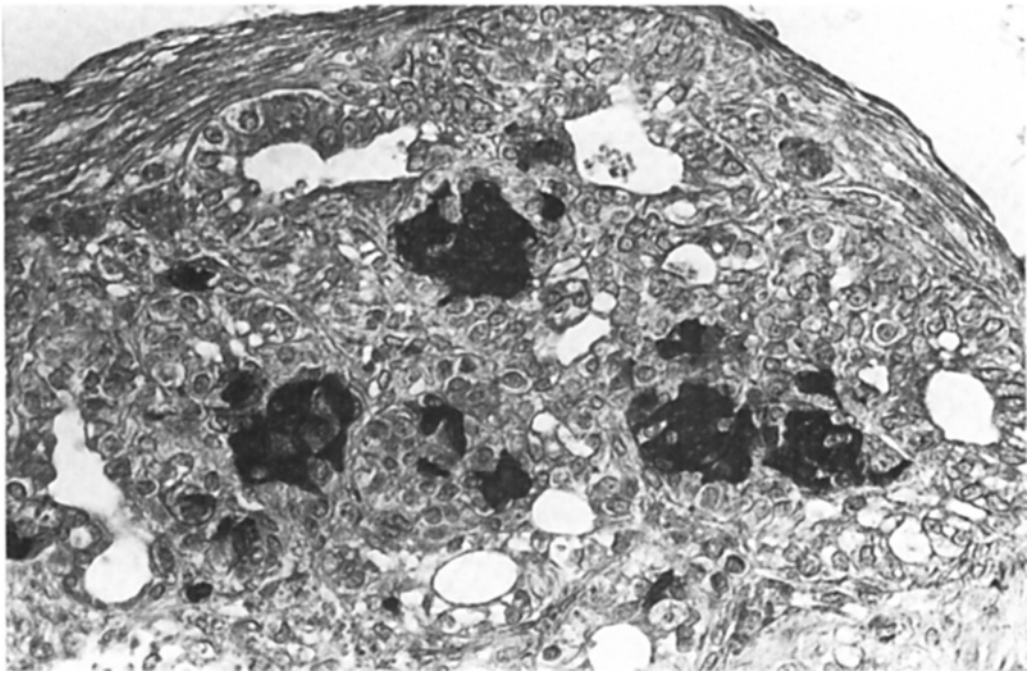

Fig. 1. Section of a human fetal pancreatic microfragment cultured for 3 days in medium RPMI 1640. Immunocytochemical staining for the demonstration of insulin. X 340.

The endocrine functions of the cultured microfragments were assessed by measuring the insulin accumulation in the culture medium (RPMI 1640 + 10% fetal calf serum) and the rate of insulin secretion from the explants when challenged with high glucose and theophylline in a perifusion system. As regards the insulin secretion during culture it was found that there was a continuous secretion into the medium although the secretory rate tended to decline slightly with time. This latter finding is in contradiction to what we and others have observed in preparations of fetal rat pancreases and may imply that the human fetal pancreas has special requirements for differentiation and growth in vitro.

The results of one perifusion experiment is shown in Fig. 2. In this and also in other experiments the explants cultured in TCM 199 (5.5 mM glucose) responded more vigorously to the acute glucose + theophylline stimulation than those cultured in RPMI 1640 (11 mM glucose). The response to the glucose challenge was however, diminished and delayed. This may reflect diffusion gradients in the cultured tissue but also the relative immaturity of the fetal B-cells. It should be noted in this context that the Millipore filter on which the fragments were located during the entire culture period was transferred to the perifusion chamber. This makes it plausible to assume that the observed dynamics of the insulin secretion were relevant for the situation in the culture system.

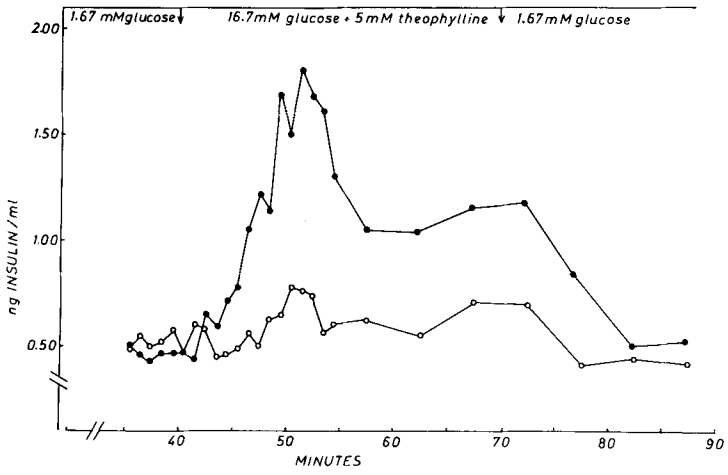

Fig. 2. Effects of high glucose + theophylline on the insulin secretion by fetal pancreatic microfragments cultured for 3 days in medium TCM 199 (filled circles) or RPMI 1640 (open circles).

Microfragments cultured for one week in RPMI 1640 have furthermore been used for studies of the effects of cryopreservation. The protocol for these experiments has been the same as that successfully applied to adult mouse islets (19), which implies freezing in a medium supplemented with 1M DMSO at a rate of 0.5°C/min. The preliminary results of these experiments suggest that the rate of protein synthesis of such cryopreserved pancreatic fragments is not different from that of the non-frozen controls. It should, however, not be taken for granted that the best conditions for cryopreservation of human fetal pancreas are the same as those observed for rodent preparations.

3. Conclusions

The combined results obtained in transplantations of endocrine pancreas in both man and animals warrant a cautious optimism for the future. The main obstacles to successful clinical use of this approach remain the immunological complications and the difficulties in obtaining sufficient pure islets for transplantation. Attempts to circumvent these problems are at present directed towards diminution of donor tissue antigenicity by tissue culture and the exploitation of the growth potential of the fetal endocrine pancreas. Should these efforts turn out to be successful islet transplantation may become a useful future therapy in selected cases of juvenile diabetes mellitus.

ACKNOWLEDGEMENTS

Final support from the Swedish Diabetes Association, the Nordic Insulin Fund, Stiftelsen Expressens Prenatalforskningsfond, The Kroc Foundation, The Medical Faculty of the University of Uppsala, The Swedish Society of Medical Sciences, Syskonen Svenssons Fond för Medicinsk Forskning and the Swedish Medical Research Council (12X-109, 16X-4527), is gratefully acknowledged.

REFERENCES

1. Andersson, A.: Survival of pancreatic islet allografts. The Lancet II:585, 1979.
2. Andersson, A., Borg, H., Groth, C., Gunnarsson, R., Hellerström, C., Lundgren, G., Westman, J. & Östman, J.: Survival of human islets of Langerhans maintained in tissue culture. J Clin Invest 57:1295, 1976.
3. Ballinger, W.F. & Lacy, P.E.: Transplantation of intact pancreatic islets in rats. Surgery 72:175, 1972.
4. Bowen, K.M., Andrus, L. & Lafferty, K.J.: Successful allotransplantation of mouse pancreatic islets to nonimmunosuppressed recipients. Diabetes 29 (Suppl. 1):98, 1980.
5. Finch, D.R.A., Wise, P.H. & Morris, P.J.: Successful intra-splenic transplantation of syngeneic and allogeneic isolated pancreatic islets. Diabetologia 13:195, 1977.
6. Gliedman, M.L., Tellis, V.A., Soberman, R., Rifkin, H. & Weith, F.J.: Long-term effects of pancreatic transplant function in patients with advanced juvenile-onset diabetes. Diabetes Care 1: 1, 1978.
7. Groth, C.G., Andersson, A., Björkén, C., Gunnarsson, R., Hellerström, C., Lundgren, G., Petersson, B., Swenne, I. & Östman, J.: Transplantation of fetal pancreatic microfragments via the portal vein to a diabetic patient.

Diabetes 29 (Suppl. 1):80, 1980.
8. Groth, C.G., Lundgren, G., Gunnarsson, R., Arner, P., Berg, B. & Östman, J.: Segmental pancreatic transplantation with duct ligation or drainage to a jejunal Roux-en-Y loop in nonuremic diabetic patients. Diabetes 29 (Suppl.): 3, 1980.
9. Hellerström, C.: Effects of carbohydrates on the oxygen consumption of isolated pancreatic islets of mice. Endocrinology 81:105, 1967.
10. Hellerström, C., Andersson, A., Björkén, C., Eriksson, U., Groth, C.G., Gunnarsson, R., Hårdstedt, C., Lundgren, G., Petersson, B., Pääbo, S., Swenne, I., Ågren, A., Östman, J.: Isolation, culture and transplantation of human pancreatic islets. Proc. Sixth Int. Congr. Endocrinol. Austr. Acad. Sci. in press.
11. Kelly, W.D., Lillehei, R.C., Merkel, F.K., Idezuki, Y. & Coetz, F.C.: Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. Surgery 61: 827, 1967.
12. Lacy, P.E., Davie, J.M. & Finke, E.H.: Prolongation of islet allograft survival following in vitro culture (24°C) and a single injection of ALS. Science 204:312, 1979.
13. Lafferty, K.J. & Woolnough, J.: The origin and mechanism of the allograft reaction. Immunol Rev 35:231, 1977.
14. Largiader, F. & Kolb, E.: A long-term functioning human pancreatic islet allotransplant. Transplantation 29:76, 1980.
15. Lorenz, D., Lippert, H. & Wolff, H.: Autotransplantation of pancreatic islets in man. Horm Metab Res, in press.
16. Mandel, T.E. & Kennedy, M.M.: The differentiation of murine thymocytes in vivo and in vitro. Immunology 35:317, 1978.
17. Mullen, Y.S., Clark, W.R., Molnar, I.G. & Brown, J.: Complete reversal of experimental diabetes mellitus in rats by a single fetal pancreas. Science 195:68, 1977.
18. Najarian, J., Sutherland, D. & Steffes, M.: Isolation of human islets of Langerhans for transplantation. Transp Proc 7:611, 1975.
19. Sandler, S., Nilsson, B., Borg, L.A.H., Swenne, I., Petersson, B., Hellerström, C. & Andersson, A.: Structure and function of cryopreserved mouse pancreatic islets. Horm Metab. Res, in press.
20. Steffes, M.W., Brown, D.M. & Mauer, S.M.: The development, enhancement and reversibility of diabetic glomerulopathy. In: Camerini-Davalos, R.A. & Hanover, B. (eds.): Treatment of early diabetes, Plenum Press, N.Y. p. 473, 1979.
21. Sutherland, D.E., Goetz, F.C. & Najarian, J.S.: Clinical segmental pancreas transplantation without duct anastomosis in diabetic renal allograft recipients. Diabetes 29 (Suppl. 1): 10, 1980.
22. Sutherland, D.E., Matas, A.J., Goetz, F. C. & Najarian, J.S.: Transplantation of dispersed pancreatic islet tissue in humans. Autografts and allografts. Diabetes 29 (Suppl. 1) 31, 1980.
23. Trimble, E.R., Siegel,.G., Berthoud, H.R. & Renold, A.E.: Control of insulin secretion in transplanted pancreatic islets. In: Bibergeil, H., Zühlke, H. & Poser, U. (eds.): Early Diabetes. Pathogenesis, Diagnosis, Prevention. Karlsburg, p. 304, 1980.
24. Ågren, A., Andersson, A., Björkén, C., Groth, C.G., Gunnarsson, R., Hellerström, C., Lindmark, G., Lundqvist, G., Petersson, B. & Swenne, I.: Human fetal pancreas. Culture and function in vitro. Diabetes 29 (Suppl. 1):64, 1980.