Lectins for Electromagnetic Purification of Islets from Humans and Large Mammals

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Introduction

For successful human pancreatic islet transplantation it remains an elusive goal to solve the problems of quantity, purity, and viability (*Hering, Bretzel* and *Federlin* 1989). Thus, preparation of islets for transplantation has required the development of a new field of biotechnology.

One prerequisite for unimpaired islet graft function is the separation from the presumably cytotoxic and highly immunogenic exocrine tissue (*Ulrichs* and *Müller-Ruchholtz:* cf. page 123 in this book; *Gray* 1989). Three different principles of islet purification are used: (A) "Hand-picking" is a method established for purification of islets from the rodent pancreas (*Finke, Lacy* and *Ono* 1979). But for the high number of islets needed for human islet transplantation this method is too time-consuming and far too laborious. (B) Density gradient centrifugation is dependent on the different densities of exocrine and endocrine tissue. Large scale purification by this method presently achieves purity degrees of approx. 40%–70% (*Lake, Bassett, Larkins, Revell, Walczak, Chamberlain, Rumford, London, Veitch, Bell* and *James* 1989; *Ricordi, Lacy* and *Scharp* 1989; *Warnock, Ellis, Cattral, Untch, Kneteman* and *Rajotte* 1989). This is partly explained by the difficulties to control the collagenase digestion process. (C) The principle of electromagnetic cell separation (EMS; *Kandzia, Scholz, Anderson* and *Müller-Ruchholtz* 1984; *Müller-Ruchholtz, Kandzia* and *Leyhausen* 1987) was further developed for large scale islet purification (*Müller-Ruchholtz, Leyhausen, Petersen, Schubert* and *Ulrichs* 1987). Magnetic microspheres (MMS) were coupled to lectins with binding specificity for exocrine tissue. UEA-I was found to be a highly selective lectin for rat (*Müller-Ruchholtz* et al. 1987) and WFA for human (*Winoto-Morbach, Ulrichs, Leyhausen* and *Müller-Ruchholtz* 1989; *Winoto-Morbach, Leyhausen, Schünke, Ulrichs* and *Müller-Ruchholtz* 1989) exocrine tissue. Lectin-coupled MMS can trap the latter in an electromagnetic field, thus providing effluent islets of a high degree of purity. Functional integrity of EMS-separated rat islets was tested in vivo (*Müller-Ruchholtz* et al. 1987) and in vitro (*Winoto-Morbach* et al. 1989).

Because of the difficulties to obtain sufficient human material we propose other species (cattle, pig and dog) to perform extensive preclinical studies and to collect data in view of the possibility of future islet xenotransplantation (*Reemtsma* 1989). This has the advantage of unlimited access to material for large scale islet separation. But for each new species used as islet donor, a particular lectin has to be found to make this approach effective for either experimental or clinical purposes. Therefore our interest was focussed on lectins with adequate specificity for pancreatic exocrine tissue of cattle, pig and dog. The characteristics of suitable lectins are the following: (a) high degree of binding specificity for the adequate mammalian tissue, (b) nontoxicity to endocrine cells, and (c) maintenance of binding specificity for exocrine cells after coupling to microspheres.

Materials and Methods

Islet preparation

Animals of both sexes were used as donors, cattle at an average age of 12–18 months, weighing 400–500 kg, pigs at an age of 6–8 months, weighing 80–100 kg, and dogs, adult beagles, weighing 10–15 kg. The pancreata were intraductally distended with prewarmed 828–30 °C) Hank's balanced salt solution (HBSS) containing 0.2% collagenase (Serva Heidelberg, Germany), 0.03% DNase type I grade II (Boehringer Mannheim, Germany) and 2% heat-inactivated fetal calf serum (Gibco Eggenstein, Germany). The volume injected was 2 ml/g pancreas sample. The digestion of the glands was performed in a continuous digestion-filtration device similar to the automated method developed by *Ricordi, Lacy, Finke, Olack* and *Scharp* (1988) and according to the method of *Hering, Romann, Clarius, Brenzel, Slijepcevi, Bretzel* and *Federlin* (1989). Crude islet preparations were stored in liquid nitrogen until further use.

Lectins (Agglutinins)

The following lectins were used: Anguilla anguilla (AAA), Bauhinia purpurea (BPA), Concanavalia ensiformis (ConA), Dolichos biflorus (DBA), Erythrina cristagalli (ECA), Griffonia simplicifolia I (GSA-I),

Table 1 Binding pattern of lectins to crude bovine pancreatic islets.

		Groups of lectins				
		1	2	3	4	5
		AAA UEA-I	DBA HPA PNA	ConA LCA LPA MPA	ECA GSA-I GSA-II SBA Succ. ConA Succ. WGA WFA WGA	BPA RCA-I
A: Lectins coupled to FITC	exo.	–	(+)/+	+	++	+++
	endo.	–	–/(+)	–/(+)	(+)/+	–
B: Lectins coupled to MMS	exo:	–	–	(+)/–	+/–	++
	endo.	–	–	–	–	–

+++ very strong; ++ strong; + medium; (+) weak; – no detectable binding; exo. exocrine; endo. endocrine.

Table 2 Binding pattern of lectins to crude pig pancreatic islets.

		Groups of lectins				
		1	2	3	4	5
		AAA	BPA ConA ECA GSA-II LPA PNA SBA	DBA HPA MPA Succ.WGA WGA	LCA Succ.ConA UEA-I	GSA-I RCA-I WFA
A: Lectins coupled to FITC	exo.	–	(+)	+	++	+++
	endo.	–	–	–	(+)	–
B: Lectins coupled to MMS	exo:	n.d.	–/(+)	(+)	+	++
	endo.		–	–	–/(+)	–

+++ very strong; ++ strong; + medium; (+) weak; – no detectable binding; n.d. not done; exo. exocrine; endo. endocrine.

Griffonia simplicifolia II (GSA-II), Helix pomatia (HPA), Lens culinaris (LCA), Limulus polyhemus (LPA), Maclura pomifera (MPA), Peanut = Arachis hypogaea (PNA), Ricinus communis I (RCA-I), Soy Bean = Glycine max (SBA), Succinyl Concanavalia ensiformis (Succ. ConA), Succinyl Wheat Germ = Triticum vulgaris (Succ. WGA), Ulex europaeus I (UEA-I), Wheat Germ = Triticum vulgaris (WGA), Wistaria floribunda (WFA). The Lectins were purchased from Medac, Hamburg, Germany.

Preparation of MMS and lectin coupling

In brief, nontoxic albumin MMS of 800 nm size were prepared. Lectins were coupled to glutaraldehyde-activated MMS by incubating them for 2 h in phosphate-buffered saline (PBS). Binding of MMS to isolated pancreatic tissue and fluorescence studies performed on isolated pancreatic tissue were studied according to a recently published protocol (*Winoto-Morbach* et al. 1989).

Results

19 different lectins were investigated in FITC- and MMS-binding studies. Each lectin that showed a strong and selective binding pattern and no loss of biological function after coupling to MMS was tested with various crude islet preparations from the species in question. The lectin-binding patterns for the 3 different species are summarized in Tables 1–3.

Crude bovine islets (Table 1)

Only two lectins, BPA and RCA-I, were suitable, according to their binding specificity to exocrine

Table 3 Binding pattern of lectins to crude dog pancreatic islets.

		Groups of lectins				
		1	2	3	4	5
		AAA PNA GSA-II	ConA ECA LPA MPA	DBA GSA-I UEA-I	HPA LCA SBA Succ. ConA Succ. WGA WFA WGA	BPA RCA-I
A: Lectins coupled to FITC	exo.	–	+	++	++	+++
	endo.	–	+	–	+	–
B: Lectins coupled to MMS	exo:	–	–	+	+	++
	endo.	–	–	–	(+)/–	

+++ very strong; ++ strong; + medium; (+) weak; – no detectable binding; exo. exocrine; endo. endocrine.

tissue fragments. FITC-labeled and microsphere-coupled lectins yielded similar results. Interestingly, neither UEA-I nor WFA, which were suitable in rat and human pancreatic tissue, were of any use in this species.

Crude pig islets (Table 2)

Three lectins, GSA-I, RCA-I and WFA, showed binding specificity to exocrine tissue fragments. In contrast to bovine tissue, but similar to results with human tissue, WFA reacted selectively with pig exocrine cells.

Crude canine islets (Table 3)

The same two lectins that had previously been found to react specifically with bovine exocrine tissue, namely BPA and RCA-I, showed the strongest selective binding to canine exocrine cells.

Discussion

Handpicking of islets, well established in rodents, is not applicable for the large quantity of purified islets needed, e.g., for human, bovine, pig, or canine islet transplantation. Density gradient centrifugation has undoubtedly been improved recently (*Lake* et al. 1989; *Ricordi* et al. 1989; *Warnock* et al. 1989), but so far it does not provide the high degree of islet purity which is considered necessary for unimpaired islet graft function (*Hering* et al. 1989; *Gray* 1989).

Functional immunogenicity studies with crude human islet preparations compared to clean islets in *in vitro* tests have clearly shown that the exocrine tissue portion contributes greatly to graft immunogenicity (*Ulrichs* and *Müller-Ruchholtz*, cf. chapter F). Immunohistological studies with human pancreas frozen tissue sections are in line with this functional data by showing large amounts of HLA-DR cells particularly within the exocrine tissue compartment. These are HLA-DR T lymphocytes, tissue macrophages otherwise known as "dendritic cells", vascular endothelial cells and duct epithelium (unpublished observation). Islets clearly contain much fewer of these cells. Thus, the EMS technique may be the technique in demand to eliminate the contaminating exocrine tissue and provide pure islets for transplantation. The feasibility of such an approach has been clearly shown in the rat system with well functioning, i.e., long-term surviving syngeneic rat islet grafts (*Müller-Ruchholtz* et al. 1987).

Preliminary EMS studies with crude human islets and the relevant MMS-coupled Wisteria floribunda lectin are similarly promising as to purity (>98%), yield (70%) and viability (>90%) of islets (*Winoto-Morbach* et al. 1989; *Winoto-Morbach* et al. 1989), provided the islets in the preparation are well digested and thus without an exocrine rim.

A different approach to achieve purity of canine islets consists of the lysis of exocrine acinar cells by complement-activating, exocrine-specific monoclonal antibodies (*Soon-Shiong, Heintz* and *Terasaki* 1988). The degree of purity has been reported to be in the range of 65–87%. These antibodies have recently been shown to be cross-reactive with human exocrine tissue (*Soon-Shiong, Heintz* and *Terasaki* 1989). Similar to our approach they are presently coupled to dynabeads and used for electromagnetic purification of human islets (*Soon-Shiong* et al., cf. chapter C). It will be interesting to compare the results of these two approaches in the near future. Monoclonal antibodies of our own production might be advantageous in view of the present high costs of lectins.

It is our present suggestion that optimal enzymatic islet digestion, followed by a pre-purification step, e.g., with density gradient centrifugation and a final purification step with the EMS technique, using either lectins or monoclonal antibodies to eliminate exocrine cells, may allow us to solve the problem of islet purity and quantity in the near future.

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Abbreviations

EMS = electromagnetic separation
MMS = magnetic microspheres
FITC = fluorescein isothiocyanate

References

Finke, E. H., P. E. Lacy, J. Ono: Use of reflected green light for specific in vitro identification of islets after collagenase isolation. Diabetes 28: 612–613 (1979)

Gray, D. W. R.: The role of exocrine tissue in pancreatic islet transplantation. Transplant. Int. 2: 41–45 (1989)

Hering, B. J., R. G. Bretzel, K. Federlin: Current status of clinical islet transplantation. Horm. Metabol. Res. 20: 537–545 (1989)

Hering, B. J., D. Romann, A. Clarius, M. Brendel, M. Slijepcevic, R. G. Bretzel, K. Federlin: Bovine islets of Langerhans. Potential source for transplantation? Diabetes 38 (Suppl. 1): 206–208 (1989)

Kandzia, J., W. Scholz, M. J. D. Anderson, W. Müller-Ruchholtz: Magnetic albumin/protein A immunomicrospheres. I. Preparation, antibody binding capacity and chemical stability. J. Immunol. Methods 75: 31–41 (1984)

Lake, S. P., P. D. Bassett, A. Larkins, J. Revell, K. Walczak, G. M. Chamberlain, G. M. Rumford, N. J. M. London, P. S. Veitch, P. R. F. Bell, R. F. L. James: Large-scale purification of human islets utilizing discontinuous albumin gradient in IBM 2991 cell separator. Diabetes 38 (Suppl. 1): 143–145 (1989)

Müller-Ruchholtz, W., J. Kandzia, G. Leyhausen: Cell separation with magnetic immunomicrospheres. In: Cellular, molecular and genetic approaches to immunodiagnosis and immunotherapy; Kano et al., Ed., University of Tokio Press (1987), pp. 181–190

Müller-Ruchholtz, W., G. Leyhausen, P. Petersen, G. Schubert, K. Ulrichs: A simple methodological principle for large scale extraction and purification of collagenase-digested islets. Transplant. Proc. 19: 911–915 (1987)

Reemtsma, K.: Xenografts. Transplant. Proc. 21: 517–518 (1989)

Ricordi, C., P. E. Lacy, E. H. Finke, B. J. Olack, D. W.Scharp: Automated method for isolation of human pancreatic islets. Diabetes 37: 413–420 (1988)

Ricordi, C., P. E. Lacy, D. W. Scharp: Automated islet isolation from human pancreas. Diabetes 38 (Suppl. 1): 140–142 (1989)

Soon-Shiong, P., R. Heintz, P. Terasaki: An immunological method of islet cell purification using cytotoxic anti-acinar cell monoclonal antibodies. Transplant. Proc. 20: 61–63 (1988)

Soon-Shiong, P., R. Heintz, P. Terasaki: Identification of novel blood group-reactive monoclonal antibodies to human acinar cells but not islets. Transplant. Proc. 21: 2622–2623 (1989)

Warnock, G. L., D. K Ellis, M. Cattral, D. Untch, N. M. Kneteman, R. V. Rajotte: Viable purified islets of Langerhans from collagenase-perfused human pancreas. Diabetes 38 (Suppl. 1): 136–139 (1989)

Winoto-Morbach, S., G. Leyhausen, M. Schünke, K. Ulrichs, W. Müller-Ruchholtz: Magnetic microspheres (MMS) coupled to selective lectins: A new tool for large-scale extraction and purification of human pancreatic islets. Transplant. Proc. 21: 2628–2630 (1989)

Winoto-Morbach, S., K. Ulrichs, G. Leyhausen, W. Müller-Ruchholtz: New principles for large-scale preparation of purified human pancreas islets. Diabetes 38 (Suppl. 1): 146–149 (1989)

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Large Scale Purification of Pig Islets of Langerhans by Fluorescence Activated Cell Sorting

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Introduction

The transplantation of islets of Langerhans into humans or into large animal models is still hampered by the fact that the purification methods which are mainly based on gradient techniques show relatively low yields (*Sutherland* 1981). Furthermore small lymphnodes and vessels can not be separated definitely from the islets (*Van Suylichem, Wolters* and *van Schilfgaarde* 1989).