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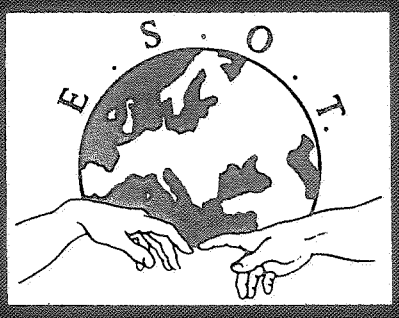
BOOK OF ABSTRACTS



HIGH YIELD PURIFICATION OF PIG ISLETS OF LANGERHANS BY FLOW CYTOMETRY. BU v Specht, M Finke, A Eckhardt, PM Feger. Dept.Surg., Univ.Freiburg, 78 Freiburg, FRG.

Purification of islets of Langerhans of large animals is still a problem because hand picking is too time consuming and gradient-techniques have the disadvantage of poor yield and lymph nodes and vessels are not always separated from the islets. The recently introduced Partec® cell sorter separates neutral red stained islets from non stained exocrine tissue as was shown for rat islets, without damaging the islets (1). We have succeeded now to separate with this technique pig islets of Langerhans in high yield. Pig pancreas was perfused immediately after removal from the animal with neutral red dissolved in Hanck's solution. The organ was transported to the laboratory on ice and perfused through the duct with collagenase (Serva 1 mg/ml) and placed in an automatic digestion chamber which was recently described by Ricordi (2). After digestion the collagenase was removed by filtration, the islets were washed and than subjected to flow cytometry. From 20 organs the median yield was 800.000 islets with a purity above 90%. Function was tested successfully by the measurement of insulin synthesis in vitro (incorporation of ^{35}S Cystein into insulin) and by transplantation into diabetic nude rats.
Transplantation of diabetic pigs are in progress and will be reported.

1 DWR Gray, W Göhde, N Carter, T Heiden, PJ Morris. Diabetes 38, 133-135, 1989
2 C Ricordi, PE Lacy, DW Sharp. Diabetes 38, 140-142, 1989

COMPARISON OF CURRENT ISLET ISOLATION TECHNIQUES IN DOGS.
M.P.M. van der Burg, H.G. Gooszen, M.J. Field, J.P. Scherft, J.L. Terpstra, F.T. van de Woude, O.R. Guicherit, M. Frölich and J.A. Bruijn.
From the Departments of Surgery, Cell Biology, Nephrology, Endocrinology and Pathology, University Hospital Leiden, Leiden, The Netherlands, and the Department of Surgery, University of Minnesota, Minneapolis, Minnesota.

The efficacy of pancreatic islet isolation must improve to allow clinical islet transplantation. The two major isolation techniques usually performed in large experimental animals were compared in terms of islet yield. Pancreatic segments were intraductally distended either by manual injection of a low-volume (1 ml/g tissue) prewarmed (38°C) collagenase solution (n=6) or by infusion of a high-volume (~4 ml/g tissue) collagenase solution at room temperature using a peristaltic pump (n=4). Subsequently the tissue was processed by stationary digestion at 38°C, teasing using forceps, syringing and filtration (mesh 400 µm). Isolated islets ranged from 25-400 µm diam. Total islet yield (mean ± SE; calculated from the volume of islets in duplicate samples and expressed as a percentage of the stereologically assessed mean pancreatic islet content) amounted to 14 ± 5% with the duct injection and 72 ± 12% with the ductinfusion method (P<.01). Next islet suspensions were purified using either Ficoll or dextran gradients. Both Ficoll and dextran purification reduced islet yield by ~50% (P<.02). Islets and acinar tissue were however better separated in dextran than in Ficoll gradients. In addition on Ficoll- as opposed to dextran-purification islet size distributions shifted to smaller diameters (P<.08), suggesting fragmentation of islets to have occured. Dextran-purified islet suspensions maintained overnight in tissue culture demonstrated intact morphology and a biphasic insulin response with a 4-7 fold increase over basal release following glucose-stimulation (from 3 to 10 mM) by perifusion (P<.001). In conclusion: as compared to manual duct injection of collagenase and Ficoll gradient purification, isolated islet yield increased fivefold using duct infusion and dextran purification. Key elements of the superior technique appeared to be a more uniform distension of the gland and the higher ratio of collagenase to tissue volume.

234

IMMUNOLOGIC AND METABOLIC CONSEQUENCES OF IN VITRO AND IN VIVO IRRADIATION OF SMALL BOWEL ALLOGRAFTS Manfred J. Stangl,M.D., Kenneth K. W. Lee,M.D., Harry L. Moynihan,B.S., Thomas K. Lee,M.D., Wolfgang H. Schraut,M.D. University of Pittsburgh, 497 Scaife Hall, Pittsburgh, PA 15261

Donor irradiation (10Gy) has been found to postpone rejection in experimental kidney and heart transplantation by reducing passenger leukocyte content. In vitro graft irradiation has also been shown to avert graft versus host disease (GVHD) in small bowel transplantation (SBT). Using the rat model of orthotopic small bowel transplantation, we sought to determine if in vitro graft irradiation prior to SBT (deactivation of graft lymphatic tissue) and in vivo donor irradiation (10Gy) 4 days prior to organ harvest (leading to profound lympho/-leucopenia) would mitigate graft rejection. The possibility of inducing intestinal radiation injury was evaluated in syngeneic donor-recipient pairs.

Group	Irradiation (10 Gy)	Survival (days)	
1 LEW-LEW (n=6)	in vivo	>180	) no radiation
2 LEW-LEW (n=6)	in vitro	>180	) damage
3 LBN-LEW (n=15)	---------	12.4±0.2	rejection
4 LBN-LEW (n=5)	in vitro	8.0±0.2	rejection
5 BN-LEW (n=6)	---------	9.5±1.0	rejection
6 BN-LEW (n=6)	in vitro	10.6±0.4	rejection
7 BN-LEW (n=6)	in vivo	10.6±0.5	rejection

Irradiation (10 Gy) does not induce lasting histologic or functional graft impairment. Irradiated grafts as well as small bowels obtained from leukopenic donors provoke a normal humoral (anti-donor antibody titers) and cellular (MLR) response of the recipient and are rejected promptly. The failure of radiopretreatment supports the concept that small bowel grafts contain a large amount of Ia^+ radioresistant cells which play a major role in the rejection process.

235

UW SOLUTION AS THE ISOLATION MEDIUM MARKEDLY IMPROVES CANINE ISLET PURIFICATION. M.P.M. van der Burg, H.G. Gooszen, O.R. Guicherit, R.J. Ploeg, J.P. Scherft, J.L. Terpstra, J.A. Bruijn and M. Frölich. From the Departments of Surgery, Cell Biology, Pathology and Endocrinology, University Hospital Leiden, Leiden, The Netherlands.

Inconsistent results with purity ranging from 10-60% following density gradient separation of pancreatic islets from acinar tissue remains perhaps the biggest obstacle to allow safe clinical islet transplantation. We sought whether the organ cold storage preservation solution developed at the University of Wisconsin (UW solution) when used as the isolation medium throughout the isolation procedure better protects pancreatic tissue as compared to standard isolation media like RPMI and Hanks' solution (HBS). Islet isolation from the canine left pancreatic limb (n=7) was performed by intraductal infusion of collagenase, stationary digestion at 38°C followed by discarding ducts and blood vessels, gentle syringing (14G) with expulsion over a 400 µm filter and vigorous shaking of trapped tissue in an ice-bath. Next tissue was spun in dextran gradients (densities 1.094, 1.085, 1.075 and 1.041 in HBS) and aspirated from the upper middle (UM: 1.085-1.075) and top layers. In Group 1 (n=4) collagenase in HBS and RPMI-1640 + 10% newborn calf serum for all subsequent steps was used. In Group 2 (n=3) collagenase in UW and UW +0.4% BSA was used instead of RPMI. Islet yield (mean ±SE; calculated from the volume of all diphenylthiocarbizone stained islets in duplicate samples, and expressed as a percentage of the stereologically determined pancreatic islet content) did not differ between groups and amounted to 72 ± 12% prior to and 38 ± 10% post purification. Purity estimated from amylase content in sonicated samples, assuming 98% acinar volume in pelleted tissue prior to purification, amounted to 32 ± 9% islet volume in Group 1 and 89 ± 6% in Group 2 ($P<.01$). It should be noted that both final purity and yield in Group 1 were calculated for tissue obtained from the dextran top layer only, since it contained 92 ± 3% of the islet volume at top + UM and amylase increased twofold at the UM layer, whereas final purity and yield in Group 2 were calculated from the combined top and UM tissue since 64% of these islets were recovered from the UM. In conclusion: it is suggested that a markedly improved efficacy of density gradient purification using UW solution as the isolation medium resulting in 90% as compared to 30% purity using tissue culture medium for isolation may be attributed to UW preservation of normal islet and acinar tissue density.