Large-Scale Purification of Human Islets Utilizing Discontinuous Albumin Gradient on IBM 2991 Cell Separator

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A new method is described for the large-scale purification of human pancreatic islets with a discontinuous gradient of bovine serum albumin formed on an IBM 2991 cell separator. Fifteen human pancreases were processed, and after density-gradient centrifugation, a mean of 2643 islets/ml pancreatic digest were recovered with a mean purity of 63% and contained in 430 μl mean vol. Viability of gradient-isolated islets was compared with that of non-density-gradient islets (handpicked) and showed no difference in function. This technique allows isolation of intact, viable human islets of Langerhans of sufficient purity for potential human transplantation. *Diabetes* 38 (Suppl. 1):143–45, 1989

One of the major problems of human islet transplantation as a potential treatment of type I (insulin-dependent) diabetes is the methodology required for rapid and effective large-scale isolation of viable islets from the human pancreas. Collagenase digestion is the standard method of releasing islets from the intact pancreas, and it has recently been demonstrated that intraductal delivery of this enzyme can produce large volumes of digest containing islets and dispersed exocrine cells (1). Although successful transplantation of this crude digest is possible in species such as dogs (2), similar studies in humans have resulted in serious complications (3). A second stage is thus required to purify the islets from the contaminating exocrine tissue. Various purification techniques have been used, including handpicking, serial sieving, and density-gradient isolation with Ficoll (1,4). Although pure islets can be obtained by handpicking, this method is unsuitable for the large numbers required for human transplantation. Islet yield can be improved by the technique of serial sieving, although there may be significant contamination in the final preparation (1). Density-gradient isolation therefore offers the best method of purifying large numbers of islets. We have recently shown that a discontinuous gradient of bovine serum albumin (BSA) improves the yield and purity of rat islets compared with Ficoll (5). To adapt this method to the purification of human islets, we had to overcome the difficulty involved in processing the large volumes of digest produced from the human pancreas. To solve this problem, we have used a machine that is normally used for processing bone marrow for transplantation that we have adapted to produce a large-volume density gradient (6). The aim of this study was thus to use BSA density gradients formed on an IBM 2991 cell separator for large-scale purification of islets from the human pancreas.

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MATERIALS AND METHODS

Preparation of pancreatic digest. Adult human pancreases were obtained from cadaveric donors. The tail segment of the pancreas was resected, and a size-4 or -5 FG cannula (Portex, Hythe, Kent, UK) was inserted into the exposed duct and ligated in place. Intraductal collagenase digestion of the pancreas was performed by a modification of the method described by Gray et al. (1). Briefly, the collagenase was delivered by peristaltic pump, and the digested pancreas was disrupted by shaking by hand. The digest was then washed in minimum essential medium (MEM; Flow, Rickmansworth, Hertford, UK), and islets were isolated either by handpicking with a finely drawn pipette or by the discontinuous BSA (Sigma, Poole, Dorset, UK) gradient, described below.

Islet isolation. The IBM 2991 cell separator (COBE, Lakewood, CO) was developed to wash units of erythrocytes and to remove the preservative agent from cryopreserved blood. The machine consists of a centrifuge bowl with a flexible membrane in the bottom, into which fits a sealed (sterile) doughnut-shaped plastic processing bag. The flexible membrane is connected to a hydraulic pump that allows liquid

TABLE 1
Results of isolation of human pancreatic islets with IBM 2991 cell separator and bovine serum albumin as density gradient

	Mean ± SE	Range
Total digest volume (ml)	11.9 ± 1.1	5–27
Digest volume (ml/g pancreas)	0.7 ± 0.1	0.4–0.9
Islets recovered (islets/ml digest)	2643 ± 486	1100–3500
Estimated purity (%)	63.6 ± 7.8	10–90
Islet volume (μl)	430 ± 110	150–900
Sterile preparation (%)	100	

to be added to or removed from the processing bag through a rotating valve during centrifugation. The sequence of events to produce a discontinuous BSA density gradient on the cell separator was as follows: the washed digest was thoroughly dispersed in 200 ml BSA stock solution (density 1.10) in a sterile 500-ml glass bottle. The digest/BSA mixture was then run into the centrifuge bag under gravity. At slow-speed centrifugation, a second layer of BSA (75 ml, density 1.063) was loaded onto the gradient with a peristaltic pump. This layer was overlaid with 75 ml MEM. The bag was then centrifuged at 900 × *g* for 20 min. After centrifugation, the sequence of events was reversed; i.e., the supernatant (MEM) was pumped off as waste, and the islet-containing (inner) interface was collected in a sterile bag. The islets were washed with MEM/BSA in 50-ml centrifuge tubes, and aliquots were taken for the appropriate assays.

Assessment of islet yield and viability. Islet counts and measurement of purity were performed as described previously by serial dilution under an inverted microscope with side illumination (7).

Measurement of insulin release of isolated islets. Aliquots of five islets were taken randomly by micropipette from the initial digest (handpicked) and from the final islet preparation (BSA isolated). The aliquots were washed in MEM and were incubated at 37°C for 1 h in MEM containing low glucose (5.5 mM) or in MEM with added glucose (20 mM). At the end of this period, the MEM was carefully aspirated and immediately frozen for later insulin radioimmunoassay (Wellcome, Dartford, UK).

Transplantation of isolated islets into diabetic nude rats. BSA and handpicked islets were transplanted into nude rats made diabetic with streptozocin (STZ; 80 mg/kg). Islets (800–1000) were transplanted into the renal subcapsular space 48 h after STZ injection. Regular blood glucose measurements were performed until graft removal (21 days).

RESULTS

Fifteen human pancreases were processed as described. The mean digest volume produced was 11.9 ml, with a yield of 0.7 ml digest/g original pancreatic tissue (Table 1). Pancreatic digests were separated by BSA density-gradient centrifugation, and after purification, a mean islet volume of 430 μl (3.6% of original digest volume) was obtained. The number of islets recovered was 2643 ± 486 islets/ml pancreatic digest. The estimated purity of this preparation was 63.6 ± 7.8%.

To compare viability of the handpicked and BSA density-gradient–isolated islets, static incubations were performed in both low- and high-glucose media (Fig. 1). There was no significant difference in insulin release of handpicked and BSA-isolated islets in response to glucose (3.3 vs. 3.4 ng · islet^{-1} · h^{-1}).

With one exception, in which only BSA-isolated islets were obtained, both handpicked and BSA-isolated islets were purified from four human pancreases and were transplanted individually into seven diabetic inbred Rowett nude rats. Diabetes was successfully reversed with islets from three of the pancreases (5 rats), with the animals remaining normoglycemic until graft removal at 21 days (8). The remaining two rats, transplanted with islets from the fourth pancreas, remained hyperglycemic until graft removal for histology at 15 days.

DISCUSSION

Attempts to treat patients with type I diabetes by transplantation of isolated adult islets of Langerhans have been largely unsuccessful because hyperglycemia could not be controlled or was only temporarily controlled in most patients (3). There are a number of possible reasons for the disappointing results. Although rejection of allografted islets has been suggested as one cause of failure, poor viability and inadequate islet dosage probably play a significant role as well.

The most common method for isolating islets involves initial collagenase digestion of the pancreas. Although transplantation of pancreatic digest (dispersed grafts) has been shown to ameliorate diabetes in the dog, this technique is inappropriate for use in humans. The reasons for this include the difficulty in transplanting large volumes of tissue and the likely inflammatory response caused by the contaminating exocrine cells. For further progress to be made, efficient means of purifying large numbers of viable islets from human pancreatic digest need to be established.

We have thus developed a technique that will isolate large numbers of islets from the human pancreas in a rapid and efficient manner. Our method (with a BSA gradient generated on the IBM 2991 cell separator) has given us an average yield of 2643 islets/ml digest, with a maximum yield of >90,000 islets, despite the fact that we only use a portion of the pancreas at this stage. Purified islets were recovered

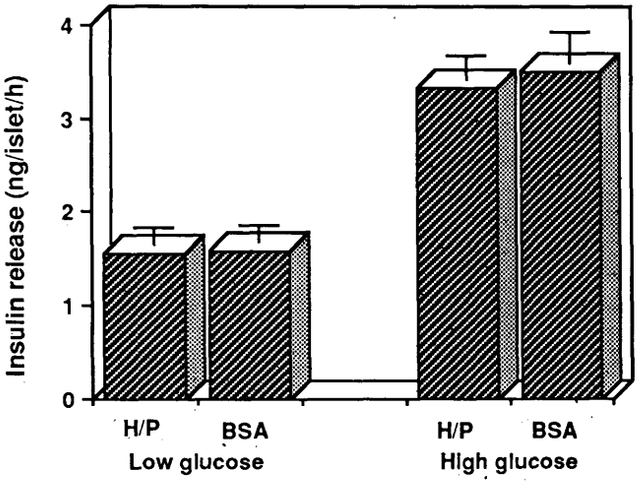

FIG. 1. Insulin release, in response to glucose, of handpicked (H/P) and BSA density-gradient–isolated human islets.

in a relatively small tissue volume (on average 430 μl), which represents only 3% of the original digest but contained >60% of the islet tissue. The high degree of islet purity obtained is important because it should facilitate pre-transplantation culture and cryopreservation of islet tissue as well as the actual transplant itself. Furthermore, the high degree of purity may be important for potential immunomodulation of the islets before transplantation.

We confirmed that the purification procedure does not damage the islets. Tests of viability both in vitro (Fig. 1) and in vivo by transplantation into diabetic nude rats have shown no difference in function between handpicked and BSA-isolated islets. An additional advantage of this procedure is that the purification takes place in a sterile enclosed system, which is essential for future transplantation of islets into diabetic patients. In conclusion, we have developed a method for the large-scale isolation of intact, viable islets of Langerhans from the collagenase-digested human pancreas.

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