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Isolation and Transplantation of Islet Tissue

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Recently, there have been remarkable advances in the area of islet tissue isolation and immuno-alteration that place islet tissue transplantation on the verge of clinical trials. This paper reviews the islet isolation advances by focusing on 5 approaches: altering the pancreas in vivo, distending and/or perfusing the pancreas in vitro, chopping the pancreas, isolating either intact islets or islet cells, and purifying the islet tissue. This review also focuses on 3 areas of islet tissue transplantation: islet tissue preparation, transplantation sites, and the prevention of rejection. Advances in these areas should solve the remaining technical problems currently preventing clinical trials.

There has been remarkable progress in the last 2 to 3 years in the area of islet transplantation that has brought us to the threshold of safe and effective islet transplantation for the diabetic patient. There have been 2 primary obstacles preventing these clinical trials since the feasibility of this approach was first demonstrated. The first has been the inability technologically to isolate and purify sufficient quantities of human islet tissue for successful transplantation results. The second has been the requirement of immunosuppressive therapy to prevent allograft rejection. There has been sufficient progress in both of these areas to expect effective clinical trials to be initiated in the near future.

Williams and Harsant [1], using bits of sheep pancreas, in 1893 were the first to attempt islet tissue transplantation in a diabetic patient. However, when Banting and Best [2] discovered insulin in 1922, most therapeutic approaches centered on the optimal administration of exogenous insulin. The cause of death for diabetic patients dramatically shifted from hyperglycemia and ketoacidosis to those of chronic complications. More recently, we have come to understand that exogenous insulin therapy does not prevent the complications of this disease. Thus, there has been renewed interest in alternative modes of therapy such as transplantation.

This paper will review the progress made in the area of islet isolation and transplantation. Sutherland also has recently reviewed this topic [3, 4].

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Islet Tissue Isolation

Moskalewski [5] in 1965 was the first to describe an enzymatic method of islet isolation. Lacy [6] in 1967 modified this method with distension of the pancreas which increased the yield. In 1972, Ballinger and Lacy [7] demonstrated the feasibility of islet transplantation by intraperitoneal implantation. Their work was independently confirmed by Reckard and Barker [8]. It soon became apparent that human islet isolation was to be a formidable barrier to clinical transplantation. Still, there have been relatively few studies whose primary function is the analysis of the entire isolation process.

The original, classic method of islet isolation being used for many studies today is a very inefficient and poorly controlled process. Starting with a procedure in which a given quantity of chopped pancreatic fragments is placed in a test tube with a given collagenase concentration, one incubates this for a given time at 37°C until the preparation "looks right." The digestion is halted at the point in time that gives the maximal amount of isolated islets. This is the process that worked for islet isolation studies and early transplantation studies. Upon examination one finds that islets released early in the process are broken down into single cells, while many islets are trapped in the pancreatic fragments at the time the digestion is stopped. Therefore, only a fraction of the available intact islets are effectively released. Applied to the human pancreas, this process simply does not work. It is quite apparent that an entirely new approach must be developed in order to solve this problem successfully. Table 1 subdivides this topic into 5 areas that each offers

Table 1. Approaches to islet tissue isolation.

Pancreas in vivo
Alteration of exocrine content
Stabilization of islet tissue
Warm ischemia time
Pancreas in vitro
Distention
Ductal, venous, arterial
Perfusion
Ductal, venous, arterial
Additives
Exocrine degranulation
Preliminary digestion
Enzyme inhibitors
Pancreatic chopping
Scissors
Automated
Islet tissue digestion
Mechanical
Enzymatic
Intact islets
Auto-isolator
Velcro
Enzyme mixtures
Islet cells
Auto-isolator
Enzyme mixtures
Islet tissue purification
Sedimentation
Density gradients
Hand-picking
Elutriation
Fluorescent activated cell sorter
Electrophoresis
Anti-islet antibodies

improvements leading to a potential solution of this problem. The rest of this section evaluates progress in each of these areas.

Pancreas in Vivo

While the pancreas is still in the donor, there are several manipulations that may improve islet yield; however, some are impractical in humans. D-1 ethionine given in multiple injections to donor rats markedly reduces the exocrine content of the gland and improves islet isolation. However, this effect is time dependent and may have widespread effects on other tissue. It is also a known carcinogen and poses problems for clinical trials. The administration of pilocarpine to the donor also improves islet yield and remains a practical approach for human donors [3, 9, 10]. Since pilocarpine degranulates the exocrine pancreas, the addition of pancreozymin and secretin to the donor may improve the degranulation even more.

Other investigators have utilized neonatal and fetal pancreas for islet isolations because of its reduced exocrine content [3]. This topic will be addressed by other authors in this symposium. The effect of warm ischemia has not been systematically evaluated for human tissue. This is a practical consideration with multiple organ donors since pancreatic and liver removal interfere with each other, increasing pancreatic ischemia time. This area needs attention with regard to human tissue.

Pancreas in Vitro

The next opportunity for modifying the isolation process comes with removal of the intact pancreas. Lacy [6] has shown that mechanical distension of the rodent pancreas increases islet yield by causing mechanical separation of islets from the exocrine tissue, thus making collagenase digestion easier. Examining the effectiveness of ductal distension in dogs and humans, we rarely found the same effect that had been observed in rodents. We turned to evaluating alternate methods of achieving effective pancreatic distension. In the dog pancreas, we found that the islets essentially exploded after arterial injection of salt solution. Both afferent and efferent arterioles are associated with the islets. The efferent arterioles have sphincters [11]. Most likely an intra-arterial injection of a salt solution ruptured the islets since the efferent islet sphincters seemed to be competent. Venous distension should provide better mechanical disruption between the islet and the exocrine pancreas since effective sphincters would protect the islets from the force of the injected fluid. We found this to be the case and documented an increase in islet yield by venous distension [11, 12]. However, we still did not get sufficient amounts of free islets to permit successful single-donor, single-recipient transplants in dogs. This entire area of rapid distension of the human pancreas needs reevaluation to determine if ductal, arterial, or venous distension is the optimal method.

The next advance in treating the pancreas in vitro came from Horaguchi and Merrell [13]. They adapted a collagenase perfusion technique used for hepatocyte preparation to the pancreas. This method employed a pre-digestion step before islet isolation. We and others have confirmed the importance of this step [14, 15]. One of the unanswered questions is the effect the endogenous enzymes may have on the collagenase digestion of the pancreas. Treating the pancreas in vitro with agents that reduce the exocrine enzyme concentration prior to collagenase digestion may resolve this question. To date, we have added pilocarpine, secretin, and pancreozymin to the ductal perfusate and have reduced exocrine enzyme concentration while increasing islet yield. This change has produced more effective

transplants in dogs [14, 16]. Yet, the less dense degranulated exocrine cells ascend to the upper Ficoll layers where the islets are located, reducing the effectiveness of this gradient approach to islet purification. There are many unanswered questions regarding the effectiveness of distension versus perfusion, ductal versus venous routes, and additional enzymes versus inhibitors that require investigation to improve this pre-digestion step.

Pancreatic Chopping

The next step in procuring islet tissue is chopping the gland. This is currently accomplished with scissors, an inefficient method. We have tried a tissue chopper with counter-rotating blades [3] but have found that it fragments the islets and is often bound by fibrous components of the pancreas. Other investigators use this type of apparatus. We have not found anything more suitable through the years. A more effective, automated device needs to be developed for a mass isolation system as clinical trials are anticipated.

Islet Tissue Digestion

The collagenase isolation of the islets has received the greatest attention from islet investigators. The original method, which is still the most commonly used, is extremely inefficient [17]. Basically, the process destroys islets that are released early in the digestion process. It also leaves much of the islet mass trapped in the pancreatic fragments. We, therefore, began to explore ways of removing the islets from the digestate as soon as they were released. This would permit complete digestion of the pancreatic fragments while protecting the separated islets from further destruction. The digestion filtration process was thus developed [10]. Chopped, pancreatic fragments were loaded into a single stainless steel screen with a pore size of 140 μ. Collagenase was loaded into the chamber holding the screen and a series of separate digestions were performed, washing the released islets out of the chamber. A bell-shaped curve of islet release was documented and reasonable yields of islets were isolated from the dog pancreas [11]. The effectiveness of the process has been confirmed by others [18, 19]. We have utilized this method for all our subsequent rat transplant studies and have recently presented a standardized protocol for the process [20].

Further examination of the initial digestion-filtration process clarified that, while it was more efficient, it was too cumbersome and inefficient for mass human islet isolation. We began a series of modifications designed to increase the efficiency and convert the process from a discontinuous to a continuous digestion-filtration process. The result of this effort is the auto-isolator [14]. This device has 5 stainless steel screens for the pancreatic fragments. It is connected to a reservoir of enzyme which is pumped into the screens and across the tissue fragments. Automatic mixing of the tissue assists the islets in passing through the screens and out of the auto-isolator where they are collected in horse sera at 4°C to reduce continued enzyme activity. The combination of all the modifications thus far described have resulted in sufficient yields of islets to permit successful autotransplants in dogs [14, 16]. Our longest graft survival is 2½ years and the recipient is still normoglycemic, although glucose tolerance tests are not completely normal. Subsequent studies have confirmed that sufficient islets are released by this method such that over 90% of single-donor, single-recipient islet autotransplants in dogs have been successful.

Lacy's group [21] is evaluating a novel way to retain the pancreatic fragments while permitting collagenase to release islets. They are using the universal fastener, Velcro® to hold onto the fibrous portion of the gland. This method seems to yield more intact islets than the auto-isolator which yields intact islets, islet fragments, and islet cells.

One of the primary problems with collagenase isolation of islets is the collagenase enzyme mixture itself. We have been unable to correlate the analysis of enzyme concentrations in any given lot of collagenase with successful islet isolation. These enzymes are the result of fermentation of *Clostridia histolyticum* bacteria. There is minimal control of the nutrient mixture used in this fermentation process and thus little control over the resulting enzyme mixture recovered from any given batch. Trial and error has been our only method of determining an effective lot.

Being unable to understand, let alone control, the variables involved in the islet isolation process, we began observing a common result of our attempts to isolate intact islets, i.e., pancreatic single cell preparations. Since we could not precisely control the digestion process to give pure, intact, isolated islets, we began exploring the possibility of purifying islet cells from pancreatic single cells. The use of trypsin or dispase to make single cell preparations of fetal or neonatal pancreas or islets had been available for some time. Moscona [22] described selective aggregation of different cell types under rotational tissue culture conditions. We evaluated the combination of pancreatic single cells and rotational tissue culture for purifying islet tissue. The original trypsin digestion of the pancreas was done

by hand using serial digestions in an Erlenmeyer flask. Between each digestion, the pancreatic fragments were repeatedly forced through a 14-gauge needle increasing the mechanical disruption and producing viable single pancreatic cells. Ficoll density gradients only partially purified the islet cells from this mixture. Rotational tissue culture using micro-Fernbach flasks demonstrated that cellular aggregates re-formed in several hours. These were solid, cellular structures by 4 days of culture and were predominantly islet cells [17]. These islet aggregates were called pseudo-islets since they did not have normal islet structure after reaggregation. Modifications of this technique have led to a standardized method of pseudo-islet formation [23]. We have recently documented by extraction studies and immuno-peroxidase staining that pseudo-islets consist of over 90% islet cells, contain all 4 islet cell types, and release insulin in vitro [24]. While the original aggregation by islet cell type is random, the different cells seems to migrate within the aggregate to domains of identical cell types with time in culture. Moscona observed this process with other cell types and called it "sorting out." While this technique established the feasibility of this approach, it would have taken 20 technicians and 2,000 flasks to prepare cells from a human pancreas. Automation was obviously needed.

Automation of this approach was begun by using trypsin in place of collagenase in a digestion-filtration screen adopting a process of serial digestion that had been used for islet isolation [11]. Ficoll was still found inefficient for obtaining islet cell purity. While we obtained islet tissue by producing pseudo-islets, the yield was still low. Larger micro-Fernbach flasks permitted many more cells to be cultured. Yet, it was obvious that a major change in technology was needed to make the pseudo-islet approach feasible for human islet cell isolation.

The development of the auto-isolator provided this opportunity. Having observed this device working well for intact islet isolation, and having seen that the digestion-filtration process would work for pancreatic single-cell preparation, modification of the auto-isolator for large-scale, single-cell production was begun. Initially, the pore size of the screens had to be reduced to permit only single cells to escape from trypsin digestion of the pancreatic fragments. The Ficoll gradients remained an ineffective method of purification. We learned of the Beckman Elutriator which separates cells by size. Fortunately, the exocrine cells are nearly twice as large as islet cells. Initial trials using the elutriator demonstrated that the larger exocrine cells were trapped in the rotor chamber, while the smaller islet cells passed through and were collected, showing the feasibility of this approach for islet purification. This machine was designed to handle aliquots of cells with equilibration time allotted within the chamber so that the 2 cell populations could separate. This was impractical for isolating the over 10^9 cells that were isolated from the canine pancreas. By setting a particle size intermediate between islet cells and exocrine cells, we were able to obtain fairly pure islet cells with the rotor when it ran as a continuous model. Since we did not permit equilibration of the different cell types, we were nevertheless still losing islet cells that were caught with the exocrine cells. However, we have now determined that successful pseudo-islet autotransplants can be done in pancreatectomized dogs using this technique [25].

With this preliminary success we began investigating how the other components of the digestion process might affect the single-cell isolation. Pilocarpine, pancreozymin, and secretin also increased the yield of islet cells as had been noted with intact islets. Using the auto-isolator, elutriator, and rotational tissue culture to form pseudo-islets, we found that 25% of the animals receiving autotransplants became normoglycemic [25]. The recipients that did not become normoglycemic or ones that reverted to hyperglycemia survived without insulin for prolonged periods suggesting that they received suboptimal islet tissue dosage. This process of islet cell isolation results in very pure islet tissue for transplantation studies, but gives a marginal amount of islet mass for a normal metabolic response.

Islet Tissue Purification

The next step in the islet tissue isolation process is the purification step. Sedimentation is the easiest method of purification but is also the most inefficient. Compared to sucrose or albumin, Ficoll gradients [6, 26, 27] proved the most effective in early transplantation studies [28]. This approach has been modified by employing green-filtered light to identify islets for hand-picking [29]. Ficoll gradients remain less efficient for human or dog tissue than for rodent tissue. We have documented the efficiency of Ficoll gradients regarding islet concentration [11, 14]. As discussed, manipulation of the exocrine enzyme concentration by pilocarpine, pancreozymin, and secretin reduces the density of the exocrine cells so that they migrate higher in the Ficoll gradient along with the islets. Thus, while exocrine degranulation increases islet yield, it decreases the ability to purify the islets by density gradients [14]. We have tried other density gradients including Percoll, Ficoll-Hypaque, and Metrisamide, but none is superior to Ficoll.

In an attempt to improve the purification process,

we have been exploring a new electrophoresis device developed by the McDonnell Douglas Corporation. Preliminary trials have demonstrated the feasibility of partially separating individual islet cell types from each other. We also have a suggestion that Ia-containing immune cells can be separated from other immune cells such as T cells using this apparatus. This electrophoresis device provides a new and potentially powerful purification process since it can separate over a billion cells in an hour and a half. The fluorescent activated cell sorter (FACS) has been shown to be able to partially purify islet cells [30]. Yet it can only process 10^6 cells per hour. Successful clinical islet transplantation would most likely require 5×10^9 purified cells.

An arrangement with McDonnell Douglas Corporation and NASA recently gave us a unique opportunity to test the device at zero gravity in space. Isolated, cryopreserved canine islet cell preparations went on board the space shuttle, *Challenger*, from August 30, 1983, to September 5, 1983. Viable islet cells returned that released insulin into the media after electrophoresis in space. Additional evaluation is in progress.

Another alternative approach in purifying islet tissue is the use of monoclonal antibodies directed against islet tissue. Schlossman [31] developed a technique for specific enrichment of a cell population based on the affinity of certain cells for antibody contained in a column. Wysocki [32] described a simpler technique called panning which involves binding the antibody to the surface of plastic dishes. The cell preparation to be purified is either passed through the column or incubated in the prepared plates. The antibody complexes with the appropriate cell types and retains them. If mouse monoclonal anti-dog or anti-human islet antibody is developed, then exocrine cells, lymphocytes, and macrophages will be eluted retaining the islet tissue. If exocrine antibody is developed, then the islet tissue and others will be elutriated with the exocrine cells retained. If antibody to the donor immune cells (anti-Ia or anti-dendritic antibody) is used, these cells can be eliminated if the preparation is in a single-cell form. By changing the solutions, the antibody complexing with the desired cell type will release its antigen, permitting recovery of the retained cells. If one has been able to produce a cytotoxic antibody for an undesirable type of cell, then incubation with complement can eliminate this cell type, resulting in purification. We have recently been producing monoclonals that have shown positive reactions to pancreatic cells that were harvested from the auto-isolator. One clone has suggested cytotoxicity to exocrine cells [33]. Many others are showing reactivity to islet tissue by cell-surface immuno-fluorescence, immuno-peroxidase staining, as well as by ELISA testing. These have been compared with 2 reference antibodies given by Eisenbarth (personal communication). Additional work is in progress to confirm that these antibodies have sufficient specificity to function as purification reagents and to determine the required quantity of antibody. Monoclonals against Ia or dendritic cells have thus far demonstrated their usefulness in the immunoalteration studies already described. It is hoped that, as purer islet preparations are available, more specific monoclonals can be produced that will be effective islet purification reagents.

Evaluation of Table 1 demonstrates the broad approach that must be developed in order to reach the goal of clinical islet transplantation trials. As we understand more of the process of islet tissue isolation, we can more effectively use this methodology in obtaining large quantities of pure, viable islet tissue for transplantation trials.

Islet Tissue Transplantation

The unique character of the islet organ as a functioning unit offers advantages in transplantation over other, more complex organs. In vitro manipulation of these grafts permits transplantation without immunosuppression. However, this graft also has to develop its own blood supply, leaving it perhaps more vulnerable to destruction during the engraftment process than an organ whose vascular anastomosis is immediate. The type of islet preparation also influences the fate of implantation, the sensitivity to rejection, and the effect on the host tissue. Table 2 describes approaches to islet transplantation focusing on the islet preparation, the transplantation site, and the prevention of rejection.

Islet Preparations

There is a wide range of preparations labeled islet transplants [3, 4]. All of the islet transplants performed in patients to date have not been true islet transplants. Rather, they have been various forms of partially digested pancreatic fragments which have essentially been unsuccessful. It would seem helpful to accept definitions of these preparations to minimize confusion as this field expands. Referring to Table 1, I would propose the following definitions:

a. Islets—Isolated and purified islets that are intact or minimally fragmented containing the normal islet cell types for any given species.

b. Islet cells—Isolated and purified individual islet cells with minimal contamination of other non–islet cell types. These may have been prepared by

Table 2. Approaches to islet transplantation.

Islet preparations
Islets
Islet cells
Pseudo-islets
Pancreatic fragments
Major fragments
Micro-fragments
Transplantation sites
Autologous sites
Intraportal
Intrasplenic
Mesenteric
Omental
Intrahepatic
Intraperitoneal
Heterologous sites
Subcutaneous
Intramuscular
Renal capsule
Intratesticular
Intraperitoneal
Prevention of rejection
Immuno-suppression
Immuno-alteration
Immuno-isolation

disassociating intact islets or by purification from pancreatic single-cell preparations. These preparations should be subdivided as to whether they contain the original islet cell constituent types or have been purified to a predominant individual islet cell type such as beta cells.

c. Pseudo-islets—Aggregates of islet tissue formed from purified islet cells originating from either method of isolation. Pseudo-islet seems appropriate since normal islet architecture has been destroyed resulting in differing islet-to-islet cell arrangements that can affect their function.

d. Pancreatic fragments—Essentially all other types of islet preparations. It may be helpful to subdivide this group based on fragment size as to larger pancreatic fragment preparations and to micro-fragments which are the islet size preparations that are now being developed.

If one accepts these definitions and reviews the literature, it is appreciated that the only islet transplants published to date have taken place in the rodent [3]. There have been no islet cell transplants. The only pseudo-islet transplants are from our laboratory [25], but other investigators are beginning to evaluate this preparation [34] in confirmation of our work in dogs [11, 17, 24], pigs [35], and humans [36]. All the rest of the transplants to date in humans or larger animals have been pancreatic fragments [3, 4]. The recent work with pancreatic micro-fragments include our own [14, 16], and that of Rajotte [15], Merrell [13], Noel [37], and Alderson and Farndon [38].

Transplantation Sites

The major objective of evaluating possible transplantation sites is to identify the safest, most efficient location for islet tissue transplantation in humans. Since the level of metabolic control required to prevent the diabetic complications remains unknown, we do not know how precisely the transplanted islet tissue must mimic normality. As shown in Table 2, an easy way to divide the potential sites is with regard to the venous effluent being either autologous, back to the portal circulation, or heterologous, directly into the systemic circulation, bypassing the liver.

The first islet transplants were into the peritoneal cavity [7, 8]. However, it was soon noted in our laboratory that islet injection into the portal vein led to embolization of the islets into the liver [39, 40] where they could become well vascularized [41]. The intraportal site has become the most commonly studied location and seems the most efficient site [3]. With the feasibility of dog transplants, it became apparent that the less purified pancreatic fragments and micro-fragments were not well tolerated when injected into the portal vein [3, 14]. While some problems resulted from large fragment size, others resulted from the acute release of pancreatic enzymes into the vascular system [42]. This has also been a problem with human transplants [43]. Our attention turned to the spleen as an alternative site after successful studies in rodents [44]. This site seems effective for protecting the recipient from the vasoactive substances in the dog model [13–15, 36, 37]. Yet, there are several problems in using this site clinically.

There has been only one definitive study evaluating the effectiveness of autologous sites over heterologous sites. Brown [45] transplanted fetal pancreatic tissue under the renal capsule and showed only a marginal response. By anastomosing the renal vein to the portal vein, he made the diabetic rat become normal. We are currently evaluating other autologous sites such as the mesentery of the small intestine and the omentum which both show initial promise. The omentum has already been shown to be practical in the rodent [46]. The heterologous site most thoroughly evaluated in islet studies has been the renal capsule. This has been especially effective in evaluating rejection studies due to the easy retrievability of the islet tissue [47]. When these sites were evaluated in comparison with the immuno-privileged site of the testes, rejection times of isolated islets were found to differ depending on whether they were placed in the spleen, liver, renal capsule, or the testes [48]. Intramuscular or subcutaneous sites have not been effective in islet studies unlike the parathyroid. The intraperitoneal site has

both autologous and heterologous venous drainage depending on where implantation takes place.

The ideal site for clinical trials is one that can rapidly provide a vascular supply for the islets, autologous venous drainage to the liver of their released hormones, and easy access both for transplantation and removal of the islet tissue. Which of these sites will best meet these criteria remains to be determined.

Prevention of Rejection

The 3 topics in this section of Table 2 will be covered in detail in other sections of this symposium. The majority of the islet immunosuppression studies have been reviewed [3]. The unique ability of this cellular graft to be manipulated in vitro provides the most distinct advantage of isolated islet tissue over segmental pancreatic grafts. Thus, the concept of immuno-alteration originally suggested by Snell [49] in 1957 is rapidly being demonstrated now for islets. Immuno-alteration approaches are essentially involved with finding a selective method of eliminating the passenger lymphoid cells, the dendritic cells, from the graft prior to transplantation.

There have now been 7 ways developed to immuno-alter the graft successfully prior to islet transplantation. The first method uses room temperature culture and an injection of anti-lymphocyte serum (ALS) to the recipient [50]. The second method uses high oxygen concentrations in culture to kill the donor immune cells selectively and permit islet survival [51, 52]. The third method uses a monoclonal antibody directed against the Ia antigens located only on the donor immune cells. This antibody cultured with complement and isolated islets eliminates the donor dendritic cells, permitting successful islet allografts [47]. The fourth method uses ultraviolet irradiation to kill the donor immune cells selectively prior to transplantation [53]. The fifth method uses an anti-dendritic cell antibody in tissue culture to eliminate the dendritic cells successfully in the islets prior to transplantation (Faustman, personal communication). The final 2 methods treat the recipient to induce some sort of tolerant state so that untreated islets are not rejected. The sixth method treats peripheral blood cells of mice with Ia antibody and complement and gives these treated blood cells to the future recipient. Following this pre-treatment program, untreated islets are accepted [54]. The seventh method uses ultraviolet irradiation to treat the peripheral blood prior to its being given to future islet recipients (Hardy, personal communication). While all of this work was done in rodents, Lacy's laboratory has recently demonstrated the presence of Ia-containing cells in dog islets [55]. We are currently evaluating immuno-alteration studies in dogs. If these techniques can be applied to dogs, it seems more likely that the human response will be the same. The final answer will have to await clinical trials. All of these methods are discussed in greater detail elsewhere in the symposium.

If the immuno-alteration studies are not applicable to the human immune system, then one could still rely on immuno-isolation systems. These hybrid artificial devices utilize semipermeable membranes to prevent cellular contact and thus rejection. They are also discussed in detail in the symposium.

The remarkable recent advances in islet tissue isolation and immuno-alteration have brought us to the threshold of effective clinical trials. Investigations in each of the 5 approaches involved with the islet tissue isolation process should produce the required amount of purified islet tissue to result in successful clinical trials. Approaching the optimal islet preparation, defining the most efficient and safest site, and manipulating the tissue appropriately to prevent rejection should confirm whether islet tissue transplantation will be the ideal way to offer diabetic patients a form of therapy that may effectively prevent the complications of their disease.

Résumé

Des étapes considérables ont été franchies récemment dans le domaine de l'isolement des îlots pancréatiques et de leur immuno-altération, ce qui permet d'envisager à brève échéance des essais cliniques de greffes d'îlots. Cet article passe en revue les progrès des méthodes d'isolement, en se concentrant sur cinq modes d'approche. Il s'agit des méthodes pour modifier le pancréas in vivo, distendre et/ou perfuser le pancréas in vitro, le dissocier, isoler les îlots entiers ou les cellules d'îlots, et purifier le tissu insulaire. Nous examinons aussi trois des questions posées par la transplantation d'îlots, à savoir le choix du mode de préparation du tissu, la sélection du site de transplantation et l'évaluation des méthodes de prévention du rejet. Des progrès dans ces domaines devraient lever les derniers obstacles qui empêchent encore à ce jour les essais cliniques.

Resumen

Notorios avances han ocurrido recientemente en el área del transplante de tejido insular pancreático, específicamente en lo referente al aislamiento y purificación de suficiente cantidad de tejido insular

humano y en lo relativo a la terapia inmunosupresiva, todo lo cual coloca al transplante de islotes al borde de la iniciación de ensayos clínicos. El presente trabajo revisa los avances logrados en el aislamiento de islotes a través de cinco enfoques diferentes, incluyendo métodos que alteran el páncreas in vivo, la distensión y/o perfusión del páncreas in vitro, la sección del páncreas en pequeños fragmentos, el aislamiento de islotes intactos o de células individuales y la purificación del tejido insular. Esta revisión también está enfocada sobre tres áreas del transplante de tejido insular: la definición de la preparación del tejido insular, la ubicación de los lugares de transplante y la evaluación de métodos de prevención del rechazo. Los avances que puedan ser logrados en estas áreas deberán resolver los problemas técnicos que todavía impiden realizar ensayos clínicos.

References

1. Williams, P.W.: Notes on diabetes treated with extract and by grafts of sheep pancreas. Br. Med. J. *2*:1303, 1894
2. Banting, F.G., Best, C.H.: The internal secretion of the pancreas. J. Lab. Clin. Med. *7*:251, 1922
3. Sutherland, D.E.R.: Pancreas and islet transplantation. I. Experimental studies. Diabetologia *20*:161, 1981
4. Sutherland, D.E.R.: Pancreas and islet transplantation. II. Clinical trials. Diabetologia *20*:435, 1981
5. Moskalewski, S.: Isolation and culture of the islets of Langerhans of the guinea pig. Gen. Comp. Endocrinol. *5*:342, 1965
6. Lacy, P.E., Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes *16*:35, 1967
7. Ballinger, W.F., Lacy, P.E.: Transplantation of intact pancreatic islets in rats. Surgery *72*:175, 1972
8. Reckard, C.R., Zeigler, M.M., Barker, C.F.: Physicological and immunological consequences of transplanting isolated pancreatic islets. Surgery *74*:91, 1973
9. Scharp, D.W., Murphy, J.J., Newton, W.T., Ballinger, W.F., Lacy, P.E.: Application of an improved technique for islet transplantation in primates and rats. Transplant. Proc. *7*:739, 1975
10. Scharp, D.W., Murphy, J.J., Newton, W.T., Ballinger, W.F., Lacy, P.E.: Transplantation of islets of Langerhans in diabetic Rhesus monkeys. Surgery *77*:105, 1975
11. Scharp, D.W., Downing, R., Merrell, R.C., Nunnelly, S., Kiske, D., Greider, M.H.: New approaches in the methods for isolating mammalian islets of Langerhans utilizing the dog pancreas, In Islet Isolation, Culture, and Cryopreservation, Ferderlin, K., Bretzel, R.G., editors. New York, Thieme-Stratton, 1981, pp. 9–31
12. Downing, R. Scharp, D.W., Ballinger, W.F.: An improved technique for the isolation and identification of mammalian islets of Langerhans. Transplantation *29*:79, 1980
13. Horoguchi, A., Merrell, R.C.: Preparation of viable islet cells from dogs by a new method. Diabetes *30*:455, 1981
14. Scharp, D.W., Long, J.A., Olack, B.J., Drummer, L.: The auto-isolator: A closed digestion-filtration device for the continuous isolation of islets. (*in press*)
15. Warnock, G.L., Rajotte, R.V., Procyshyn, A.W.: Normoglycemia after reflux of islet-containing pancreatic fragments into the splenic vascular bed in dogs. Diabetes *32*:452, 1983
16. Long, J.A., Britt, L., Olack, B.J., Scharp, D.W.: Autotransplantation of isolated canine islets. Transplant. Proc. *15*:1332, 1983
17. Scharp, D.W., Downing, R., Merrell, R.C., Greider, M.: Isolating the elusive islet. Diabetes *29*[Suppl. 1]:19, 1980
18. Shibata, A., Ludvigsen, C.W., Naber, S.P., McDaniel, M.L., Lacy, P.E.: Standardization of a digestion-filtration method for isolation of pancreatic islets. Diabetes *25*:667, 1976
19. Orsetti, A., Puech, A.M., Zouari, N., Guy, C., Passebois, F.: Isolement d'ilots de Langerhans intacts au moyen de chambres de digestion filtration. J. Ann. Diabetol. Hotel Dieu [Paris], 66, 1977
20. Scharp, D.W., Long, J.A., Feldmeier, M., Olack, B.J., O'Shaughnessy, S., Swanson, C.: Automated methods of mass islet isolation. In Methods in Diabetes Research, Larner, J., Pohl, S., editors. New York, John Wiley, (*in press*)
21. Lacy, P.E., Lacy, E.T., Finke, E.H., Yasunami, Y.: An improved method for the isolation of islets from the beef pancreas. Diabetes *31*:109, 1982
22. Moscona, A.A.: Recombination of dissociated cells and the development of cell aggregates. In Cells and Tissues in Culture, Willmer, E.N., editor. New York, Academic Press, 1955, pp. 489–529
23. Scharp, D.W., Long, J., Feldmeier, M., Olack, B., O'Shaughnessy, S., Swanson, C.: Automated methods for isolating and purifying islet cells from the pancreas. In Methods in Diabetes Research. Larner, J., Pohl, S., editors. New York, John Wiley, (*in press*)
24. Scharp, D.W., Merrell, R.C., Gingerich, R.L., Greider, M.H., Britt, L.D., Downing, R., Feldmeier, M.M., Kiske, D.: The formation and characterization of canine pseudo-islets. (*in press*)
25. Scharp, D.W., Long, J.A., Feldmeier, M., Olack, B.J., Stojeba, P.: Use of the auto-isolator in large-scale production of canine pseudo-islets for transplantation. (*in press*)
26. Jonsson, A.R., Moran, N.C.: Comparison of several methods for isolation of rat peritoneal mass cells. Proc. Soc. Exp. Med. Biol. *123*:886, 1966
27. Lindall, A., Steffes, N., Sorenson, R.: Immunoassayable insulin content of subcellular fraction of rat islets. Endocrinology *85*:218, 1969
28. Scharp, D.W., Kemp, C., Knight, M., Ballinger, W.F., Lacy, P.E.: The use of Ficoll in the preparation of viable islets of Langerhans from the rat pancreas. Transplantation *16*:686, 1973
29. Finke, E.H., Lacy, P.E., Ono, J.: Use of reflected green light for specific *in vitro* identification of islets following collagenase isolation. Diabetes *28*:612, 1979
30. Pipeleers, D., In't Veld, P., Maes, E., Van De Winkel, M.: Glucose induced insulin release depends

on functional co-operation between islet cells. Proc. Natl. Acad. Sci. USA *79*:7322, 1982
31. Schlossman, S.F., Hudson, L.: Specific purification of lymphocyte population on a digestible immunoabsorbent. J. Immunol. *110*:313, 1973
32. Wysocki, L.J., Sato, V.L.: "Panning" for lymphocytes: A method for cell selection. Proc. Natl. Acad. Sci. USA *75*:2844, 1978
33. Long, J.A., Adair, W.S., Scharp, D.W.: An immunological approach to islet cell purification. J. Cell Biol. *95*:4061, 1982
34. Schroder, D., Wegner, U., Besch, W., Zuhlke, H.: Characterization pseudo-islets formed from pancreatic islet cell suspensions of neonatal rats. Mol. Cell. Endocrinol. *32*:179, 1983
35. Britt, L.D., Stojeba, P.C., Scharp, C.R., Greider, M.H., Scharp, D.W.: Neonatal pig pseudo-islets: A product of selective aggregation. Diabetes *20*:580, 1981
36. Scharp, D.W., Feldmeier, M.M., Rajotte, R.V., De Schryver, K., Bell, M.: Human pseudo-islet formation, culture, and preservation. Diabetes *29*[Suppl. 2]:18A, 1980
37. Noel, J., Rabinovitch, A., Olson, L.: A method for large-scale, high-yield isolation of canine pancreatic islets of Langerhans. Metabolism *31*:184, 1982
38. Alderson, D., Farndon, J.: Transplant. Proc. (*in press*)
39. Kemp, C.B., Knight, M.J., Scharp, D.W., Lacy, P.E., Ballinger, W.F.: Transplantation of isolated pancreatic islets into the portal vein of diabetic rats. Nature *244*:447, 1973
40. Kemp, C.B., Knight, M.J., Scharp, D.W., Ballinger, W.F., Lacy, P.E.: Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. Diabetologia *9*:486, 1973
41. Griffith, R.C., Scharp, D.W., Hartman, B.K., Ballinger, W.F., Lacy, P.E.: A morphologic study of intra hepatic portal vein islet isografts. Diabetes *26*:201, 1977
42. Traverso, L.W., Gomez, R.R.: Investigation of a pancreatic shock factor obtained from the canine pancreas. Proc. Soc. Exp. Med. Biol. *168*:245, 1981
43. Walsh, T.J., Eggleston, J.C., Cameron, J.L.: Portal hypertension, hepatic infarction, and liver failure complicating pancreatic islet auto transplantation. Surgery *91*:485, 1982
44. Feldman, S., Hirshberg, G., Dodi, G., Raiznor, M., Scharp, D.W., Ballinger, W.F., Lacy, P.E.: Intrasplenic islet isografts. Surgery *82*:386, 1977
45. Brown, J., Mullen, Y., Clark, W.R., Molnar, I.G., Heininger, D.: Importance of hepatic portal circulation for insulin action in streptozotocin-diabetic rats transplanted with fetal pancreas. J. Clin. Invest. *64*:1688, 1979
46. Yasunami, Y., Lacy, P.E., Finke, E.: A new site for islet transplantation: A peritoneal-omental pouch. Transplantation *36*:181, 1983
47. Lacy, P.E., Finke, E.H., Janney, C.G., Davie, J.M.: Prolongation of islet xenograft survival by *in vitro* culture of rat megaislets in 95% O_2. Transplantation *33*:588, 1982
48. Bobzien, B., Yasunami, Y., Majercik, M., Lacy, P.E., Davie, J.M.: Intratesticular transplants of islet xenografts (rat to mouse). Diabetes *32*:213, 1983
49. Snell, G.D.: The homograft reaction. Ann. Rev. Microbiol. *11*:439, 1957
50. Lacy, P.E., Davie, J.M., Finke, E.H.: Prolongation of islet xenograft survival without continuous immunosuppression. Science *209*:283, 1980
51. Bowen, K.M., Prowse, S.J., Lafferty, K.J.: Reversal of diabetes by islet transplantation: Vulnerability of the established allograft. Science *213*:1261, 1981
52. Faustman, D., Hauptfeld, V., Lacy, P., Davie, J.M.: Prolongation of murine islet allograft survival by pretreatment of islets with antibody directed to Ia determinants. Proc. Natl. Acad. Sci. USA *78*:5156, 1981
53. Lau, H., Reemtsma, K., Hardy, M.: Pancreatic islet allograft prolongation by donor-specific blood transfusions treated with ultraviolet irradiation. Science *221*:754, 1983
54. Faustman, D., Lacy, P.E., Davie, J.M., Hauptfeld, V.: Prevention of allograft rejection by preimmunization with donor blood depleted of Ia-bearing cells. Science *217*:157, 1982
55. Gebel, H.M., Yasunami, Y., Dieckgrafe, B., Davie, J.M., Lacy, P.E.: Ia+ bearing cells within isolated canine islets. Transplantation *36*:346, 1983