Pancreatic Islet Transplantation-Factors That Determine Insufficient Long-Term Function and Strategies to Overcome Such Limitations

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Graft 2002; 5; 86
DOI: 10.1177/1522162802005002006

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Introduction

Transplantation of isolated islets of Langerhans is being regarded as an attractive alternative treatment to current options of daily insulin injections in Type I diabetic patients. However, a successful breakthrough in this therapeutic approach suffers from limitations set by the insufficient long-term function of the islet allografts and the general shortage of human donor organs to provide sufficient islet allografts for an increasing number of diabetic patients. The International Islet Transplant Registry (ITR) states that only 8% of the 200 recipients who received an allograft between 1990 and 1997 still carry a functioning graft and are withdrawn from exogenous insulin 12 months after transplantation.[1] This stands in marked contrast to nearly 90% 1-year function of pancreatic full organ grafts.[2] This result is disappointing and indicates that clinical islet allotransplantation is still in its infancy, despite the commendable efforts of the centers involved to continuously improve results. The aim of this article is to review some major factors that may be highly influential to the poor long-term function of the islets and to address strategies that could be helpful in overcoming such limitations.

Factors Contributing to Poor Long-Term Function of the Islets

The Islet Isolation Technique

The half-automated enzymatic digestion of the donor pancreas is the common approach to release islets from the intact tissue,[3] but it cannot prevent a certain degree of damage to peripheral islet cells, including the beta cells. The new species-specific collagenases, for example, LiberaseHI (HI, human islet),[4] may act more gently than conventional collagenases on the connective tissues that separate exocrine from endocrine cells. Yet the principle of controlled tissue destruction was basically not changed while the enzymes' quality was gradually improved. Damaged islet cells may induce local nonspecific inflammatory processes by activating macrophages, which in turn may then destroy islet cells via the cytotoxic cytokines interleukin-1 or tumor necrosis factor α.[5,6] Our own experiments (unpublished data) have repeatedly shown macrophages accumulating near the islets as early as 24 h after syngeneic or allogeneic transplantation into the portal system of the liver. Thick fibrotic layers developed around such islets that otherwise would have functioned normally for more than a year. It is difficult to imagine how cell damage can be prevented during isolation. However, some very recent results may provide the solution for this problem. When a highly purified, functionally active fraction of a commercial collagenase was used,[7] instead of the usual enzyme mixture to isolate porcine islets, damage in the peripheral islet cells was decreased significantly. This was demonstrated by staining the fresh islets with fluoresceine diacetate and propidium iodide. Some in vitro experiments are now in progress to evaluate the degree of macrophage and subsequent fibroblast activation, comparing a con-

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ventional collagenase—a mixture of numerous enzymes—and the purified single enzymes. Whether such inflammatory reactions are efficiently inhibited by those "classical" drugs, which are used to suppress the recipient's immune reactions against the allogeneic islets, is still unknown.[8] Steroids certainly have this potential,[9] but for other reasons they may not be the most favorable drugs. Therefore, the main research interest should be the evaluation of the mode of action of the inflammatory processes, its strengths, and the concepts of inhibiting them, in order to significantly prolong the long-term function of islet allografts.

ISOLATION:

Enzymatic digestion of the donor organ (pancreas) to release isolated intact islets of Langerhans.

The Number of Transplanted Islets

Although the total number of islets in a human donor organ is still a matter of debate, the full transplanted pancreatic graft should have sufficient functional potential, that is, insulin-producing Langerhans islets. In our histomorphological studies, we estimated that a complete porcine organ, weighing just over 200 g, contains approximately 2.4 million islets.[10] Assuming that only 25% of all islets are available after isolation, i.e., enzymatic digestion of the donor organ, namely those that are "capsulated" to an extent of > 75% of their surface, the total number would be 300,000 islet equivalents (IEQ) for an organ section of 100 g. This represents the standard donor tissue in most laboratories. Supposing that the total number of islets is similar to the amount in the human organ, an islet allograft of 300,000 IEQ would represent a mere one-eighth of the insulin production as compared with the production potential of a full graft. This is the major disadvantage of the isolated islets compared with the intact islets within the solid pancreas. It is quite likely that the individual islets of such an islet allograft would hardly withstand the long-term stress of increased insulin production. Another problem is that functional stress can alter the immunogenicity of the isolated islets and thus increase the danger of accelerated graft rejection. For instance, rat islets that were cultured for 4 days in medium containing 4 mg/ml glucose expressed increased levels of major histocompatibility complex class I. Such islets were more immunogenic toward allogeneic CD4+ T cells via the direct and indirect pathway of antigen recognition than islets that were kept in medium containing 1.5 mg/ml glucose (our unpublished data). To decrease the functional stress, the number of islets was continuously increased in clinical transplantation, often by pooling islets from 2 or even > 8 donor organs,[8] and by treating recipients with additional exogenous insulin for a certain time period after transplantation.[8] This beneficial effect of insulin-induced normoglycemia on transplanted islets was shown with a 14-day posttransplantation exogenous insulin treatment in a syngeneic mouse model.[11] Currently, the ITR recommends transplanting ≥ 6,000 IEQ/kg body weight,[12] which would make a total of 450,000 IEQ for a patient weighing 75 kg. However, even this number may not be enough. Recently, the Edmonton Group documented their clinical success after increasing the number of islets to > 11,000 IEQ/kg body weight.[13] It is difficult to imagine how the dilemma of insufficient number of human islets can be overcome other than by isolating and transplanting xenogeneic, that is, porcine, islets.

Immunosuppression after Islet Transplantation

Allogeneic islets are either transplanted simultaneously with or after an allogeneic kidney into a diabetic patient.[1] Thus, immunosuppressive therapy was adapted to the requirements of the allogeneic kidney, assuming that the requirements for the allogeneic islets were more or less identical.[1] However, it was never thoroughly questioned whether the immunosuppressive regime suited the islets. It cannot be excluded that the reduced survival rates after 12 months may be partly due to an islet-inadequate immunosuppression. There is some evidence that tacrolimus, cyclosporine A, and steroids are diabetogenic,[14,15] at least when given in higher doses. They may be unsuitable to suppress immunoreactions against the allogeneic islets. Therefore, the Edmonton Group changed the immunosuppressive regime in their clinical islet transplantation program. They completely avoided cyclosporine A and steroids; tacrolimus was given at low dosage in combination with daclizumab, a monoclonal antibody against interleukin-2 receptor, and rapamycine.[13] On the basis of this new protocol, islet allograft recipients were still normoglycemic after the 1st observation period of 12 months. It must be taken into account, however, that other criteria of

this protocol have changed as well. For instance, the cold ischemic time of the donor organ was reduced to less than 8 h, the number of islets was nearly doubled, and the diabetic patients were transplanted at a time point when they did not yet require a kidney allograft. The long-term beneficial effects of this new islet transplantation protocol have yet to be shown by the ongoing multicenter study.

Strategies to Overcome the Limitations

Further Improvement of Enzymatic Pancreas Digestion

The problem of sufficient enzyme activity and tissue-specificity did not loose its actuality. It is disappointing that after 2 decades of research it remains unclear which component(s) of collagenase is required to successfully isolate islets. The production of Liberase[4] with specificity for human,[16,17] porcine,[18] and canine tissue[19] was a long-awaited step in the right direction; however, our experiences clearly indicate that batches of Liberase still vary remarkably in activity. This means that each new batch has to be tested in advance, which is money- and time-consuming. Some Liberase batches seem to be even highly toxic to beta cells, a problem that can be solved by adding Pefabloc®, a trypsin inhibitor, to the enzyme solution.[20,21] In purifying enzyme fractions of sufficiently high activity and in producing recombinant collagenases,[22] such problems should finally be successfully solved, resulting in long-term allograft function.

INFLAMMATION

Reaction of the body and its tissues against damaging stimuli.

Using the Xenogeneic Transplantation to Increase the Number of Islets

There will never be enough human islet allografts to treat the increasing number of diabetic patients. The most likely substitute for the human islets are the porcine islets.[23,24] Despite the infections that may be connected with xenogeneic islet transplantation,[25] the isolation of porcine islets seems to be particularly difficult and thus a number of laboratories have retreated from this task. Experiences of our group with porcine tissue over the past decade showed that

1. islets can be successfully isolated from 2- to 3-year-old hybrid pigs with approximately 5000 IEQ per gram of organ;
2. individual isolations result consistently in up to 15,000 IEQ/g organ, the equivalent of a total of 1.5 million IEQ for a donor organ of 100 g; and
3. prospective histomorphological screening of the donor organ prior to the isolation procedure is necessary.

We use only donor organs with an average islet size > 200 µm, which is consistent for 80% of the donor organs of 2- to 3-year-old hybrid pigs and 35% of the donor organs of hybrid pigs 6 to 7 months old. On the basis of this prescreening, the percentage of highly successful isolations (> 4000 IEQ/g organ) increased from 48% (without screening) to 65% (with screening) in 2- to 3-year-old pigs, and from 15% to 35% in 6- to 7-month-old hybrid pigs. The fact that a sufficient number of islets can now be repeatedly isolated, not only from old but also from young pigs (given that Liberase shows the necessary high and constant quality), is a tremendous innovation for xenotransplantation.

Islet Transplantation without a Life-Long Immunosuppressive Therapy

If one of our aims is to transplant the islets at an early stage after diagnosing the disease, the diabetic patient, the young and otherwise healthy patient, should not receive immunosuppressive therapy after transplantation, or immunosuppression should be time limited. One way to prevent the need for drug treatment is the immunoisolation or immunoprotection of the islets.[26] Particularly, the concept of encapsulating islets in biocompatible materials has recently lead to long-term success, that is, in pig islet to rat.[27] Our studies using the microencapsulation technique showed similar findings. Thirty percent of the Wistar rats became normoglycemic for more than 240 days (the longest period of normoglycemia is now > 390 days) after receiving barium-alginate-encapsulated porcine islets without further drug treatment. The porcine islets had better integrity, functionality, and viability. This may be based on the use of alginates with a higher degree of chemical purity and biocompatibility, when compared with alginates used more than a decade ago.[28,29] Although still preliminary, these data clearly show that it is possible to achieve long-term functionality encapsulating islets with-

out immunosuppression. This 1st long-term success in small animals allows us to address basic questions that may be essential for the successfully long-term islet function.

Conclusions

Transplantation of isolated intact islets of Langerhans is an attractive therapeutic alternative to the daily insulin injections necessary to treat severe forms of diabetes. Despite the remarkable success recently achieved in clinical islet allotransplantation,[13] the lack of human donor organs remains the most serious limitation in providing this type of therapy. There are no solutions to this dilemma. This limitation is in part due to the success of all pancreas allotransplantation. Xenogeneic tissue, and in particular porcine islets, could replace human tissue within the next years—at least as long as beta cells, generated from embryonic or ductal stem cells, or from immortal and engineered cell lines, are not yet available for clinical purposes. The concept of xenogeneic islet transplantation is attractive and based on (a) increasing success in isolating sufficient numbers of vital islets from the porcine pancreas, (b) increasing availability of highly purified and thus biocompatible encapsulation materials, and (c) increasing positive reports of long-term survival/function of encapsulated porcine islets in xenogeneic small animal models without immunosuppressive therapy. The last point in particular is a strong boost to all who wish that islet transplantation would become clinical routine in order to achieve normoglycemia and thus reduce or delay the onset of the secondary complications of diabetes.[30]

LIBERASE:

A mixture of enzymes to disintegrate endocrine from exocrine tissue in the donor pancreas.

Acknowledgment

Research work performed by the author's group is supported by the Federal Ministry of Education and Research funds supplied to the Interdisciplinary Center for Clinical Research (IZKF) of the University of Würzburg (research project grant number 01 KS 9603).

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