Lilly Lecture 2002

Islet Transplantation: A Brave New World

Camillo Ricordi

It has indeed been a distinct honor to be the recipient of the 2002 Outstanding Scientific Achievement Award, and I was happy to accept it, on behalf of the entire islet transplant field, as there have been many surgeons, physicians, and scientists who have worked as a team, moving this field forward. I was born the year the Lilly Lecture was established, 1957, and since then, this is the first time that the award goes to islet transplantation, representing indeed a very important recognition to all of us in the field. Therefore, I'd like to outline the major steps in the development of islet transplantation, from a hypothesis, to its clinical application, through what we call translational research, bench to bedside. At the end, some of the challenges ahead, including novel immunomodulatory strategies, tolerance induction, and the development of alternative sources of insulin-producing tissue, are discussed.

The challenge, as you may predict, is diabetes. A syndrome well known since over 3,500 years ago, even if the term "diabetes" was coined in the first century by Arataeus from the Greek "siphon" or "pipe-like." Arataeus described diabetes as "a melting down of flesh and limbs into urine." It took over 3,500 years to link diabetes to the pancreas, and until insulin was discovered, diabetes remained a devastating, deadly disease, often treated by starvation. The discovery of insulin, by Banting and Best in 1922, changed the course of history in the treatment of the disease, and suddenly children with diabetes could be offered a new chance at life. While definitely a life-saving breakthrough, insulin treatment still cannot fully prevent chronic complications, and intensive insulin treatment to improve metabolic control has paralleled an increased risk of severe hypoglycemia (1). The hypothesis that replacement of the endocrine pancreas by transplantation of insulin-producing tissue could represent a better and more physiologic solution compared with exogenous insulin treatment is definitely not novel (2,3). Pancreatic islets can clearly restore metabolic control after transplantation, preventing the development of chronic complications. Islets not only make their own insulin, but they are capable of a closely controlled and perfectly timed insulin release. If an islet transplant is not destroyed by the recipient's immune system, the insulin-producing cells could keep glucose levels in the normal range and function for an entire lifetime.

The English surgeon Watson Williams was the first to attempt transplantation of pancreatic fragments in 1893, from a sheep to a 15-year-old boy. This was a time in which it was still under debate whether the pancreas was capable of producing a sugar-destroying substance. This attempt, which was in fact also the first islet xenograft, preceded the discovery of insulin by almost 3 decades (4). In 1929, Brancati, in Italy, was able to demonstrate preserved islets 1 month after transplantation of pancreatic fragments, after he had induced acinar athrophy, by pancreas duct ligation. However, the real father of islet transplantation is Paul E. Lacy, who was the first to describe the method for isolation of islets from rodent pancreata in 1969 (2) and who a few years later also first demonstrated successful islet transplantation in rodents (3).

Why did it take so long to get human islet isolation developed to a level sufficient to obtain enough islets from human pancreata to reverse diabetes following transplantation in a recipient? It was because the human pancreas is very different from the pancreas of rodents and small animals, where islet isolation methods were initially developed. Lack of suitable reagents for enzymatic dissociation of the human pancreas, together with pancreas consistency, tissue composition, and donor variables, imposed a formidable challenge to the extraction of the hundreds of thousands of islets that are distributed throughout the human pancreas. In the 70s and 80s, human islet isolation techniques were highly traumatic to the islet tissue and equally ineffective. Everything that could be found in a kitchen was tried, from mincing with scissors, to food processors, blenders, choppers, forcing the pancreas through needles of decreasing sizes, and using modified meat grinders. When in 1984 I proposed a radically different approach to my mentor in Milan, Professor Guido Pozza, he gave me the unique opportunity to develop the concept by joining the best center for islet isolation, which in the 80s was Washington University in St. Louis, and more specifically the laboratories of Paul E. Lacy and his associate, David Scharp. At that time, the St. Louis group was still using a meat grinder, renamed the "tissue macerator," that reminded me of one of those used by Eli Lilly to grind pancreata in the early days of insulin production (5). Initially, the idea of loading the entire pancreas in a chamber and obtaining free islets at the outlet without any significant mechanical disruption was

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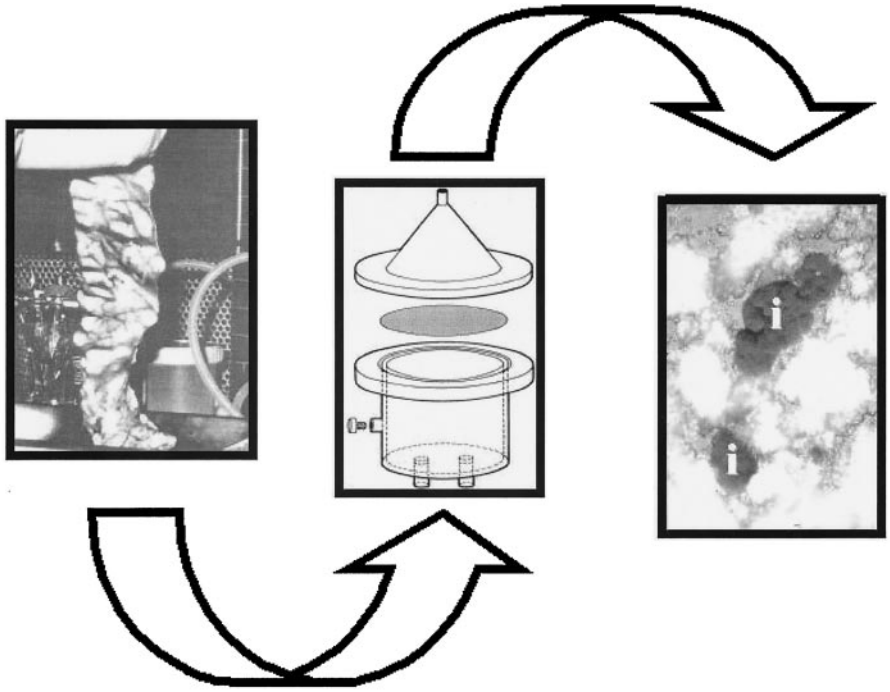

FIG. 1. The concept introduced by the automated method was to progressively disassemble the pancreas, after enzymatic intraductal perfusion (left), into fragments of decreasing sizes, until cell clusters of the volume range of islets are released. A constant flow through a digestion chamber (center) allows for the released islets to pass through a screen (right, i: islet stained with dithizone) and be collected in separate compartments, where further enzymatic action is blocked by cooling and dilution.

considered a little heretical (Fig. 1), and I was assigned a different project on porcine islet isolation, using the "tissue macerator," where I learned to appreciate even more the fragile characteristics of porcine pancreatic islets. Over 1 year later, in 1986, the initial transition between the "tissue macerator" and the automated method occurred. We initially tested the approach using rodent and porcine pancreata, until my big chance presented itself, when a research human donor pancreas shipped to us by NDRI (then the National Diabetes Research Interchange) was discarded because of poor quality and prolonged ischemia time. I still remember the adrenaline surge I experienced when Dr. Lacy allowed me to recover the organ from the trash container and test the new method. However, my best accomplishment in 1986 was not the introduction of the automated method for human islet isolation but was actually convincing the most beautiful girl in St. Louis, Valerie Grace, to marry me. On the laboratory front, the automated method rapidly replaced all previously tested procedures (6,7). Briefly, the following steps are required for human islet isolation with the automated method (Fig. 2). Initially, the human pancreas is carefully dissected from the duodenum. Main and accessory ducts are identified, clamped, and divided. A cannula is then placed in the pancreatic duct to allow for an enzyme blend that includes collagenase to distend the organ. The pancreas is placed into a chamber, and a continuous digestion process is started to progressively disassemble the organ into frag-

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Automated Method for Isolation of Human Pancreatic Islets

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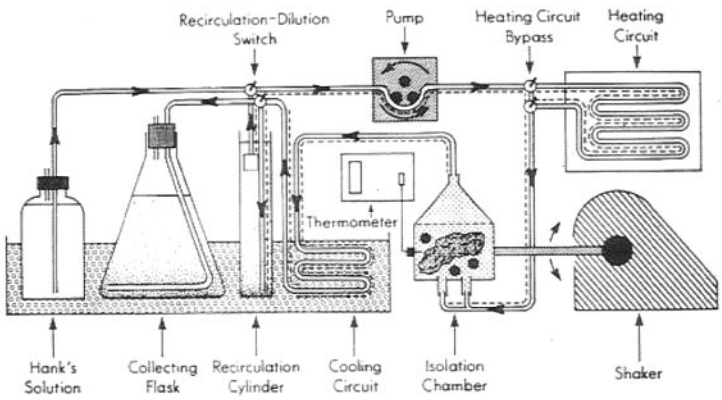

FIG. 2. Schematic diagram of the automated method, as it was originally published in *Diabetes*.

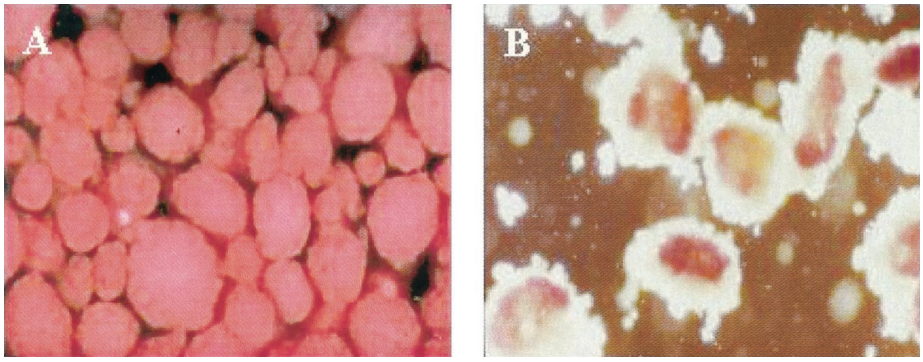

FIG. 3. Human islets after density gradient separation. The less dense layers generally contain the highest purity in islets (*A*), while "mantled" islets can be retrieved in layers with a relatively higher density (*B*).

ments of decreasing sizes. A heating circuit activates the enzymes in the chamber, while continuous flow through the chamber allows the islets that are progressively released to be collected. Cooling and dilution protect the islets leaving the chamber from further enzymatic action. This process can be monitored using transparent chambers and taking samples from the chamber outlet. At the time of development of the method, dithizone (diphenylthiocarbazone) was not yet introduced to confer the characteristic red stain to the islets (8), and identification of the islets to monitor the progression of the digestion process sometimes required considerable experience. At the end of the process, the pancreatic cell clusters are collected in separate containers, while the fibrous network of ducts and vessels is retained in the chamber by the screen. The final step is a purification step that utilizes density gradients to separate the small islet fraction from the predominant exocrine and nonislet tissue. For density gradient centrifugation, we initially used specially made large syringes, with a removable plunger, that could fit the lab's refrigerated centrifuge. More recently, specialized cell processors (COBE 2991 Cell Processor; COBE, Lakewood, CO) have been used for the purification step (9,10). After purification, the top layers generally contain highly purified islets, while less pure islet layers and "mantled" islets generally migrate within more dense interfaces. "Mantled" islets, which are islets surrounded by a thin rim of exocrine tissue (11), are not undesirable, and in fact, there is no need to aim at perfectly purified islets (Fig. 3). The main concern is to maintain the volume of tissue infused in the recipient portal vein, within acceptable limits, generally less than 5–7 ml.

The younger the donor age, the more difficult it is to obtain perfectly purified islets (12). In the case of a few-months-old pancreas donor, the entire pancreas is so small, there is no need to perform the final purification step, as most cell clusters contain islets and probably islet precursors. The automated method has been significantly improved over the past 15 years, thanks to the collaboration among key groups of investigators in St. Louis, Miami, Milan, Giessen, Edmonton, Minneapolis, and others, who should collectively share the credit for the progress of the method as we are using it today in most clinical trials of islet transplantation.

Once we obtained purified human islets, we needed to prove that they were viable, functionally competent, and able to reverse diabetes, initially in animal models. For this purpose, immunosuppressed murine recipients (13) or, more frequently, athymic mice (14,15) that are unable to reject human tissues, can be used. Following transplantation of aliquots of human islets beneath the renal capsule of diabetic mice, it is in fact possible to document normalization of glucose levels. In addition, a nephrectomy of the kidney bearing the islet graft will result in a return to the diabetic state, confirming that the human islets were responsible for maintaining normoglycemia. In addition, this allows us to perform histochemical studies of the renal subcapsular islets, for example, staining for glucagon, insulin, and somatostatin, to assess morphologic integrity of the transplanted islets. The human islet transplant in the nude mouse model system remains today the more reliable test to assess in vivo the functional competence of human islet preparations (16,17).

Proof of function in humans has been definitely more challenging than proof of function in nude mice. In 1988, I returned to Milan to set up and start the clinical islet transplant program at San Raffaele Institute, but before we were ready to start, transplant pioneer Thomas Starzl asked me to join the University of Pittsburgh to head the cell transplant program, giving me 24 h to decide, and 1 week to move. In Pittsburgh, I met Daniel Mintz and Rodolfo Alejandro, who also were invited to join Starzl's Team, just in case one of us was unable to deliver the best islet isolation technology. In the record time of 2 months, we set up the Pittsburgh human islet isolation lab, while the news arrived that the St. Louis group had a patient off insulin following human islet transplantation. This was indeed encouraging, proving that islets obtained with the automated method could reverse diabetes, but we were disappointed by the limited time of function, as we learned that the transplant failed shortly after the reported 12 days of insulin independence (18).

On 10 January 1990, we performed the first of a successful series of six islet allografts that resulted in prolonged insulin independence in patients who had the entire pancreas surgically removed. This was the first unequivocal evidence of long-term reversal of diabetes following human islet allotransplantation, with up to 5 years' insulin independence (19–21). The success of the Pittsburgh trial introduced the use of FK-506 (Prograf) in human islet allotransplantation in a steroid-free immunosuppressive protocol. In these initial cases, the islets were transplanted immediately after isolation. The unprecedented results of long-term insulin independence in the recipients were considered a breakthrough that resulted in a significant enthusiasm in the field, with several centers beginning or resuming clinical islet transplant protocols.

Within a few months, Milan, Miami, Edmonton, St. Louis,

and Minneapolis reported successful islet transplants in patients with type 1 diabetes (4). During the same period, we demonstrated for the first time histologic survival of intrahepatic human islets following liver biopsies (22–24). In subsequent observations, we have shown persistence of intrahepatic islets for years, indicating that islet cells can indeed survive long term in the human liver in the absence of rejection or recurrence of autoimmunity.

This indeed turned out to be a formidable challenge in the 90s. In fact, when we started performing islet allografts in patients with type 1 diabetes, the success rate decreased significantly (20). The international registry clearly showed that a significant portion of the islet transplants failed within the first 2 months, and only ~35% of them were still working at 1 year, and an even inferior rate of insulin independence of about 10%.

In 1993, I was recruited by Dr. Mintz to the University of Miami to take the lead of the cell transplant center and eventually of the Diabetes Research Institute. During these challenging years in islet allotransplantation, encouraging results came from Dr. Sutherland's group in Minnesota, confirming the superiority of the automated islet isolation method in obtaining higher numbers of islets that were successfully autotransplanted (25). In the meantime, Rodolfo Alejandro in our center and others established that the few islet allografts that continued to function long term in patients with diabetes were able to normalize the HbA_{1c} in the absence of severe hypoglycemic episodes (26), revitalizing our efforts to get the job done. An important advancement was accomplished by the production of enzyme blends with low endotoxin content so that finally, in 1996, all reagents used in human islet isolation could be considered within FDA guidelines (27).

The role of endotoxins in islet graft failure was clearly defined in recent studies by Luca Inverardi's group (28), indicating that the relevance of endotoxin contamination was beyond safety, as islets could adsorb endotoxins through the LPS receptor and evoke inflammatory events at the transplant site.

Using the isolation technology so far developed, including the use of endotoxin free reagents (27) and improved peritransplant recipient treatment, the Giessen group headed by Dr. Hering and Dr. Bretzel was able to completely prevent early islet graft loss with 100% initial graft function and a 40% insulin independence at 1 year after transplantation (29). A remarkable series of islet transplants was also subsequently reported by the Milan group, with achievement of insulin independence in 60% of the recipients of human islet allografts (30), and an additional major step forward in islet transplant outcomes came when James Shapiro and the Edmonton group introduced a steroid-free, rapamycin-based protocol of immunosuppression, with rapid infusion of islets immediately after isolation, and using two or more pancreas donors to obtain sufficient numbers of insulin-producing cells (31). Thirty-three patients became insulin-independent following this protocol, with a high rate of insulin independence that is still over 70% at 2 years' follow-up. The price for this more effective but also more powerful immunosuppressive protocol was paid in terms of complications, reminding us that the final goal in islet transplantation remains the elimination of chronic recipient immunosuppression.

After the Edmonton Trial, the islet transplant field still faced significant challenges. We have recently introduced a novel islet culture protocol to avoid the requirement for immediate islet transplantation, as originally described in the Edmonton trial (32,33). Besides logistics, several advantages of culture include improved safety, decreased immunogenicity, and the ability to achieve therapeutic levels of immunosuppression in the recipients before the islets are actually infused.

Another recent improvement in islet transplant outcomes was obtained thanks to the introduction of oxygenated perfluorocarbons in pancreas preservation as originally described by Kuroda and Matsumoto in experimental models. We have already successfully tested pretransplant culture and PFC for pancreas preservation in our most recent Miami Trial of islet transplantation (34). We also used an immunosuppressive strategy similar to that introduced by the Edmonton group.

Fifteen consecutive patients became insulin-independent for up to 1 year using this protocol, including three patients transplanted by Dr. Goss, Dr. Brunicardi, and collaborators at Baylor College of Medicine in Houston, with islets prepared in Miami and shipped to Houston (35). For the first two transplants, our team went to Houston to assist with the islet infusion. In our experience, it took generally two infusions to fully reverse diabetes, even though a few patients were able to discontinue insulin after a single islet cell infusion. Continuous glucose monitoring data indicated dramatic improvement following islet transplantation and completely off insulin (Fig. 4). The mean amplitude of glycemic excursions also normalized, even after the first islet infusion. This islet transplant procedure is performed in interventional radiology, under local anesthesia. A cannula is placed in the liver through percutaneous catheterization of the portal vein, and the islet preparation is loaded in a blood transfusion bag that is then connected through an IV infusion set. The procedure at this point is very simple, being just an infusion by gravity, similar to a blood transfusion, with the difference that the islets are infused in the portal vein. We have introduced the bag infusion technique to avoid possible complications that occasionally occurred when islets were infused using a syringe. The bag allows to control the portal pressure continuously. Since the infusion occurs by gravity, the portal pressure can never be higher than the height at which the bag is placed during the infusion.

Insulin independence following islet transplantation has now been achieved by several groups in North America and Europe. Besides, of course, the Edmonton team and our experience in Miami and Houston, the NIH has started a program in collaboration with our institute in Miami. Minneapolis also has an excellent program. Philadelphia and now also Boston, St. Louis, and Seattle have started islet transplant activities, as well as Zurich, Giessen, Geneva, and Milan in Europe. All of these centers have achieved insulin independence following islet transplantation in type 1 diabetes over the last 12-month period.

One of the major challenges that we are facing in the immediate future is to develop strategies for recipients' immunosuppression and immunomodulation with agents that are not toxic to the transplanted islets. In fact, we know well that steroids can induce graft failure (36), but

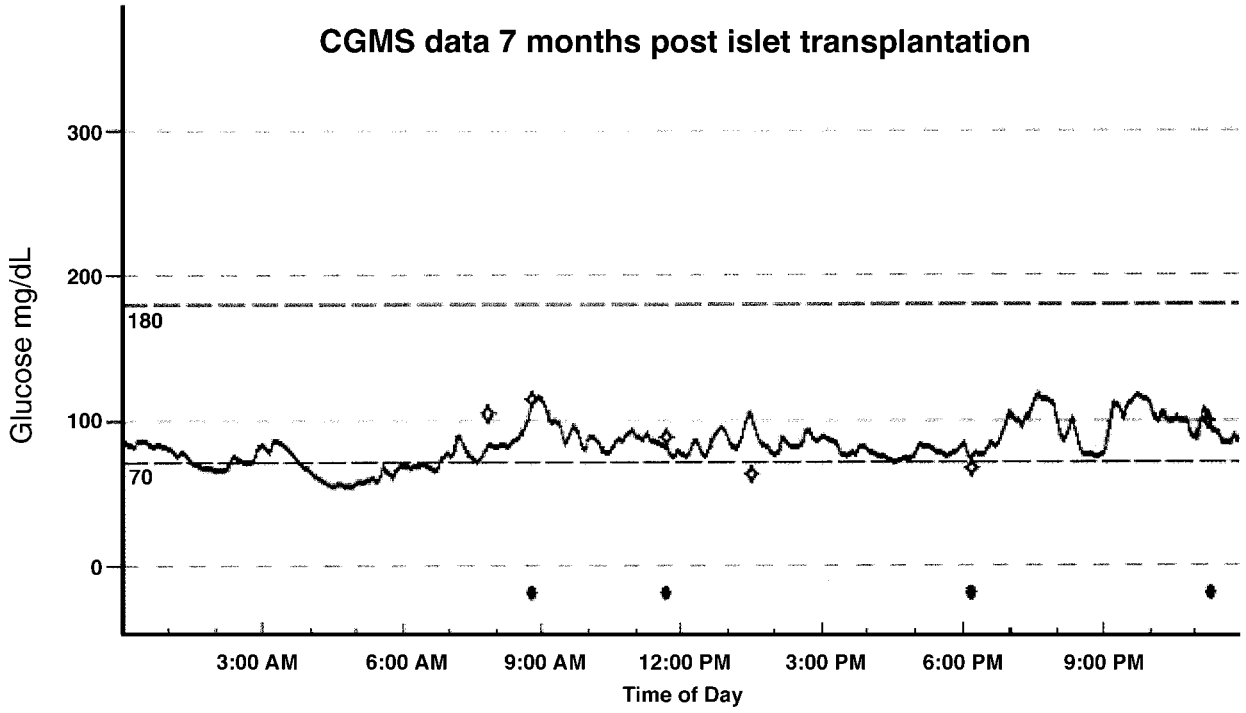

FIG. 4. Continuous glucose monitoring system (CGMS) data from a patient with brittle type 1 diabetes, who was insulin-independent since receiving an islet transplant 7 months before the test.

we also know that even calcineurin inhibitors alone, such as FK506 (37) or cyclosporin, can be deleterious to islets, even in the absence of steroids. The diabetogenicity of calcineurin inhibitors in steroid-free protocols was recently confirmed by our group in clamp studies performed in liver transplant recipients treated with low-dose maintenance FK506 or cyclosporin A (38). The definition of immunosuppressive/immunomodulatory strategies that do not include diabetogenic drugs will be critically important to improve long-term islet transplantation outcomes and to minimize the number of islets required to reverse diabetes. In fact, in studies coordinated by Norma Kenyon, we have clearly shown that the use of a nondiabetogenic immunosuppressive strategy, based on co-stimulatory blockade with anti CD154 monotherapy, can parallel progressive improvement of posttransplant islet function. First- and second-phase insulin secretion in response to intravenous glucose tolerance tests was observed to improve with time during the first year posttransplant, reaching levels that were comparable with those of a native pancreas.

Clearly, for islet transplantation to become widely applicable in the treatment of diabetes, we have to develop better strategies for tolerance induction to the transplanted insulin-producing cells, which will be clinically relevant and will not introduce significant risks for the recipients. The concept of inducing tolerance to transplanted tissues and organs has been well known since the 50s, when Sir Peter Medawar was awarded the Nobel Prize for his pioneer research, demonstrating that the infusion of donor bone marrow–derived cells into neonatal recipient can result in a state of donor-specific nonresponsiveness (39). This allowed later transplantation of organs and tissues from the same donor without immunosuppression. Anthony Monaco has pioneered the clinical application of this concept in adult recipients of kidney allografts (40) but was unable to duplicate the success previously obtained in animal models.

We have demonstrated how the induction of donor-specific tolerance in experimental models of donor bone marrow transplantation was associated with repopulation of cells that appear of dendritic morphology and that could be detected in all recipient tissues analyzed, including the skin, brain, and thymus (41). These antigen-presenting cells were thought to potentially play a key role in the process of tolerance induction (42).

More recently, Alberto Pugliese at our institute has shown that antigen-presenting cells with a dendritic cell phenotype express insulin and other islet autoantigens (43). Dendritic cells could therefore be involved, both in the process of tolerance induction to alloantigens as well as promoting self-tolerance. Tolerance induction in type 1 diabetes is critically important, not just to protect transplanted islets from immune rejection (44,45) but also to prevent recurrence of autoimmunity. It is, in fact, well known that bone marrow transplantation and chimerism induction can either protect from or transfer diabetes (46–48). These strategies have a dual protective effect, with the potential for inducing both tolerance to the transplanted islets and restoration of self-tolerance, therefore protecting from recurrence of autoimmunity.

The association of chimerism with organ transplant acceptance and tolerance in the clinical setting has been highly debated in the last decade. What we described in 1992 was that after successful clinical organ transplantation, donor bone marrow–derived cells migrate into recipient tissues (49), similarly to what we have observed in rodents (41). A two-way traffic of immune cells was unveiled, not just recipient immune cells migrating into transplanted organs but also an opposite migration of

bone marrow–derived cells that are contained in transplanted organs and that following transplantation migrate into recipient tissues (49). If these two immune systems learn to coexist in what is called "stable chimerism," tolerance can be achieved.

During my Pittsburgh years with Drs. Starzl, Trucco, and collaborators, we described this phenomenon in patients who were long-term transplant recipients and discontinued the use of immunosuppression without rejecting the transplanted organs. When tested through blood samples and tissue biopsies, these tolerant patients always revealed the presence of donor-derived bone marrow cells in their tissues (50,51). We have continued donor bone marrow infusion studies for tolerance induction in Miami, in collaboration with the organ transplant program, headed by Drs. Tzakis and Miller. The results have been very encouraging (52), especially in kidney transplant recipients, who received donor bone marrow infusions at the time of the kidney transplant. In these patients, we have observed protection from chronic rejection, now over 6 years after kidney transplantation (53–55).

Nonetheless, for tolerance induction in clinical islet transplantation, we most likely need to increase the levels of chimerism, since in our initial experience in trials of islet and donor bone marrow progenitor cell infusions, we were unable to discontinue immunosuppression without losing the islet grafts (56). However, we cannot obtain higher level of chimerism using the harsh conditioning regimens that have been tested in animal models and that were mainly based on lethal or sublethal radiation conditioning of the recipients to eliminate most of the recipient bone marrow and make space for the donor bone marrow cells. Clearly, the risks associated with these approaches are not acceptable for treatment of diabetes, and we now need to develop safer strategies.

In this direction, we have recently introduced a very promising and safe approach to increase the level of chimerism and induce tolerance without the risks of harsh radiation conditioning. This consists in a novel targeted bone marrow minimal conditioning strategy, based on the delivery of minimal doses of radiation, specifically targeting the bone marrow, through a bone-seeking compound, Lexidronam, coupled to a radioisotope, Samarium. Using these molecular "smart bombs," we were able to obtain high levels of chimerism with a minimal, safe radiation dose (57). This very promising strategy has already been successfully tested in experimental skin transplantation and is now under evaluation in islet transplantation.

Selective monoclonal antibodies could also play a key role in the process of tolerance induction (58). Some of these antibodies that are currently under investigation target the so-called co-stimulatory pathways; co-stimulation is required for T-cell activation. In the absence of co-stimulation, tolerance can be more easily achieved. We have extensively studied one of these agents, anti-CD154, in islet transplantation. Blockade of the CD40–CD154 interaction with this antibody has multiple effects not just at the level of B- and T-cells but also at the level other cells that could be involved in early recognition and destruction of the transplanted islets, such as dendritic cells, macrophages, and endothelial cells. Aldo Rossini's group was the first to show tolerance induction to islet allografts in mice using anti-CD40 ligand (59), and more recently, he applied this strategy in combination with donor-specific cell transfusions. More recently, Dr. Luca Inverardi and collaborators in our group have shown the efficacy of this strategy also in the NOD autoimmune diabetes model system (60).

We have demonstrated the preclinical efficacy of anti-CD154 monotherapy in nonhuman primates. These studies were headed by Norma Kenyon in collaboration with David Harlan. For the first time, we had an agent that could be used as monotherapy to prevent islet transplant rejection in a very relevant preclinical model (61,62). In addition, as I mentioned before, this agent was not diabetogenic and allowed for improvement of islet function with time. Another very promising strategy is the combination of co-stimulatory blockade with blockade of the CD45 pathway. CD45 plays a crucial role in the regulation of lymphocyte activation signals, and preliminary studies we have performed with Giacomo Basadonna and collaborators at Yale (63) have shown significant promise. Several other monoclonal antibodies and tolerogenic strategies are currently under evaluation in preclinical model systems, and I am confident that very soon we will see some of them in clinical practice.

To catalyze and synergize efforts in tolerance induction, including efforts in islet transplantation, NIH recently established the Immune Tolerance Network under the leadership of Jeff Bluestone. The first multicenter trial of islet transplantation to reproduce the results of the Edmonton Group is currently in progress, and in the near future we will see tolerogenic strategies being tested through this unique resource.

Finally, we will need to develop new sources of insulin-producing tissue. In fact, if tolerance induction becomes a reality, we could only treat less than 1% of patients with diabetes, based on the current availability of human donor pancreases. To treat the majority of patients with diabetes, we need to develop alternative strategies (64). Initially, we could surely improve and maximize pancreas utilization from cadaver donors, which is currently very inefficient. We should also try to minimize islet loss before and after isolation so that we could use single donors and maybe even one donor for multiple recipients. If we are successful in expanding islet β-cells in vitro, we may be able to use a portion of the pancreas from living donors. However, the final answer will probably come either from animal islet sources or from genetically engineered human cell lines, such as those Christopher Newgard eloquently outlined in last year's Lilly lecture, or by using human islet cells derived from embryonic stem cells or even adult stem cells.

Several strategies to improve in vivo function after transplantation are based on maximizing islet survival and prevention of apoptosis, such as by induction of heme oxygenase-1, as shown by Pileggi, Inverardi, and collaborators (65). Another novel strategy includes the use of protein transduction domains. Dr. Pastori and collaborators in our group have shown very recently that it is possible to protect insulin-producing cells using this unique technology (66), which allows protective and regulatory proteins to diffuse into cells without the need for permanent genetic modifications of the insulin producing cells, as it would occur with gene therapy strategies.

Progress has been reported also in the field of adult β-cell proliferation, including a recent study by Hayek's group, indicating that hepatocyte growth factor used in conjunction with selected matrices could induce proliferation of human adult β-cells (67), as demonstrated by co-localization of the proliferation marker BrdU and insulin when the growth factor was used. Recent studies by Stewart and collaborators at the University of Pittsburgh support this concept, demonstrating that transgenic mice overexpressing hepatic growth factor generated islets that were much bigger than control islets in normal littermates (68). In these studies, evidence of β-cell proliferation was associated with maintenance of β-cell differentiation markers, such as Glut2 and glucokinase.

Animal islet sources and encapsulation technologies are also strategies that could play a role in providing an unlimited supply of insulin producing cells. We and others have shown that it is possible to very effectively extract islets from the porcine pancreas (69) using methods similar to what I described above for human islet isolation. Animal insulin-producing cells could become an option if successful strategies using protective, semipermeable membranes or devices become available. Some form of immunoisolation will probably be required for pig to human islet transplantation, since achievement of tolerance across such xenogenic barriers will be much more challenging.

Major efforts are under way studying stem cells and pancreatic cell development. At our institute, we have started a program under the direction of Helena Edlund from Sweden to try to define the very complex upregulation and downregulation pathways that characterize normal β-cell development, as well as to develop unlimited sources of human β-cells, starting from β-cell precursors.

Another very important addition to our institute has been the program of cell biology and signal transduction, under the direction of Per-Olof Berggren, also from Sweden, that will introduce unique new tools for defining and monitoring metabolic pathways within islets and β-cells. Cell biology and signal transduction studies will allow us not only to better characterize human islet preparations before transplantation but also to follow and characterize the generation of mature islet cells from β-cell precursors.

This concludes my lecture on what has been for me an extraordinary 20 years of bench-to-bedside, translational research in islet transplantation, a success story that has rewarded perseverance and team work, as it is only through helping each other and sharing all the progress we make that we can speed up the search for a cure.

I would like to thank my mentors, Guido Pozza, Paul Lacy, Thomas Starzl, Andreas Tzakis, and Daniel Mintz for their invaluable support and guidance over the years. I'd also like to thank my three Research Associate Directors at the Diabetes Research Institute, Rodolfo Alejandro, Norma Kenyon, and Luca Inverardi, who are truly an outstanding example of commitment and dedication to our mission. I would also like to thank Luigi Meneghini, Ron Goldberg, and Jay Skyler, who are the three Clinical Associate Directors of the Diabetes Research Institute. I would not have received the 2002 Outstanding Scientific Achievement Award if not for Jay Skyler, who casually asked me how old I was in an elevator a few months before the deadline for nominations. I then learned that it was to determine whether I was still eligible for this award. I would like to thank the numerous colleagues who wrote wonderful letters of support and the outstanding faculty at the Diabetes Research Institute at the University of Miami Transplant Program and at the many institutions that have been working with us over the years. I would like to give a special mention to David Harlan, who started a program of islet transplantation at the National Institutes of Health in collaboration with our institute in Miami. Also, I would like to thank James Shapiro and Jonathan Lakey in Edmonton and Bernhard Hering in Minneapolis for their continuous teamwork with us to improve the field of islet transplantation and for collectively having contributed to most of the progress that I have described in the field of clinical islet transplantation. I would also like to acknowledge and thank the institutions that have supported and continue to support our work, especially NIDDK, who supported our institute over the past 20 years, and also NCRR, NIAID, JDRFI, ADA, IFW, NDRI, Friends United, the Iacocca Foundation, and most recently the Immune Tolerance Network, the Collaborative Islet Transplant Registry, and the Islet Cell Resources Network. We could not have done our work, and we wouldn't be here today, without the support of the Diabetes Research Institute Foundation and its President, Robert Pearlman. The DRIF is our single largest supporter of the work in Miami. A special mention should go to the Ramon Poo family, who has been helping us develop and distribute worldwide the equipment required for islet cell separation. Also, my deepest gratitude goes to the AFL-CIO unions, who donated the building and continue to support our efforts at the Diabetes Research Institute, and to the Stacy Joy Goodman Foundation, who established my endowed chair and continue to support other key programs at the institute. On a more personal note, I would like to thank my family: my parents for their guidance and support through the school years; our three children, Caterina, Eliana, and Carlo, who are all champions and the best kids a father could hope for; my wife, Valerie, without whom I wouldn't have received this award, as she allowed me to work while running our family, and raising our children with an unequal load, compared to my parenting contribution.

Finally, I would like to dedicate this Award and the 2002 Lilly Lecture to the memory of Stacy Joy Goodman. Stacy Joy had diabetes and was 17 when she did not wake up one morning because of severe hypoglycemia. She, as many other kids, is a continuous reminder that insulin is not enough and that our search for a cure must continue stronger than ever.

We will get this job done, and this is not a prediction. It is a promise!

Thank you all.

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