

Current Advances and Travails in Islet Transplantation

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The successful demonstration that insulin-producing β -cells can be isolated (in the form of cell clusters called islets containing β and other endocrine and nonendocrine cells) from a recently deceased donor's pancreas, then transplanted into subjects with type 1 diabetes, and thereby restore, at least temporarily, insulin-independent normoglycemia has firmly established the important "proof of concept." Even so, worldwide efforts to advance the therapy for widespread applicability have served to focus attention on the hurdles yet to clear. This review will briefly describe the present state of the art and succinctly define the research problems being attacked along with some recent advances that demonstrate significant progress.

Since Paul Lacy's early rodent experiments in the 1960s established that pancreatic islets could be isolated from one animal and transplanted into a diabetic recipient to restore normoglycemia (1), investigators have pursued efforts to develop the therapy for clinical use. After years of development in various animal models and efforts to improve human islet isolation techniques (see [2–4] for reviews with a historical perspective), the first patient achieving short-term insulin independence was reported by the group at Washington University in St. Louis. That advance was based on new islet isolation technology utilizing islets pooled from several donors, intensive insulin treatment in the peritransplant period, and induction immunosuppression with antithymocyte globulin (ATG) to avoid glucocorticoid therapy (5). The development of new immunosuppressive drugs that allowed patients to remain off glucocorticoid therapy while awaiting subsequent islet infusions (because most recipients require islets from two or more donors) enabled the group in Edmonton to optimize the clinical islet transplantation procedure (6). The approach allowed the group to conclude that about 12,000 islet equivalents per recipient body weight (in kilograms) was required to restore insulin-independent normoglycemia (6) and sparked intense international interest and effort.

Current estimates are that ~400 individuals have received allogeneic isolated islets since 1999 (7), with ~40 centers actively engaged in further developing the therapy. The Edmonton case series remains the world's largest, and

its data demonstrate that approximately two-thirds of the recipients enjoy insulin independence 1 year after receiving their final islet infusion (again, most recipients require islets from two or more donors). Unfortunately, islet function decreases over time such that by 5 years post-transplant, less than 10% remain insulin independent (8). On the other hand, the majority continues to display islet allograft function and, with it, decreased insulin requirements, less frequent hypoglycemia, and overall improved blood glucose control.

While other groups have reported incremental advances (i.e., predictable insulin independence using islets from a single donor, although typically from an ideal donor into a small recipient) (9), the Immune Tolerance Network multicenter trial results were as follows: less than half achieved insulin independence at 1 year and <15% remained insulin independent at 2 years (10). Further, recipients required (on average) islets from 2.1 donors, less than half of the pancreases donated for islet isolation yielded a product suitable for transplant, and for those recipients classified as insulin independent, blood glucose control was not normal for many using the American Diabetes Association criteria (10). Aside from the imperfect success, islet recipients experienced a small number of procedure-related complications (e.g., intraperitoneal bleeding, portal vein thrombosis, and gallbladder puncture).

Islets, though only small cell clusters, obey the same immunological laws that govern solid organ transplantation, i.e., allogeneic islets trigger immune-mediated rejection that must be controlled with immunosuppressive drugs, which are associated with an increased risk for declining kidney function, hyperlipidemia, infectious complications, and risk for malignancies. Also, if immunosuppression is stopped, recipients become immunologically sensitized against islet donor tissue antigens, and because islets from multiple donors are typically required, finding a suitable donor for subsequently required transplant therapy may prove difficult (e.g., should the patient develop kidney failure) (11,12). Although islets sharing HLA antigens with a recipient's previous kidney allotransplant may weaken the anti-islet donor immune response (13), repeatedly administering islet-associated alloantigens to recipients of previous allogeneic islets can jeopardize β -cell survival (14). Clearly, experience has identified problems to overcome (15), including the need to develop better assays for monitoring both anti-islet autoimmune and alloimmune responses (Table 1).

Assays for immunological monitoring. T-cell assays now can, with reasonable accuracy, identify anti- β -cell immune responses in individuals with type 1 diabetes compared with nondiabetic control subjects (16). The best validated assays measure T-cell proliferation in response to diabetes-related antigens. One such assay uses pre-defined diabetes-related peptide or protein antigens and

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includes exogenous interleukin (IL)-2 in the assay medium (17), whereas another group uses antigens eluted from gel-fractionated human islet cell proteins (18,19). Neither assay has been validated for its ability to monitor anti- β -cell immunoreactivity following an islet transplant, however.

Monitoring cellular-mediated immune reactivity using parameters like granzyme B, perforin, and Fas ligand can predict deteriorating islet allograft function. Indeed, studies correlating islet allograft recipients' C-peptide production with cytotoxic lymphocyte mRNA levels determined with real-time PCR have shown that the cytotoxic lymphocyte gene mRNA levels are increased 25–203 days before hyperglycemia and loss of insulin independence (20,21), with granzyme B most reliably indicating ongoing graft loss. Unfortunately, such transcriptional immune correlates do not specify whether the type of immune reaction against the islet allograft represents recurrent autoimmunity and/or alloimmunity. Experience from solid organ transplantation (kidney, liver, and heart) has taught that patient management is critically dependent on rapid and validated assays to monitor graft function and antigraft immune responses; yet at present, no such assays have been validated to monitor islet rejection.

Monitoring anti- β -cell autoimmunity. Assays monitoring anti- β -cell autoimmunity after islet transplantation correlate with progressively deteriorating β -cell function, whereas the absence of both allo- and autoreactivity has been associated with successful islet allograft outcome (22). Further, assays that measure anti-islet cellular autoimmunity (performed before a patient receives an islet transplant) are associated with delayed insulin independence, less C-peptide production during the first year after transplantation, and more rapid return to insulin dependence (23). Notably, in this study islet allograft outcome did not appear to be influenced by either anti- β -cell autoantibody levels before or after islet implantation or assays measuring cellular alloreactivity. Recent HLA-A2^{insulin} tetramer staining assays that focus on CD8⁺ (cytotoxic) T-cells (at least for the ~50% of Caucasians carrying the HLA-A2 allele) have proved useful to detect insulin-specific T-cells correlating with recurrent autoimmunity and subsequent graft failure in islet transplant recipients (24). The importance of assays capable of monitoring the anti- β -cell immune response has recently been highlighted; the IL-2 receptor-blocking antibody strategy commonly used for induction immunotherapy before the islet allograft infusion has been associated with increased IL-7 and IL-15 serum concentrations and with the homeostatic proliferation of memory T-cells reactive against islet autoantigens, e.g., autoreactive GAD65-specific T-cell clones (25). Although recent studies enrolling kidney allograft recipients have found that anti-IL-2 receptor-based induction regimens are not as effective as ATG-based depletion strategies to prevent allograft rejection (26), it is not known whether such a depletion-based strategy would better protect allogeneic islets transplanted into a host with anti- β -cell autoimmunity. Clearly, such studies should be done.

Autoantibody titers and their relevance to graft function. Although autoantibodies have proven most useful for predicting onset of type 1 diabetes, their predictive power in the islet transplantation setting is controversial. A correlation between increasing GAD65 and insulinoma-associated protein 2 (IA-2) autoantibody titers and graft loss as a result of recurrent autoimmunity has been

reported in pancreas transplantation (27,28). With regard to islet transplantation, some have reported earlier islet graft failure in autoantibody-positive compared with autoantibody-negative recipients (29,30), whereas other investigators have found no such association (23,31). This may in part be attributed to different immune suppressive regimes and graft composition and transplantation procedures.

Alloimmune responses. Most islet allograft recipients develop antidonor antibodies (11,12), typically after immunosuppressive medications are tapered due to either reduced islet allograft function or intolerable immunosuppressant agent toxicity, but islet allograft failure has also been correlated with increased alloantibody titers (32). Presence of specific antidonor alloantibodies should exclude patients from receiving islets from donors expressing the recognized HLA allodeterminants (i.e., those with a positive crossmatch) because they predict graft failure (11,23). Assays detecting recipient antidonor T-cell reactivity also correlate with graft failure in recipients of islet-alone allografts (22,23). Cytokine profiles also correlate with islet allograft fate (23) in that those skewed toward a regulatory phenotype were found in insulin-independent recipients, but not in insulin-requiring recipients. In particular, circulating IL-10 (a cytokine associated with regulatory T-cells) inversely correlated with proliferation in allo-mixed lymphocyte cultures and with alloreactive cytotoxic T-cell precursor frequency. These results imply that immune monitoring may provide surrogate markers to guide immunosuppressive agent dosing in the future.

Innate immune system effects on islet allograft survival. As much as 50–60% of the transplanted islets may be lost in the early posttransplant period (33), thereby contributing to the need to transplant islets from multiple donors to achieve insulin independence. Islets express tissue factor (TF)—a 47 kDa transmembrane glycoprotein that initiates the extrinsic coagulation system and is pivotal for activation of the intrinsic pathway. Vascular injury exposes TF to soluble coagulation proteins and triggers clotting (34). In addition, TF binds to factor VIIa and thereby activates a number of intracellular signals that culminate in cell proliferation, diapedesis, and inflammation (35). The intravascular infusion of isolated islets results in TF-stimulated nonspecific inflammatory and coagulation pathways (36–40) promoting a so-called instant blood-mediated inflammatory reaction (IBMIR) that is detrimental to islet survival (41–44) and may delay islet revascularization and engraftment (45). IBMIR has been reported in pigs after intraportal islet transplantation (46) and in human islet allotransplantation (36,46,47). Administering a humanized anti-TF-specific monoclonal antibody (CNTO 859) (48) to nonhuman primate islet allograft recipients given a marginal islet mass significantly enhanced engraftment and function (49). The recent demonstration that potent inhibitors of inflammation, including α 1-antitrypsin (50) and imatinib (a tyrosine kinase inhibitor) (51), can restore euglycemia in NOD mice with incipient diabetes further supports the critical importance of limiting innate inflammatory events in the early posttransplant period. The original Edmonton protocol has been modified in several ways; e.g., most centers now culture isolated islets to decrease tissue factor expression and administer anti-inflammatory tumor necrosis factor- α monoclonal antibody therapy peritransplant, recipients are now typically treated with heparin postislet infusion,

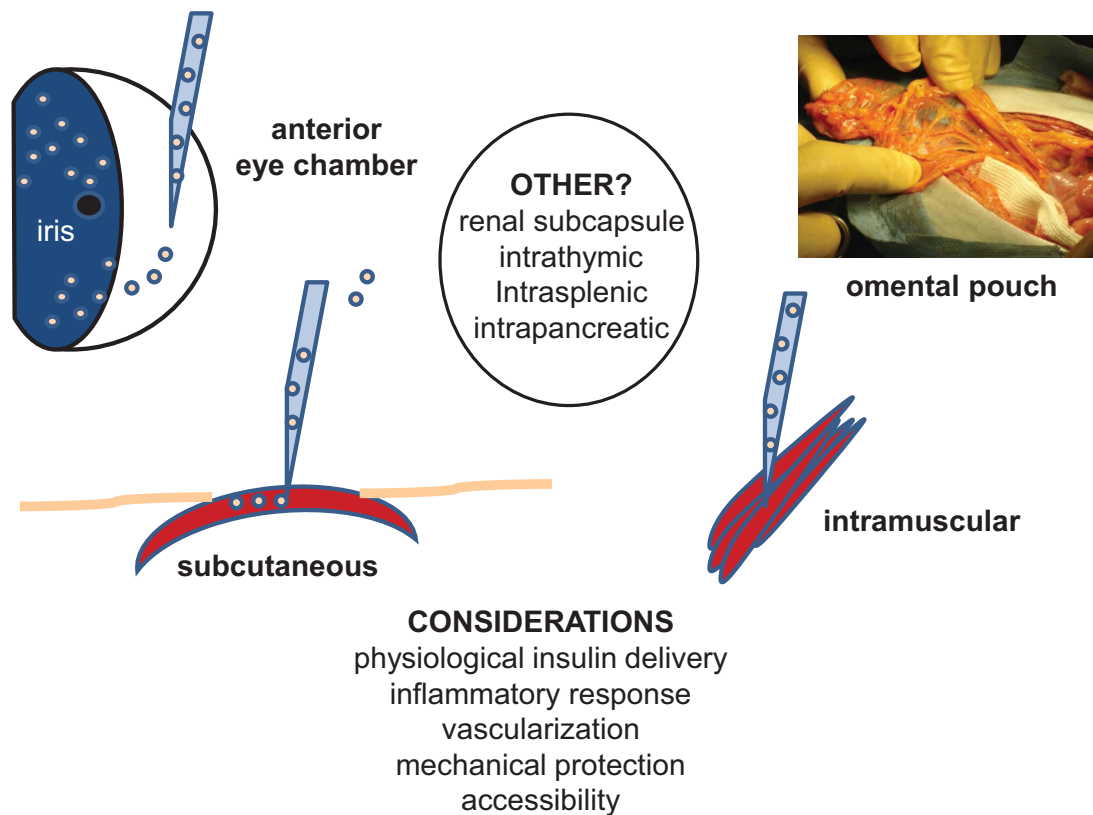


FIG. 1. Potential alternative islet implantation sites. Efforts directed to promote improved islet graft function and survival have led to studies testing alternative implantation sites, mostly in preclinical animal models, although the intramuscular route has shown some promise in the clinic (72). Recent pig model studies (73) have suggested promise for endoscopic transplant of the islets into the gastric submucosal space.

and most teams now try to minimize sirolimus exposure. These and other changes may improve outcomes like those recently reported from the University of Minnesota (52), but objectively identifying which factor or group of factors that may have resulted in the improved outcome is still difficult due to the small number of subjects who are reported in such studies.

Safely targeting the anti- β -cell immune and inflammatory responses with either drug- or regulatory cell-based strategies has proved a major challenge. In addition to the toxicity associated with usual immune suppression discussed above, several agents appear to interfere with immune tolerance, and all drugs currently used clinically to prevent islet allograft loss adversely affect β -cell function and glycemia control (4). Specific issues with current regimens are as follows: sirolimus impairs engraftment (53), interferes with angiogenesis (54), induces insulin resistance (55), and inhibits β -cell replication (56), while it, as well as corticosteroids, tacrolimus (57), and mycophenolate mofetil (MMF), decreases insulin transcription and translation (rev. in 4). Lastly, a recent study suggests that MMF also inhibits β -cell neogenesis (58). The need for different strategies to prevent allograft rejection and/or recurrent islet autoimmunity is currently debated. In the most widely studied rodent models of type 1 diabetes (i.e., the NOD mouse and BB rat), immunosuppression that readily controls allograft rejection is unable to protect against recurrent autoimmunity. In contrast, after clinical pancreas transplantation both allo- and autoimmune responses are controlled by standard immunosuppression. The notion that autoimmunity in human type 1 diabetes can be controlled by a standard immunosuppression (e.g.,

low-dose cyclosporine A) is supported by clinical studies (59). Many novel immunotherapies are under development, yet most are directed at controlling alloimmune responses (60), whereas the anti- β -cell autoimmunity preexisting any therapeutic transplant efforts in subjects with type 1 diabetes may well pose particular impediments. For example, immunotherapies that prevent autoimmune diabetes in preclinical models have been less effective when tested in humans shortly following disease onset (61–63).

An additional consideration for enhancing outcomes in islet transplantation is identification of alternative implantation sites (Fig. 1). Infusing islets into the liver via the portal vein has been the site of choice for clinical islet transplantation and is the only site that has routinely demonstrated success in large animal models. The reasoning has been that the pancreas normally secretes insulin into the portal vein, intrahepatic islets avoid the systemic hyperinsulinemia observed in some pancreas allograft recipients, the portal blood is oxygenated (albeit at lower than arterial tensions) such that the isolated islets are exposed to oxygen until they can revascularize, and the portal vein can be accessed using a minimally invasive procedure. Disadvantages of the portal vein include the aforementioned IBMIR, higher levels of immune suppression in the portal circulation that may impair islet engraftment, vascularity, or function (4;53–58;60), periportal steatosis (64,65), and an inability to routinely biopsy the transplanted islets because they are dispersed within the liver. Isolated islets have been infused via the celiac artery into nonhuman primates, reasoning that the arterial tree could be more safely accessed and that intra-arterial islets

TABLE 1
Immune correlates of type 1 diabetes and islet allograft function

Immune marker	Correlate	Comment	References
Autoimmunity			
Islet autoantibodies	Baseline prediction	<ul style="list-style-type: none"> ● Progressive islet graft failure occurs significantly earlier in autoantibody-positive than in autoantibody-negative type 1 diabetic recipients of intrahepatic islet allografts. ● Insulin independence was not achieved in patients with baseline autoantibody elevations and was significantly less frequent in patients who seroconverted. ● Patients with thyroid peroxidase autoantibodies before islet transplantation develop Graves disease after tapering of immune suppression. 	(27), (28), (92)
	Seroconversion	<ul style="list-style-type: none"> ● Autoantibody levels had no significant association with outcome. ● Insulin independence was not achieved in patients with autoantibody elevations and was significantly less frequent in patients who seroconverted. 	(92), (95)
T-cell autoreactivity	Baseline prediction	<ul style="list-style-type: none"> ● Patients without preexisting T-cell autoreactivity became insulin independent compared with none of the patients reactive to both GAD and IA-2 before transplantation. ● Cellular islet-specific autoimmunity associates with clinical outcome of islet cell transplantation under ATG-tacrolimus-MMF immunosuppression. 	(23), (98)
	Disease recurrence	<ul style="list-style-type: none"> ● Tight correlation between human β-cell allograft recipient's metabolic outcome and assays of peripheral blood cellular auto- and alloreactivity suggests a causal relationship. ● Subsequent islet implantations can reduce alloreactivity for repeated HLA mismatches. 	(22)
	Homeostatic expansion	<ul style="list-style-type: none"> ● T-cell depletion therapy results in expansion of memory (islet autoreactive) T-cells. 	(13), (14)
Cytokines	γ -Interferon	<ul style="list-style-type: none"> ● γ-Interferon production in ELISPOT associates with type 1 diabetes. 	(25)
	IL-10	<ul style="list-style-type: none"> ● IL-10 production distinguishes control subjects from subjects with type 1 diabetes and associates with delayed onset of type 1 diabetes. 	(94)
Alloreactivity			
Alloantibodies	Baseline prediction	<ul style="list-style-type: none"> ● Pretransplant HLA antibodies are associated with reduced graft survival after clinical islet transplantation. 	(11)
	Seroconversion	<ul style="list-style-type: none"> ● Monitoring panel reactive antibodies in immunosuppressed subjects has little clinical value in islet transplant recipients. The implications of allosensitization after discontinuation of immunosuppression need to be evaluated to define the clinical impact in this patient population. 	(12), (95)
	Seroconversion after immunosuppression discontinued	<ul style="list-style-type: none"> ● Incidence of antidonor HLA alloantibodies posttransplant rises abruptly in subjects weaned completely from immunosuppression and is a cause for potential concern. 	(11)
T-cell alloreactivity	CTLp	<ul style="list-style-type: none"> ● Informative correlate depending on immunotherapy. ● Secondary to recurrent autoreactivity? ● Regulatory alloreactivity associated with outcome. 	(22), (93)
Cytokines	γ -Interferon	<ul style="list-style-type: none"> ● Positive association with mixed lymphocyte reaction (MLR) assay. 	(23)
	IL-10	<ul style="list-style-type: none"> ● Marker of protection/preservation/tolerance. 	(23), (96)
Cytotoxic T-cell genes			
Granzyme B	Loss of function	<ul style="list-style-type: none"> ● Granzyme B was the most reliable indicator of ongoing graft loss. The results suggest that, when taken into consideration with other clinical parameters, CTL gene expression may predict islet allograft loss. 	(20), (95)
Perforin and Fas-L	Increased insulin needs	<ul style="list-style-type: none"> ● The decreased expression of perforin and Fas-L in patients with long-term type 1 diabetes might contribute to the inability to maintain normal levels of peripheral tolerance, which is essential for protection from autoimmune disease. 	(20), (95)

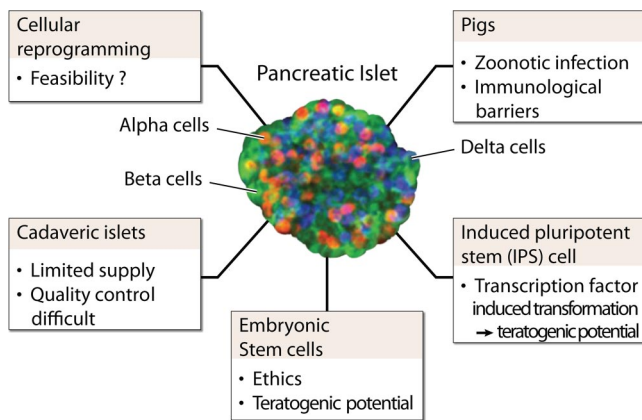


FIG. 2. Recognizing the tremendous disparity between the islet number that can be isolated from cadaveric donors and the potential recipient population, many investigators are working to develop a renewable and cost-efficient source of islets or islet-like clusters. Each strategy has potential advantages but also unique problems to overcome.

might be more readily revascularized, but islet function was promptly lost (66). The highly vascular omental pouch of diabetic dogs has been successfully used as a site for autologous islet implants (67–69), and N.S.K. and colleagues (70) have demonstrated the feasibility of this site for allogeneic islet implant in cynomolgus monkeys. Experimental efforts testing the pancreas as an implantation site have also been reported in animal models (71). Recently, implanting a child's autologous islets into an intramuscular site has been reported to decrease her insulin requirements and help maintain euglycemia (72). Identification of scaffolds, gels, matrices, and devices that can enable exploitation of alternative sites is an active area of investigation.

Cells capable of physiologically regulated insulin secretion. Assuming techniques can be developed to safely protect insulin-producing cells once they are implanted into the diabetic individual, a widely applicable strategy will require a renewable β -like cell source. In the U.S. alone, over 22 million people have diabetes (type 1 diabetes or type 2 diabetes), and yet the country produces only about 8,000 organ donors each year. Because at present only approximately half of the isolation efforts yield islets suitable for transplant (10) and because recipients usually require islets from multiple donors (10), only ~2,000 subjects in the U.S. could benefit from an islet transplant each year. Efforts to expand the pancreas donor pool (e.g., including non-heart-beating donors [74]) improve isolation techniques to more likely yield transplantable islets from each pancreas, and strategies to decrease a recipient's islet requirements, even when combined, will only marginally improve the current disparity between islet supply and potential recipients. Further, while recent promising efforts report the transplant of islets isolated from a living donor (75), other studies reporting long-term metabolic consequences for those donating half their pancreas considerably temper any optimism that living islet donors can fill the insulin-producing cell void (76).

Several groups are therefore pursuing strategies (Fig. 2) designed to use renewable sources for the insulin-producing cells; e.g., xenogeneic islets (predominantly from pigs), cells induced to differentiate from embryonic stem (ES) cells (or the related inducible pluripotent stem cells), or cells "reprogrammed" from their initial phenotype into β -like cells.

Pig islets offer many advantages as a renewable islet source. Pigs have large litters, and the animals mature quickly; glucose set points for insulin release are similar in pigs and humans; pig insulin was used clinically for decades insuring its safety; and the widespread use of pigs for agricultural reasons minimizes animal rights concerns that may exist for other potential xenogeneic sources (15). Further, some investigators have transplanted isolated pig islets to diabetic nonhuman primates and thereby restored temporary near-normal glycemia to the immunosuppressed recipients (77,78). Factors limiting this xenogeneic islet source include particular species-specific difficulties associated with islet isolation and to-date only theoretical zoonotic infectious concerns; i.e., the species is known to harbor certain pig endogenous retroviruses (PERVs), and some have suggested that a large pig tissue inoculum, especially if placed in an immunosuppressed host, may support adaptation of the pig virus for human cells. More importantly, pig tissues express a cell surface moiety (galactose α 1,3 galactose) against which humans have high-titer antibodies leading to accelerated and reinforced rejection. The latter problem is being attacked through the creation of genetically altered pigs (79,80).

Considerable excitement surrounds reports that human ES cells can be cultured *in vitro* under conditions that support differentiation into definitive pancreatic endoderm and even β -like cells, except that such *in vitro*-produced cells fail to secrete insulin in a glucose-regulated fashion (81). However, when definitive pancreatic endoderm is implanted into immunoincompetent mice, many of the cells differentiate into β -like cells that release insulin in response to glucose (82). Unfortunately, some of the implanted cells also display teratogenic potential, and it is not yet possible to select the desired cells from the undesired ones. Clearly, regulatory agencies such as the U.S. Food and Drug Administration would and should insist on strategies to overcome this shortfall. Lastly, unless ES cell lines can be established for all potential HLA haplotypes, the β -like cells produced from a particular ES line would face immune destruction from both antiallogeneic (unless the ES haplotype completely matched the recipient) and autoimmune processes. Recent progress with somatic cell nuclear transfer in the nonhuman primate (83) provides one potential solution for creating ES cells for any individual from a mature cell's nucleus taken from that individual, assuming moral/ethical issues can be worked out.

Another potential solution to overcome the alloimmune response has been offered by recent successes to create ES-like cells from fully differentiated somatic cells, so-called induced pluripotent stem (IPS) cells. This advance raises the possibility that each individual could serve as his or her own stem cell source to create new β -like cells. Unfortunately, at present the process of dedifferentiating such somatic cells requires transfection with potentially cell-transforming transcription factors like *c-myc*, and most strategies utilize viral vectors that integrate into the genome and thus further increase concerns that such cells may display malignant potential. Recent reports have shown that nonintegrating viral vectors can promote IPS cell generation, whereas others are conducting studies to avoid transcription factors altogether (84,85). By utilizing each individual's own cells to create new β -like cells, one anti- β -cell immune response (alloimmunity) is eliminated while another is quite possibly exacerbated (autoimmunity); i.e., multiple anti- β -cell T-cell clones exist in the

individual with type 1 diabetes, and each T-cell recognizes major histocompatibility complex (MHC)-restricted β -cell antigenic peptides. For any given individual with type 1 diabetes, all such autoreactive T-cells would be able to recognize β -cells created from that same individual's IPS cells because the β -cells would express all the appropriate MHC restriction elements. Indeed, recurrent anti- β -cell-specific CD8 T-cell-mediated reactivity associated with loss of islet allograft function has been shown in cases where the donor shares HLA class I alleles with the recipient (86).

Lastly, transfecting rodent pancreatic acinar cells with an adenoviral vector mixture driving temporary expression of the transcription factors (Pdx-1, Ngn-3, and Maf A) appears to convert those mature pancreatic cells into β -like cells without an intermediate dedifferentiated state, so-called lineage reprogramming (87). Ongoing studies are exploring whether more readily accessible cells (e.g., cultured hepatocytes) might be similarly reprogrammed with these (or other) transcription factors. The facts that only transient vector-driven transcription factor expression is required to reprogram the cells and that the strategy avoids the dedifferentiated cell state may decrease the transformation potential, but recurrent autoimmune destruction would remain a problem.

DISCUSSION

Progress developing renewable cellular sources capable of physiologically regulated insulin secretion, assays for monitoring the immune response against those cells, and therapies to preserve those cells' function once transplanted have all converged to bring into clearer focus the long-dreamed of "finish line" (i.e., curing diabetes by correcting the afflicted individual's insulin deficiency). That said, prudence dictates that investigators begin planning for the end-game strategy to start clinically testing cell transplant-based strategies. The process will not be fast or trivial, and yet we argue that "fast-track" approaches should be considered with great caution, especially with regard to stem cell-based approaches. For instance, most would agree that the University of Pennsylvania's unfortunate gene therapy experience not only contributed to that study's first enrollee's premature death but that the entire gene therapy field was set back (88). We offer the following thoughts for forward progress.

In most developed countries, pancreas transplantation is the only accepted procedure to achieve normoglycemia. For pancreas transplantation, established techniques exist to procure the donated organ (or part of it) from both living and diseased donors and long-term graft function is similar to other whole-organ allografts. However, the pancreas transplant procedure is limited by additional risks related to the organ's exocrine enzyme production. While therapy using isolated human islets may be on the brink of becoming an accepted clinical therapy for a small type 1 diabetic subgroup with most severe hypoglycemia unawareness and while isolated islets enjoy an advantage over the intact pancreas in that the exocrine component is removed during the islet isolation process, those same isolation procedures impose ischemic and mechanical damages and thereby induce undesired cellular stress responses. Moreover, the injection of the cells into the blood stream is unique, and it is now generally accepted that only 10–20% of the islets transplanted survive the procedure and contribute to the recipient's metabolic

control. And although some mechanisms underlying the substantial islet loss have been discussed, much remains unknown. Data obtained from rodent models suggest that these limitations can be overcome. Whether they can be successfully translated to larger animals and to humans is the focus of several ongoing studies.

Taking into consideration the enormous recent improvements in the type 1 diabetes treatment, the ultimate indications for islet transplantation could only be justified if there were almost no side effects related to the procedure and the immunosuppression/tolerance protocols applied. One could argue that there will be no need for xenogeneic or stem cell-derived β -like cells until robust immune tolerance or protection can be induced without severe side effects. The many forces conspiring to impair islet (or islet-like cell clusters) function or survival (Fig. 3) are all rich sources for study because most, if not all, will need to be overcome.

Testing therapies designed to restore anti- β -cell immune tolerance. For therapies designed to generally weaken the anti- β -cell immune response or augment immunoregulatory processes, we point out that most therapies working in one T-cell-mediated disease process also generally work when applied to a different T-cell-mediated illness. Given that the prognosis for individuals with recent-onset type 1 diabetes is now outstanding (excess mortality of $\sim 0.1\%$ per year [89]), the potential immunotherapy should have a safety profile known to not exceed that rate. For instance, the anti-VLA4 antibody (natalizumab) is estimated to carry with it 1 in 1,000 risk for progressive multifocal leukoencephalopathy such that its use in subjects with recent-onset type 1 diabetes might be unwise. Even individuals with long-standing type 1 diabetes sufficiently severe to be listed for a solitary pancreas transplant have an annual mortality of 1–2% (90). Further, using islet transplantation as a model to test new immunomodulatory approaches is complicated by our present inability to reliably predict islet allograft rejection from either allo- or autoimmune processes and by our inability to reverse an early rejection episode. The current need for islets from two or more donors and the resulting allosensitization raise additional concerns. Lastly, many immunotherapeutic agents appear to directly influence β -cell function, vascularity, survival, and/or proliferative capacity (4;53–58;91). Consequently, one might argue that efforts directed to test therapies designed to promote immune tolerance should await the ability to promote long-term insulin independence to recipients receiving islets isolated from a single donor. In the meantime, novel immunomodulatory therapies could, in general, be first tested in another setting such as kidney transplantation where disease prognosis is worse, techniques for following the antigraft immune response exist, one donor's tissue suffices to restore the recipient's lost organ function, the immunological barrier can be determined (from the HLA mismatch score and by avoiding autoimmunity), and effective rescue therapies exist should the experimental immunotherapy fail. On the other hand, a transplanted kidney is a life-saving procedure, and some have questioned the ethics of testing new immunotherapies when effective ones exist. Further, acute rejection episodes are considered a surgical emergency and a potential threat for both the patient and the graft. Also, any immunomodulatory therapy will eventually need to be evaluated in individuals with autoimmune type 1 diabetes. These imponderable variables lead most to conclude that individual

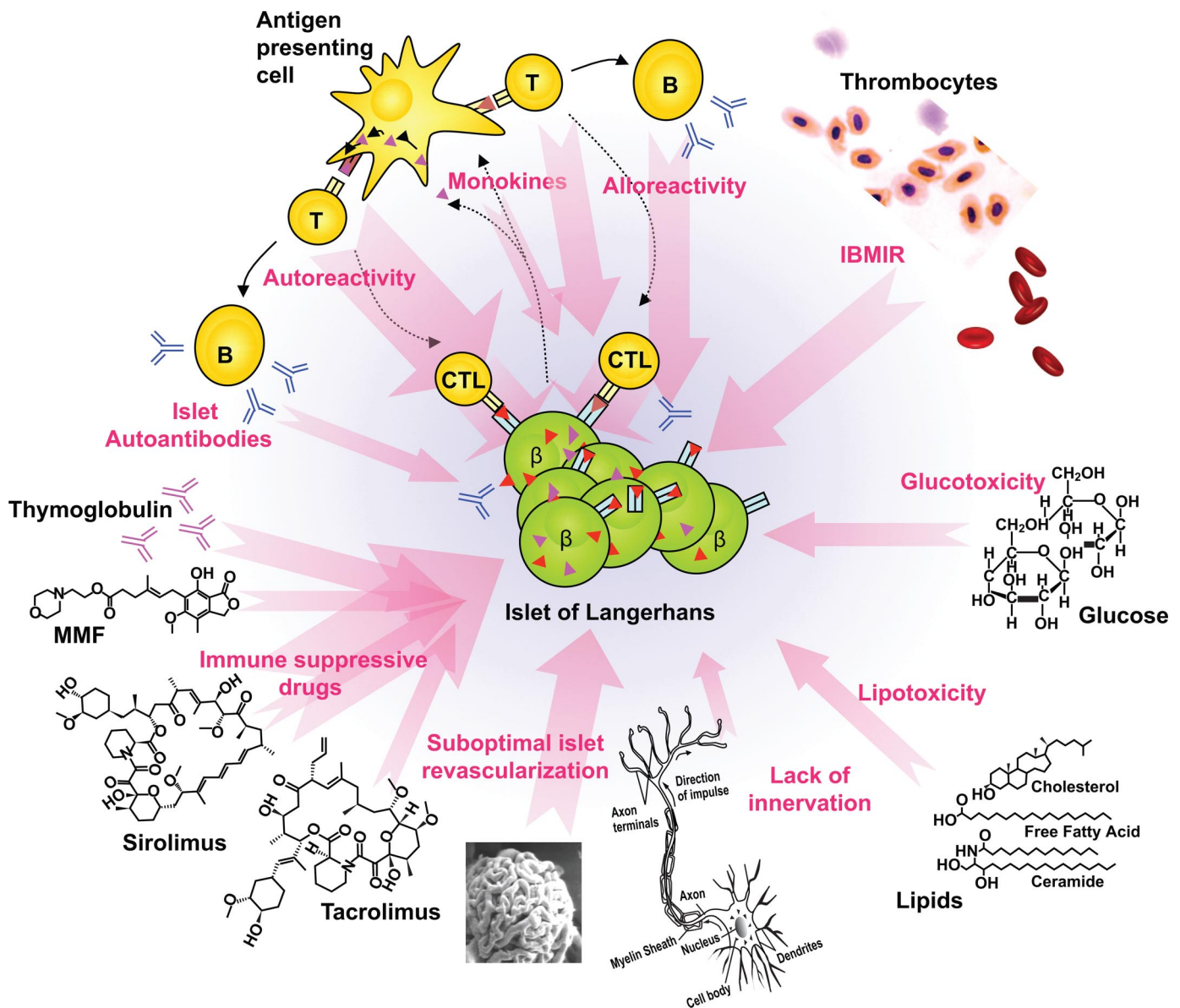


FIG. 3. Factors limiting islet graft function and survival. Present understanding is that transplanted islets (or islet-like clusters) face myriad overlapping forces all conspiring to limit graft function and/or survival. Effective strategies to develop islet transplantation for widespread clinical application will likely require effective countermeasures for each identified problem.

investigators should pursue specific protocol plans as guided by external peer review, their local institutional review board, and proper informed consent from the potential protocol participant.

Testing the cellular source for physiologically regulated insulin secretion. In view of the potential adverse effects associated with xenogeneic cells (zoonotic infection) or cells engineered in vitro, most investigators agree that testing of the insulin-producing cells in a chronic large animal (ideally nonhuman primate) model should be performed if possible. One disadvantage of the nonhuman primate model is that anti- β -cell autoimmunity has not been reported in the species.

Lastly, as discussed throughout this review, because investigators are attempting to safely manipulate the immune system to prevent it from killing transplanted insulin-producing cells, it only makes sense for scientists to develop techniques to better measure the immune processes that affect the transplanted insulin-producing cells

and to be able to quantify the insulin-producing cell mass in vivo. If successfully achieved, these techniques will become of immense importance not only for the development of replacement therapies for type 1 diabetes but also for early interventions (e.g., immune intervention for those at risk to prevent the disease or drugs to stimulate β -cell function and proliferation in those recently diagnosed) aiming to prevent clinical overt diabetes (type 1 or type 2).

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