The New York Diabetes Association's 48th Annual Scientific Meeting, the American Diabetes Association's 48th Annual Advanced Postgraduate Course, and the Meeting of the Naomi Berry Diabetes Center of Columbia University

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This report covers the New York Diabetes Association's 48th Annual Scientific Meeting, October 2000; the Meeting of the Naomi Berry Diabetes Center of Columbia University, December 2000; and the American Diabetes Association's 48th Annual Advanced Postgraduate Course, January 2001, New York, New York. Topics include diabetic retinopathy, diabetic nephropathy, the cell biology of insulin action, and islet cell transplantation.

Diabetic retinopathy

At the 48th Annual Scientific Meeting of the Gerald J. Friedman Symposium on October 28, 2000, in New York, NY, Lloyd P. Aiello, Boston, MA, discussed potential pharmacologic treatments of diabetic retinopathy. Diabetic retinopathy is a major cause of blindness and visual loss, acting via two complications, proliferative retinopathy, which leads to retinal hemorrhage and scarring with the inevitable development of traction detachment, and that of macular edema, which is caused by abnormalities of retinal vessels. Panretinal photocoagulation, the existing treatment, is less effective for macular edema and can be expected to lead to some degree of visual loss; it may also lead to severe complications when performed incorrectly. The original hypothesis that diabetic retinopathy is caused by abnormal production of growth factors stimulated by retinal hypoxia was made more than 50 years ago by I.C. Michaelson. Growth hormone and IGFs play a permissive role, fibroblast growth factor may act synergistically, and vascular endothelial growth factor (VEGF) appears to be the major mediator. The expression of this glycoprotein is increased by hypoxia, and its levels are particularly high in patients with active proliferative retinopathy. VEGF increases retinal permeability in a dose-related fashion at levels encountered clinically in patients with retinopathy.

These considerations suggest a number of potential approaches to treatment. Prevention of hypoxia with glycemic control, antioxidants, and AGE inhibitors might directly protect the retinal vessels. Angiotensin II (A2) is an important signal for VEGF secretion, with ACE inhibitors and A2 receptor blockers (ARBs) existing as candidates for treatment via this mechanism. Also, the action of VEGF at its receptor can be antagonized, with receptor blockers having been shown to decrease neovascularization in rat, pig, and primate models of diabetic retinopathy. A final approach is to block the cascade of cellular activities in which VEGF participates. Integrin and metaloproteinase antagonists may play a role here. Protein kinase C (PKC) activation is a major mediator of the effect of hyperglycemia on vascular dysfunction, of the effect of vascular dysfunction on hypoxia, and, in turn, of the effect of hypoxia on VEGF and the effect of the actions of VEGF. An important agent under study is the PKC-β inhibitor LY333531, which can be administered orally and eliminates VEGF-induced increases in retinal blood flow, permeability, and neovascularization in animal models (1). In a study of 29 patients with diabetes of <10 years' duration, a dose-related normalization of circulation time and retinal blood flow was demonstrated. More than 600 patients have been studied for periods over 6 months without evidence of adverse effects. An ongoing study of 942 patients will be completed during the coming year to show whether the agent can be used to prevent proliferative retinopathy and macular edema.

VEGF has many signaling path interactions with other growth factors. Hepatocyte growth factor (HGF) levels are increased in serum of patients with diabetes and in the vitreous of patients with diabetic retinopathy. HGF increases retinal permeability and increases migration of retinal epithelial cells, and it may prove to play an important role in pathogenesis. Another important factor is the interaction of diabetic retinopathy with hypertension and with cardiovascular disease. Hypertension is an independent risk factor, associated with a threefold increase in proliferative retinopathy, that possibly acts via the mechanical stretch of medium and large arteries. Aiello noted that arterial wall stretch may increase expression of both VEGF and kinase insert domain-containing receptor (KDR), a VEGF receptor, in a dose-related fashion. Another

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Abbreviations: A2, angiotensin II; ARB, A2 receptor blocker; CAP, Cbl-associated protein; ERK, extracellular signal-regulated kinase; ERV, endogenous retrovirus; ESRD, end-stage renal disease; GLP, glucagon-like peptide; HGF, hepatocyte growth factor; IL, interleukin; IRS, insulin receptor substrate; JNK, c-Jun NH_2-terminal kinase; KDR, kinase insert domain-containing receptor; MAPK, mitogen-activated protein kinase; PI3K, phosphatidlyinositol 3-kinase; PKB, protein kinase B; PKC, protein kinase C; PPAR, peroxisome proliferator–activated receptor; VEGF, vascular endothelial growth factor.

connection between hypertension and VEGF is A2, which increases KDR levels. The ARB canderesartan blocks this increase while not affecting stretch-mediated KDR expression. In a hypertensive rat model, KDR and VEGF increased, with normalization achieved by administration of captopril or canderesartan. This may explain the findings of the EUCLID Study (EURODIAB Controlled Trial of Lisinopril in Insulin-Dependent Diabetes Mellitus) (2) and the U.K. Prospective Diabetes Study (3), which demonstrated that blood pressure treatment improved retinopathy.

Treatment to suppress VEGF action may ameliorate other adverse effects of hypertension as well as those in retinopathy. Aiello noted, however, that VEGF administration has been suggested as a treatment of peripheral and coronary ischemia. Interestingly, VEGF and its receptor show decreased expression in the heart in animal models of both diabetes and the insulin resistance syndrome, and preliminary studies suggest this to occur in humans, in contrast to the overexpression seen in the eye and kidney. There is, then, danger that VEGF inhibitors might have adverse effects under circumstances of ischemia, and while VEGF agonists might worsen retinopathy, it demonstrates the importance of carefully assessing any proposed treatment.

Aiello was asked why rapid control of glycemia may exacerbate retinopathy, and he noted that rapid glycemic control may induce VEGF expression in vitro. Further, he was asked about the relationship of pericyte loss to retinopathy and whether this was discordant with the VEGF data. He replied that VEGF is mainly produced by endothelial cells and that its effect is most apparent at a late stage of retinopathy, whereas the pericyte loss begins to play a role in much earlier stages of retinopathy.

Diabetic nephropathy

Eli Friedman, New York, NY, presented an optimistic perspective on diabetic nephropathy, which he suggested "will no longer be a concern [in properly treated patients] by the end of this decade." An important current finding is the realization over the past decade that the glomerular mesangium is "the hot zone," with "accumulation of an amorphous material that blocks up the filtration pores." Friedman reviewed studies of the potent antioxidant nitecapone (4) and the AGE blocker aminoguanidine, showing evidence of prevention of nephropathy. Diabetic nephropathy progresses from hyperfiltration, with pathologic findings of glomerular enlargement with mesangial expansion, to microalbuminuria through a rise in creatinine to end-stage renal disease (ESRD) in type 1 and type 2 diabetes (5,6). Clearly, hyperglycemia is the cause of diabetic nephropathy, with reversal by pancreatic transplantation after 10 years. Furthermore, if a kidney from a nondiabetic donor is transplanted into a patient with type 1 or type 2 diabetes, or even glucocorticoid-induced diabetes, diabetic nephropathy develops. Blood pressure treatment was once considered problematic below diastolic levels of 120, but beginning with Parving's studies two decades ago, we have come to a realization that the optimal blood pressure is no more than 130/80. Friedman's own goal is 125/70, a level he finds possible to reach in about half of patients. With ACE inhibitors, as well as adjunctive treatment, as seen with vitamin D analogs and erythropoeitin, the treatment of diabetic patients with ESRD now contrasts dramatically with the early experience with dialysis, which showed little benefit among patients with diabetes (7). Patients with diabetes now comprise 35–45% of ESRD patients on dialysis. Currently, the major problem for these individuals is not the dialysis, but the increase in frequency of concomitant illnesses, with retinopathy, lower-extremity ulcers, stroke, and cardiovascular disease frequently developing. Ongoing follow-up to assess the coronary circulation and regular ophthalmologic evaluations are important. Of note is the finding that once nephropathy becomes established, it shows more rapid progression in patients with type 2 rather than type 1 diabetes, among both younger and older patients, which is in opposition to the usual belief that type 2 diabetes is more benign. Friedman pointed out that 90–99% of nephropathy is caused by type 2 diabetes (8).

Cell biology of insulin action

David Thomlinson, Manchester, U.K., discussed mitogen-activated protein kinases (MAPKs) as glucose transducers in diabetic neuropathy. Several features of nephropathy differ from other complications. Neuropathy is length-dependent in a stocking-glove distribution, with disorders of both neurons and supporting cells. There is evidence of nerve function abnormality long before clinical diagnosis, and it is possible that by the time of manifestation there is no reversibility.

Hyperglycemia clearly causes diabetic neuropathy, with potential mechanisms including hyperosmolarity, the polyol pathway, oxidative stress, and posttranslational macromolecular glycation. These processes interact, with hyperosmolarity and oxidative stress affecting polyol metabolism and oxidative stress increasing AGE formation. The polyol pathway involves the activation of aldose reductase to metabolize glucose to sorbitol, consuming large amounts of NADPH, which is then not available for glutathione regeneration. The superoxide anion, a major cause of intracellular oxidant stress, is "scavenged" by superoxide dismutase to H_2O_2, with glutathione peroxidate needed for subsequent H_2O formation. With depletion of cellular glutathione stores, excess H_2O_2 accumulates, leading to oxidative damage to DNA and other cellular elements.

A major factor in the development of irreversible cell phenotype changes after chronic exposure to hyperglycemia involves three MAPKs: extracellular signal-regulated kinase (ERK), the major insulin-responsive MAPK, which is involved in control of the cell cycle, c-Jun NH_2-terminal kinase (JNK), and p38. ERK is activated directly by extracellular signals, while JNK and p38 are typically activated by cellular stresses. These form part of a cascade, with MAPK kinases activating MAPK, thereby leading to changes in specific transcription factors. Assays using paired antibodies to nonphosphorylated regions of the kinase, thus allowing measurement of total kinase levels, and to phosphorylated regions, thus allowing measurement of the level of activated kinase, give a ratio indicative of the degree of activation. Using these techniques, ERK and JNK have been shown to be hyperactivated in neurons of diabetic rats, with an increase in the active total ERK and an increase in both total and activated levels of JNK. Immunocytochemical study has shown levels of all three MAPKs to be present in the neurons rather than in the supporting cells of these rats. Thomlinson hypothesized that MAPKs act as transducers of oxidative stress and high-glucose effects on the cells.

A number of relevant presentations were made at a meeting at the Naomi Berry Diabetes Center of Columbia University, New York, NY, in December, 2000. Morris J. Birnbaum, Philadelphia, PA, showed that insulin mediated effects on organismal growth, with fascinating data suggesting that the "primitive" growth response to IGF-1 and insulin is a coordinated anabolic response with an antiapoptotic element, which may include increased islet formation. The "modern" metabolic response to insulin may be seen as an adaptation of this pathway to increase storage of nutrients used by the organism for growth. He hypothesized that insulin and IGF-1 signaling through the serine protein kinase Akt and protein kinase B (PKB) evolved as a mechanism to sense nutritional abundance and to instruct the organism to respond appropriately.

Insulin in lower vertebrates and invertebrates mediates response to the nutritional state. In the fruit fly *Drosophila melanogaster*, the enzyme Akt increases organismal growth, so that nutritional deprivation leads to the development of a normally patterned but smaller fly. In fish and amphibians, insulin diverged to have two actions: involvement in organismal growth and a role in the repletion of energy stores. Akt plays a role in other anabolic effects of insulin, including a cytokine-activated antiapoptotic effect, actions on protein and glycogen synthesis, and effects on the generation of nitric oxide.

In *Drosophila*, insulin activates an Akt analog, Dakt. This subsequently leads to phosphorylation of p70 S6 kinase, an important effector in these cell types, like a kinase present in mammalian cells. In the intact fly, overexpression of active Dakt or the insulin receptor increases cell size of the photoreceptors of the eye, with little or no change in cell number. Birnbaum studied Akt regulation of cell size in mammalian cells. In type 2 diabetes, β-cells secrete less insulin, and the β-cell mass does not expand appropriately in response to increased demand. This may be attributable to the inability of the β-cell to appropriately suppress apoptosis, which is compatible with a defect in the Akt pathway that leads to both peripheral and β-cell defects of type 2 diabetes. In a mammalian model, overexpression of Akt increases β-cell mass 8- to 10-fold, particularly increasing islet number, with new islets budding from the pancreatic ducts. These islets are functional, leading to increased insulin production and improved glucose tolerance, and they decrease the severity of experimental diabetes induced by low-dose streptozotocin, a type 2 diabetes model with increased apoptosis.

Alan Saltiel, Ann Arbor, MI, discussed insulin-mediated compartmentalization of intracellular glucose metabolism. Insulin receptor substrate (IRS)-1 and phosphatidylinositol 3-kinase (PI3K) mediate synthesis of glucose transporters and their membrane translocation. Insulin also affects protein synthesis, glucose and lipid metabolism via protein phosphatases, cell growth via serine kinases, and gene expression/transcription. One concept for control of these various actions is that of compartmentalization, as signals initiate insulin action segregated into different compartments physically located in specific domains of the plasma membrane. Caveoli, small invaginations of the plasma membrane, are areas of altered membrane lipid. Caveoli are examples of structures referred to as "lipid rafts," defined by their lipid content, and are highly enriched in signaling molecules, which suggests that they act as signaling organelles. Studies of tyrosine phosphorylation in these domains show that the proto-oncogene c-Cbl is specifically tyrosine phosphorylated under influence of Cbl-associated protein (CAP) in response to insulin, after which it translocates into a lipid raft in these structures. Cbl attaches near a protein in the caveoli, caveolin. CAP is expressed in insulin-sensitive tissues, particularly fat, muscle, and liver, and is induced upon differentiation of preadipocytes into adipocytes and by activation of peroxisome proliferator–actived receptor (PPAR)-γ in vitro and in vivo (the CAP promoter contains a functional PPAR response element), with PPAR-γ activators increasing Cbl phosphorylation. This is the only gene involved in insulin signaling directly activated by PPAR-γ. CAP may recruit Cbl to the area of the insulin receptor for phosphorylation, after which the CAP-Cbl complex dissociates from the insulin receptor and migrates to the caveoli. The NH_2-terminal of CAP contains a domain showing 80% sequence identity to the gut protein sorbin. Flotillin, another protein, is a homolog of epidermal surface antigen and a member of a family of adapter proteins involved in functions of the cytoskeleton, which acts as the binding site in the caveoli for phosphorylated Cbl-CAP. If CAP is structurally modified, translocation of GLUT4 to the plasma membrane cannot occur, suggesting that the insulin receptor–IRS–PI3K pathway works with the insulin receptor–Cbl pathway.

Salteil next described a non-PI3K pathway of insulin action, enumerating some of the many cellular proteins involved. Insulin induces the association of Cbl with c-Crk-II, an adaptor protein that interacts with the nucleotide exchange factor C3G and catalyzes the exchange of GTP for GDP, a potential mechanism for activation of G proteins. Modification of CAP also blocks the translocation of C3G to caveoli. Furthermore, insulin activates the protein TC10 in a PI3K-independent fashion, which is blocked by inhibitors of the C3G pathway. Thus, C3G may also be an exchange factor for TC10. TC10 is a Rho family GTPase that is highly expressed in fat and muscle and activated by insulin via a COOH-terminal "CAAX Box" sequence, which allows it to undergo tandem acylation. Salteil showed that TC10 localizes with caveolin in adipocytes, and its activation by insulin appears to involve translocation of Crk/C3G to the caveoli. He concluded that two groups of signals are involved in glucose transport, PI3K and CAP-Cbl. These pathways embody distinct functions, with PI3K leading to release of the GLUT4 vesicle and CAP-Cbl regulating the docking and fusing of the vesicle with the plasma membrane. An alternative hypothesis is of differing pools of insulin receptors in the lipid rafts, one set involved in PI3K activation and the other set interacting with flotillin and activating Cbl-CAP.

Morris White, Boston, MA, discussed transgenic approaches to elucidation of the molecular physiology of insulin signaling. The insulin response includes glucose uptake, amino acid uptake, acetyl-CoA metabolism to fatty acids, glucose storage in glycogen, inhibition of pyruvate metabolism to glucose, activation of the Na-K pump, protein synthesis, gene expression, DNA synthesis, apoptosis, and effects on β-cell function. The insulin/IRS-1 signaling cascade involves the receptor activation and autophosphorylation, recruiting IRS proteins composed of docking and phosphorylase subunits. The activation of PI3K causes a subse-

quent phosphorylation cascade, which leads to effects on glucose transport and glycogen synthesis via PKB/Akt, as well as effects on protein synthesis via other phosphorylation enzymes. The Akt system leads to activation of a group of nuclear transcription factors, some of which are also activated by MAPK.

IRS-1 is a large protein with 20 tyrosine phosphorylation sites that interact with the enzymes that mediate biological responses. IRS-2 is a similar molecule, and it appears to be the main player in regulation of nutrient homeostasis as well as being involved in hypothalamus-ovarian function. IRS-3 and IRS-4 are present in specific tissues and mediate other aspects of insulin action.

By studying mice lacking insulin receptor, IRS-1, and IRS-2 alleles, in various combinations, one can assess glucose metabolism in various tissues. Mice lacking one of each allele maintain normal glucose levels but require fivefold higher insulin levels, whereas intermediate levels of insulin are required with defects in only one or two of the genes. IRS-2 knockout mice, but not those without the other IRSs, show development of diabetes in males at 10 weeks of age. Recognizing that type 2 diabetes requires multiple defects, in insulin secretion as well as insulin action, White speculated that the IRS-2 knockout mice may show defects in both insulin action and insulin secretion. Indeed, IRS-1 knockout mice show greater degrees of hyperinsulinemia than mice lacking IRS-2, suggesting that they have greater insulin secretory capacity, as reflected by increased pancreatic β-cell mass. In contrast, mice lacking IRS-2 show a decrease in islet size and total β-cell mass. IRS-2 then appears to have effects on β-cell growth and survival. Other actions include effects on brain growth and reproductive function. Interestingly, mice lacking one allele of the IGF-1 receptor and one IRS-2 receptor have decreased β-cell mass, whereas this is not seen in mice lacking one allele of the insulin receptor and one allele of the IRS-2 receptor. White suggested that activation of the IGF-1 receptor mediates IRS-2 promotion of β-cell development and growth. α-Cell development is not compromised in these models. It appears that cell survival rather than growth is actually more strongly affected, with studies of apoptosis showing that IRS-2 mediates an IGF-1 effect "to keep cells alive." In a study with fascinating clinical implications, White studied the reproductive characteristics of female mice completely lacking IRS-2, showing that these mice have decreased fertility, with inhibition of ovarian follicle development in a fashion similar to that seen in human polycystic ovary syndrome, with dense interstitial tissue, thickened capsule, and small immature follicles. Interestingly, these mice also show decreased hypothalamic response to leptin administration, suggesting a role of IRS-2 in normal leptin sensitivity. The appetite-suppressing effect of leptin, then, appears to require normal insulin sensitivity, suggesting a potential vicious cycle in which obesity-induced insulin resistance may lead to leptin resistance and, hence, to further obesity.

Islet transplantation

Jonathan Lakey, Edmonton, Canada, began a symposium on islet cell transplantation at the American Diabetes Association's 48th Annual Advanced Postgraduate Course in New York, NY, January 19–21, 2001, by recalling the work of Oscar Minkowski and Joseph von Merring in 1889, who showed that the pancreas was involved in diabetes, and Watson-Williams' attempts in 1894 at pancreas transplantation for treatment of diabetes. Methods of insulin delivery that can, in principle, reverse type 1 diabetes include implantable "artificial pancreas" devices and transplantation, either of a whole or segmental pancreas graft, of which ~1,500 are performed yearly, with ~75% successful. Islet isolation and infusion into the portal vein, lodging in the liver, was performed 405 times from 1974 to 1999; 267 of these cases were performed in patients with confirmed type 1 diabetes, but with only 12 and 8% being insulin-independent at 1 week and 1 year, respectively. Most of these patients were treated with cyclosporin, azothiaprim, and steroids. This "cocktail" was modified in the Edmonton Protocol (9), which used sirolimus and tacrolimus, which have a strong complementary effect (10), and antilymphocyte globulin. Islets comprise only ~3% of the pancreas, so a variety of systems have been used for their separation and purification. The Edmonton approach uses gentle mechanical dissociation of enzymatically digested pancreas fragments, with an automated "cell processor" to separate islets from pancreatic exocrine tissue based on size and density. Fetal calf serum, used in many centers, was not used in Edmonton, so as to avoid xenoantigen formation. Functional assays are used to assure that insulin-producing cells are present. "At the end of the process," Lakey said, "we have this teaspoon full of cells that we can take to the clinic." The islets are not cultured, but are transplanted immediately, using transhepatic portal vein catheterization. At the time of the lecture, 15 C-peptide–negative patients with an average age of 39 years and diabetes duration of 31 years had been transplanted for 3–24 months. Patients were selected because of reduced hypoglycemic awareness with at least two severe episodes of hypoglycemia. None had renal failure. Two transplants were given to all patients, with the daily insulin requirement decreasing from ~0.6 to 0.2 U/kg after the first, and with insulin independence achieved after the second transplantation in most patients. Donor age averaged 44 years (range 17–71), cold storage was required for a mean of 5.5 h (1–20), and each donor provided 415,000 islets of 72% purity, in a volume of ~4 ml, with ~300,000,000 cells administered per transplant. Of these, 24% were β-cells, 10% were α-cells, and >30% were cells positive for ductal and exocrine markers. The "critical mass" of islets needed for insulin independence was ~10,000 islets/kg body wt. HbA_{1c} decreased from 8.9 to 5.9% at 3 months and to 5.6% at 6 months. Two of the initial procedures were complicated by bleeding, which has been avoided subsequently with a decreased heparin dose. Oral ulcers were initially a problem and were decreased with a different sirolimus formulation. One patient developed renal insufficiency from tacrolimus, responding to an alternative immunosuppressive regimen. Lakey discussed a multicenter trial in progress with six centers in the U.S., three in Europe, and the Edmonton center, with one goal to improve islet isolation so that a single donor, rather than two donors, will be sufficient in the future. Given the important toxicities of these agents, the development of tolerance to allow transplantation without drugs is another future goal. Lakey stressed that islet transplantation cannot be considered a cure for diabetes and that it is not now an option for type 2 diabetes. Moreover, he emphasized that children will not be trans-

planted under the protocol. He noted that only one of every six pancreases potentially available from cadavers are actually being recovered, and that although younger donors are desirable for whole pancreas, older donors can be used for islets. Engineered β-cells, stem cells, cells derived from ductal tissue, and xenogenic islet transplants may be future sources.

David Scharp, Irvine, CA, Chief Scientific Officer of Novocell, Inc., pointed out the immense discrepancy between the 5,000 organ donors in the U.S. annually and the 750,000 patients in U.S. with type 1 diabetes who would potentially benefit from islet transplantation. Although it may be possible to grow islets or genetically engineer islets, he stated that consideration must be given to the use of xenografts. Primates are endangered species and have a higher insulin requirement than humans; therefore, they would not be a good source. The mature cow makes insulin in response to fatty acids rather than glucose, and, therefore, also may not show optimal characteristics. Scharp suggested that porcine and human β-cells respond in a similar fashion, making this a feasible source. Interestingly, islets from fish can be genetically engineered to make human insulin, and may also be suitable. However, preformed antibodies exist in humans to antigens present in many other vertebrates, having potential for an immune response to all species other than primates. Immune rejection mechanisms for xenografts are complex and not well understood. Furthermore, they vary from species to species, so that effective immunosuppression regimens are difficult to formulate. These regimens can be categorized into hyperacute rejection due to preformed antibodies to blood vessels not seen with islets, which are nonvascular; accelerated and acute rejection involving cell-mediated immune responses occurring over 2- to 3-day and 7- to 10-day periods; and chronic rejection, with a variety of poorly characterized mechanisms.

When unencapsulated porcine islets are administered to diabetic Sprague-Dawley rats, there is a several-day period of restoration of euglycemia, followed by acute rejection. The T-cells causing allograft rejection are CD3/4, CD3/8, and Th-1 type–positive, whereas for xenografts, acute rejection involves CD3/4 but not CD8 Th-2 T-lymphocytes and involves many more cytokines and other cells, such as eosinophils. Rather than involving interleukin (IL)-1 and IL-2 alone, IL-4 and IL-5 are also involved in xenograft rejection. Complex immunosuppressive regimens are presently required to prevent xenograft rejection. Genetic engineering may offer an approach allowing replacement of pig antibody targets with human proteins to prevent acute rejection. An alternative approach is the creation of an immunoisolation barrier around transplanted islets. Scharp's company, Novocell, has used polyethylene glycol for the encapsulation process to allow a very thin coating around each islet with control of pore size. Encapsulated porcine islets placed intraperitoneally in diabetic Sprague-Dawley rats can produce lasting maintenance of euglycemia without evidence of immune rejection. Similar studies in diabetic primates have shown that euglycemia can be maintained for a several-week period. An important challenge for encapsulation is the biocompatibility of the polymer with the host for prevention of rejection while allowing islet nutrition and function. Clinical trials are being planned for transplantation of encapsulated human islets into patients with type 1 diabetes by using donor-specific bone marrow for tolerance induction.

An important risk concerns the transference of zoonoses, such as swine influenza, vesicular stomatitis, encephalomyocarditis, and the prion-based illness bovine spongiform encephalitis. Beyond these known illnesses, there is the risk of a recombinant virus that could lead to the development of new diseases. Such viruses may be controllable and definable by donor surveillance. The porcine endogenous retrovirus (ERV) is a single RNA strand enclosed in a glycoprotein envelope derived in part from the infected host cell. Inside the host cell, the RNA is copied into the host DNA as a permanent insertion. ERV in host DNA may direct a cell to make copies, which then can circulate within the host. Most species, including human, have such ERVs. Each porcine cell in nature has 50–100 viral genome copies, some only partially complete, which are passed to all daughter cells. Scharp described the potential problem that the porcine ERV may combine with human ERV to form a new virus capable of both causing new human disease and transmitting to other humans. This scenario occurred in the development of feline leukemia virus, derived from a bird ERV combined with that of cats.

For thousands of years, humans have cohabitated with pigs without disease of this sort. Hundreds of patients have received porcine serum, skin, or heart valves, many with immunosuppression and showing evidence of chimerism with porcine cells existing in their bodies (12). It is possible that the porcine ERV does not have the ability to infect human cells in vivo, that human cells lack an appropriate receptor, or that human immune defenses against porcine ERV are sufficient to prevent disease. Porcine ERV has been shown to infect human tumor cells in vitro and to infect immunodeficient mice, though without causing disease, based on current evidence. The preformed antibodies that cause xenorejection may represent a human defense mechanism. Presently, it is uncertain whether total cessation of xenograft studies is advisable or whether carefully controlled, limited, and protected studies should be carried out in selected patients. A number of products are planned for clinical trials, particularly in the area of neurodegenerative diseases, but Scharp noted that many research groups have halted diabetes product development at this point.

Susan Bonner-Weir, Boston, MA, discussed approaches to growing β-cells and human islets. There is a linear increase in β-cell mass with body weight in Lewis rats through 2 years of age, initially by neogenesis and replication of pre-existing cells, and after 15 months largely by hypertrophy of existing cells. β-Cells replicate, and new β-cells are formed by ductal precursor cells. Patches of newly formed insulin-producing cells derived from pancreatic ducts can be seen in humans, particularly with obesity. There is also a normal process of apoptosis of β-cells, resulting in a reduction of their number. Bonner-Weir asked, "Can we tweak the duct cells in vivo and in vitro to make new β -cells?" Glucagon-like peptide (GLP)-1 has trophic action increasing β-cell mass, and studies with the GLP-1 analog Exendin-4, which has a longer half-life, show improved glucose tolerance in rodent models and humans, with evidence of a long-term effect in increasing β-cell mass. In a 90% pancreatectomy rat model, Exendin administration lowers glucose levels and increases β-cell mass

by 40%. Glucagon positivity, a marker of β-cell neogenesis, is increased in normal rat pancreas with 1 week of Exendin treatment. In addition to duct cells, there are pluripotent "true" stem cells in adult pancreas, comprising ~1 in 100 cells of the columnar ductal epithelium. However, facultative stem cells, arising from dedifferentiation of duct cells, appear to be responsible for islet neogenesis. Bonner-Weir suggested that exocrine cells currently discarded in the pancreas islet separation process could be used for islet production. She showed studies of cultivation of human islet buds in tissue culture, with a 10- to 15-fold increase in insulin content, and with expression of neurogenin3 mRNA seen early in the genetic cascade of embryologic islet development. The duct cells show GLP-1 receptors; the use of this agent to enhance the process, as well as stimulation with other growth factors, offers a promising approach.

Christopher Newgard, Dallas, TX, the founder of the company BetaGene, Inc., discussed the potential for use of genetically engineered β-cells. Human islet progenitors could create insulin-producing cell lines that make large amounts of insulin and respond to the appropriate physiologic cues. One option would be to produce a cell line expressing both insulin and the machinery that controls insulin secretion, including GLUT2, glucokinase, the ATP-sensitive K^+ channel, and a voltage-gated Ca^{2+} channel. However, although one could use genetic engineering to install such components into preexisting cell lines, it might be difficult to get optimal glucose-sensing and insulin production; in addition, xenografting might cause sensitization and transmit illness. "The goal," Newgard said, "is to produce human cells that perform this function." He described a system aimed to identify characteristics of clones that show good versus poor insulin response to glucose. Candidate characteristics are genes that are preferentially expressed in β-cells. These include glucagon, which is produced at an intermediate stage in β-cell maturation, and the transcription factors NKX6.1 and NKX3.2, the latter preferentially expressed in the glucagon-producing cells and the former in the glucose-responsive cells. An approach termed "subtractive hybridization" analyzes differences between strongly responsive and poorly responsive β-cell populations and may allow identification of additional marker genes. Another approach is to analyze biochemical differences. Using ^{13}C-enriched glucose, nuclear magnetic resonance spectroscopy allows assessment of metabolic differences between glucose-responsive and glucose-unresponsive cells. No differences are seen in ^{13}C entering the tricarboxylic acid cycle, but pyruvate recycling appears to occur only in the responsive populations.

Immunoprotection of transplanted cells may be an option to eliminate the requirement for lifelong immunosuppression. Newgard described studies with encapsulation of engineered β-cell lines within a membrane that prevents access of T-cells to the transplanted tissue but allows insulin and waste products to be eliminated. He showed a retrievable encapsulation device that has been used in streptozotocin-induced diabetic mice. However, such a membrane would still allow access by low–molecular weight immune system mediators, such as cytokines, and it is therefore necessary to make the engineered cells resistant to such destruction. A strategy has been devised to select insulinoma cell lines resistant to IL-1β and γ-interferon by use of using multiple incubations to select for cytokine-resistance. These cells show a markedly increased expression of the transcription factor Stat-1α; overexpression of this factor reproduces the cytokine resistance, suggesting a direction for further investigation.

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