Multifaceted Therapeutic Approaches for a Multigenic Disease

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Diabetes is a severe chronic disease that affects ~200 million individuals worldwide, with extremely debilitating effects and considerably high health care costs. The two major classes of diabetes, known as type 1 (previously known as insulin-dependent or juvenile-onset diabetes) and type 2 (non-insulin-dependent diabetes), share common symptoms such as hyperglycemia and the development of long-term complications, but they differ in many aspects, including their etiopathogenesis. New insights suggest that overlapping factors, formerly considered typical hallmarks of each specific type, can coexist in the same diabetic patient, making it difficult to support a sharp distinction between the two classes and, more importantly, to adopt appropriate therapeutic solutions. In type 1 and type 2 diabetic subjects, but even more in patients with combined types, multiple genetic factors play a role in determining susceptibility or resistance to the disease, and perhaps also the time of onset, the severity of the symptoms, the possibility of developing complications and, ultimately, the response to therapy. In this review, the therapeutic treatments currently under investigation, as well as the curative strategies envisioned for future applications, are reanalyzed considering the multifaceted and complex aspects of a continuum that can be just defined as “diabetes.”

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The number of people affected by diabetes is dramatically rising in the modern societies on our planet (1). Several factors contribute to this higher incidence, including improved nutritional availability and aging, together with changes in lifestyle, characterized by extremely reduced physical activity, all contributing to an increased prevalence of obesity. The number of cases of diabetes worldwide in the year 2000 approached 170 million. This number, which includes all types of diabetes, is estimated to double by 2030 (1). In 1997, the American Diabetes Association issued new diagnostic and classification criteria (2). Type 1 diabetes (the syndrome resulting from β-cell destruction, usually leading to insulin deficiency) was subdivided into two subcategories: type 1A, or immunomediated diabetes, and type 1B, or idiopathic diabetes. Type 2 diabetes still defined the disease resulting from a progressive insulin secretory defect on the background of insulin resistance, accounting for the large majority of generally adult diabetic patients. Other specific types of diabetes were added, including ones due to genetic defects in β-cell function and genetic defects in insulin action, along with diseases of the exocrine pancreas, and drug-induced or chemically induced diabetes. Finally, the type of diabetes diagnosed during pregnancy, gestational diabetes mellitus, was considered independently. Approximately 5 million people from all age-groups have type 1 diabetes, representing ~2–3% of the world’s population of diabetic subjects. Europe contains the highest estimated number of type 1 diabetic subjects (1.27 million), followed by North America (1.04 million) and southeast Asia (0.91 million) (3). In the U.S., over 30,000 new cases of type 1 diabetes are diagnosed every year (4). Type 2 diabetes remains the most prevalent form of diabetes and typically a disease of adult age. However, a novelty of our time is the surprisingly increasing number of young individuals becoming obese and sharing type 2 diabetes symptoms (5). This new syndrome accounts for 8–45% of new pediatric cases of diabetes in some U.S. centers (6). Gestational diabetes occurs in ~1–6% of pregnancies (7), usually resolves after delivery, and represents a risk factor for developing type 2 diabetes at an older age. Finally, ~12% of type 2 diabetic case subjects also show characteristic autoimmunity signs normally seen in type 1 diabetic patients, suggesting the definition of an intermediate type of diabetes, which alludes to the overlapping between type 1 and type 2 diabetes symptomatology (8).

ETIOPATHOGENESIS OF DIABETES

Type 1 diabetes is an autoimmune disease that most frequently manifests in genetically susceptible children and adolescents (9). Type 1 diabetes is considered to be a disease where several genes determine susceptibility or resistance. Because many loci have now been found to be associated with diabetes, and there is evidence for epistatic interactions among other different loci, the prevailing opinion is that type 1 diabetes is a polygenic disorder (10). Because, however, the concordance between monozygotic twins is only ~50% (11), known and unknown environmental factors must also contribute to the disease manifestation.

The autoimmune component of this serious disorder is responsible for the selective destruction of the insulin-producing cells (i.e., β-cells) of the endocrine pancreas (12,13). β-Cell destruction occurs via the activation of autoreactive T-cell clones able to recognize and specifically attack the islet β-cells. With time, the immunemediated destruction of β-cells causes, in turn, severe insulin deficiency. Whereas autoimmune diabetes shares with other autoimmune disorders the mechanisms that lead to tissue damage, it is characterized by its own specific autoantigens. Autoimmunity can derive from defective central and/or peripheral tolerance (9). In healthy
individuals, the maturation of bone marrow–derived T-cells takes place in the thymus (4,9). In the thymus, the immature T-cells undergo a process of selection finalized at the identification of self- and non–self-reactive clones, so that the former can be blocked in the thymus, while the latter are released into the bloodstream. In the thymus, self-antigens (i.e., antigenic peptides derived from proteins of our own tissues) are presented to the immature T-cells by major histocompatibility complex (MHC) molecules. If the affinity between the MHC molecule/self-peptide and the T-cell receptor is high, the T-cell is negatively selected and will not be allowed to leave the thymus and circulate in the bloodstream. The low affinity of the T-cell receptor for both MHC molecule and peptide deprives instead the T-cell of a positive signal, hence promoting the retention of the clone in the thymus, where it eventually dies. Only the clones able to properly (i.e., with sufficient affinity) recognize the MHC molecule on the antigen-presenting cell while showing no or low affinity for the self-antigen receive the positive signal for maturation and avoid the negative selection. They are the T-cells that will circulate in the periphery, protecting our body from non-self foreign invaders (9). A serious problem arises when the affinity for the MHC molecule is high but the affinity for the self-peptide results to be erroneously low, due to the presence of an MHC molecule unable to mediate a correct antigen presentation. This autoreactive T-cell clone escapes negative selection and reaches the bloodstream, with the potential to become activated and to initiate an immune reaction toward the self-antigen it was set up to recognize (i.e., autoimmunity) (4,9). Environmental factors, such as common viral infections, can trigger, even aspecifically, quiescent autoreactive T-cell clones and consequently activate the autoimmune destruction of the islet β-cells (14,15).

Defective antigen presentation has been found to be associated with some genetic polymorphisms (10). The human MHC or HLA complex and the insulin gene (INS) are considered to be the loci (IDDM1, IDDM2, respectively) that contribute the most to type 1 diabetes susceptibility. Their link to type 1 diabetes, first proposed by epidemiological studies (16,17), has been statistically confirmed with genomic screening association and linkage studies (18–23).

Because the HLA locus accounts for 40–50% of the genetic susceptibility to type 1 diabetes and the INS locus for only ~10%, the existence of additional susceptibility loci can consequently be hypothesized (24). Fifteen additional loci, with more evidence for two genes, CTLA-4 (25–27) and SUMO4 (28), are currently under intense investigation.

The HLA complex, lodged on the short arm of chromosome 6, was first considered as a player in the diabetes etiopathogenesis when some HLA class I antigens (i.e., HLA-B8, B15), detected by serological typing, were found to be associated with the disease in an affected sib-pair analysis (29).

The most influential single hereditary factor in type 1 diabetes susceptibility, consisting of a single amino acid of the β-chain of a HLA class II molecule, was identified only in the late 1980s (30,31). The principal genetic susceptibility component was found to be any allelic form of the HLA-DQ molecule that lacks a charged amino acid at position 57 of its β-chain. Conversely, resistance to the disease is associated with the inheritance of HLA-DQ alleles containing a charged aspartic acid at the same position (Asp-57) (30,31). HLA class II molecules are known to play a major role in antigen presentation in the thymus, in which autoantigen-derived peptides are presented by the antigen-presenting cells to immature T-cells. Predispensing HLA-DQ alleles seem to be ineffective at properly binding and presenting autoantigen peptides in the context of thymic selection. In contrast, protective HLA-DQ molecules promote tolerance to β-cell molecules by eliciting a more efficient negative selection. This function of the HLA class II molecules has been confirmed in the nonobese diabetic (NOD) mouse (32). The NOD mouse spontaneously develops type 1 diabetes with etiopathogenetic characteristics very similar to the disease in humans (33). In the mouse, the histocompatibility H2 I-A molecule corresponds to the HLA-DQ molecule, while the predisposing human non-Asp57 molecule is called H2 I-Ag (7) (34). NOD mice that express transgenic class II molecules other than I-Ag (7) do not develop diabetes (35). Transplantation of allogeneic bone marrow from strains that do not develop diabetes prevents the occurrence of diabetes in NOD mice as well (36–38).

Thus, the susceptibility status can be correlated with impaired peptide presentation to T-cells, leading to an inefficient negative selection of self-reactive T clones and possibly to the reduction in positive selection of regulatory T-cells (4,39,40). However, an inappropriate self-antigen presentation can also contribute to the insufficient expression of self-proteins in the thymus (41,42). This is considered the best explanation of why the variable number of tandem repeats (VNTR) region present in the promoter of the insulin gene is the locus linked to the disease (IDDM2) and not the insulin gene itself. Evidence of different insulin expression can be found by comparing diabetes-resistant mouse strains with NOD mice (43). Insufficient self-antigen expression in the thymus of the NOD mouse seems to be directly translated in inadequate self-antigen presentation.

Unfortunately, technical limitations do not permit to identify the pool of autoreactive T-cell clones circulating in the blood of pre-diabetic individuals (44). Nonetheless, autoantibodies against β-cell markers (e.g., GAD, IA-2, and insulin), developed as an effect of the destruction of the β-cells and consequent release of antigens so far unknown (i.e., ignored) to the immune system, can be measured in the serum (45). High titers of one or more of these autoantibodies are present in 85–90% of individuals with type 1 diabetes at initial diagnosis compared with 0.5% of nondiabetic individuals (46). Autoimmunity, however, does not account for the whole population of type 1 diabetic subjects. Approximately 10% of type 1 diabetic subjects constitute the subgroup of type 1B, characterized by lack of β-cell autoimmunity markers, i.e., no autoantibodies in the serum, and fluctuating insulinopenia (47). In the majority of cases, maturity-onset diabetes of the young (MODY) results from genetic defects in β-cell function characterized by an autosomal-dominant inheritance (47). Few mutations in MODY genes (48) have been described in subjects initially classified as having type 1 diabetes but lacking high-risk HLA alleles and β-cell–specific autoantibodies. Other cases of atypical diabetes have been described in African-American children (47,49).

Also, it has been reported that insulin resistance, the typical hallmark of type 2 patients, is present in a type 1 diabetic population, and it appears to confer a more prominent risk for cardiovascular diseases (50). On the other hand, 12% of type 2 diabetic subjects show signs of
autoantibody production, and this testifies to an autoimmune component present and active also in these patients (8).

Classic type 2 diabetes is a metabolic disorder with a phenotype characterized by hyperglycemia and glucose intolerance in association with hallmarks such as insulin resistance and obesity (51). Type 2 diabetes is a very heterogeneous disorder caused by a concurrent deficit of insulin secretion or insulin action and insulin resistance. Hyperglycemia is the result of the reduced ability to absorb and use glucose in the muscles and in the liver or is consequent to higher hepatic gluconeogenesis. Conditions such as obesity and those created by prolonged use of certain types of drugs can enhance insulin resistance. A familiar trend is evident in type 2 diabetes so that some genes should play a role in predisposing individuals to insulin resistance and obesity, although only a few polymorphisms have been found that are associated and can be considered predisposing to the disease. These few and not strongly linked polymorphisms affect insulin-signaling pathways such as insulin receptor substrate 1 (52,53) or affect energy metabolism such as uncoupling protein 2 (54). Other genes encode for scavenging enzymes of reactive oxygen species, which seem to be insufficiently expressed in diabetic subjects (55).

Insulin resistance is a common pathogenetic feature of type 2 diabetes; however, hyperglycemia would not develop in the absence of a simultaneous impairment in insulin secretion. Defective insulin secretion results in turn from functional and survival faults of the β-cells. If genetic susceptibility might be at the basis of impaired insulin secretion and increased insulin resistance, chronic exposure to hyperglycemia and to high levels of circulating free fatty acids certainly contributes to both functional and survival defects (56). There is evidence that, in type 2 diabetic subjects, defective metabolic endocrine function of the pancreas is not only restricted to insulin but involves glucagon as well. Fasting plasma glucagon levels are higher in type 2 diabetic subjects than in normal individuals and defective feedback follows an oral glucose challenge. This results in a reduction of the insulin-to-glucagon ratio. Glucagon stimulates hepatic glucose output. In the presence of an altered relative proportion of circulating insulin and glucagon, basal endogenous glucose concentrations will remain higher, causing fasting hyperglycemia and an excessive postprandial glucose rise. Both β- and α-cell dysfunctions should be the target of any therapeutic approach (56).

The cellular and molecular mechanisms that cause β- and α-cell dysfunction are not fully characterized; however, a perturbation in the relative proportion of these islet cell subpopulations has been observed (57,58). More studies are necessary to clarify in detail differences in function and survival rate between α- and β-cells.

The β-cell mass is reduced, mainly because of apoptosis, since differentiation and maturation are not impaired (59). Accelerated lipolysis resulting in elevated plasma free fatty acid levels, a very common feature in the insulin-resistant state, may be important as well. In clinical settings, it has been shown that an association exists between declining β-cell function and progressively worsening hyperglycemia (60). The role of incretins in maintaining normal glucose homeostasis and in the pathophysiology of obesity and diabetes is now largely debated (61–63).

**NATURAL THERAPY: REGENERATION AND ENDOCRINE PANCREAS PLASTICITY**

The most effective recovery of the pancreatic tissue is observed in response to β-cell injury, insufficient functional β-cell mass, or increased metabolic demand. It is generally sufficient to maintain a physiological metabolic balance to indirectly prevent the occurrence of overt disease. This physiological compensatory ability of the pancreas is also known as “endocrine pancreas plasticity” and is considered a physiological response to particular conditions. Typically, endocrine pancreas plasticity is most evident in cases of obesity and in pregnancy. Increases in β-cell mass may occur through increased β-cell replication, increased β-cell size, decreased β-cell death, and differentiation of possibly existing β-cell precursors (64).

It has been shown that occasional insulin-producing cells can be found embedded in normal pancreatic ducts. However, these cells are few and far between (65). The number of these duct-associated endocrine cells physiologically increases as a consequence of severe insulin resistance, particularly in obese individuals and during pregnancy (66,67). Similar histological changes are observed under conditions of tissue injury and repair after partial pancreatectomy, duct ligation, cellophane wrapping of the gland, or interferon γ overexpression driven by the insulin promoter (68–72). The presence of peripheral insulin resistance consequent to obesity initially induces an increase of β-cell mass that can reach 150% of its normal size (73). At the time of type 2 diabetes diagnosis, however, β-cell mass is already reduced to 50% of the normal pancreatic endocrine content (74).

In type 2 diabetes, even in the absence of autoimmunity, a reduced β-cell mass seems to indicate that the endocrine pancreas is unable to compensate by adjusting β-cell size (hypertrophy), volume, and number (hyperplasia) or through functional compensatory mechanisms such as changes in thresholds of response to glucose stimulation (56). Phenomena such as glucotoxicity or lipotoxicity consequent to chronic exposure to hyperglycemia and to circulating fatty acids may also contribute to modulating the rate of β-cell mass loss. In vitro studies have demonstrated that exposure of β-cells to hyperglycemia has deleterious effects on the endocrine pancreatic function. Elevated glucose concentrations cause continued oxidative stress, which in turn can cause even more deleterious effects, particularly on β-cells. A predisposed background can account for the negative effects of even a moderate increase in glucose concentration or insulin secretion.

The physiological equilibrium between lost and newly generated β-cells can be dramatically altered in both type 1 and type 2 diabetes. However, in type 1 diabetes, the action of the autoreactive T-cell against the β-cell is quite specific and leads to an insufficient insulin production; this instance is normally described as autoimmunity. Once T-cell destructive activity overwhelms the regenerative capacity of the organ, the number of β-cells progressively decreases until they become too few to maintain the glucose homeostasis of the entire body. The time of transition over this metabolic threshold becomes immediately evident with the presentation of the characteristic signs of the clinical onset of type 1 diabetes. During the course of the disease, even if the regenerative properties of the pancreas remain functional, the continued presence of diabetogenic autoreactive T-cells consistently nullifies the reparative effort (75).
IATRISTIC THERAPY: MEASURES TO REPAIR THE FAILURE OF THE NATURAL HEALING PROCESS

There are a few possible ways to help the pancreas to activate and successfully repair Β-cell injuries or the organism to compensate for a defective hormone production or action. There is a difference between type 1 diabetes, where the Β-cells are ultimately destroyed and insulin production blunted, versus type 2 diabetes, where metabolic compensation can be achieved with even relatively simple changes in diet and exercise.

When all the physiological responses become insufficient, hyperglycemia is established and exogenous interventions are required. The replacement of insulin is the life-saving option for both type 1 and type 2 diabetic patients. However, the Diabetes Control and Complications Trial and subsequently the Epidemiology of Diabetes Interventions and Complications study have shown the limitations of insulin replacement therapy and observed that even well-controlled glycemia does not completely prevent the occurrence of secondary complications, although it can slow their development (76,77).

One of the most effective ways to prevent the development of type 1 diabetes is targeting the leading cause of it: autoimmunity. Patients with autoimmune diabetes require years to completely deplete the pool of native Β-cells and develop hyperglycemia. The only feasible way to assess the presence of Τ-cell infiltrates in the pancreas, a condition known as insulitis, and the most reliable marker of autoimmune diabetes, is achieved by histological evaluation of pancreatic biopsies. The risk associated with performing biopsies of the pancreas does not ethically justify a broad use of this approach in humans, not even in selected populations of individuals carrying genetic susceptibility markers or with significant titers of islet autoantibodies. These conditions alone cannot reliably predict the development of overt diabetes. In animal models, the progression of autoimmunity can be slowed or prevented, and, in fewer but promising studies, improvements can be achieved even after diabetes onset. However, because of the difficulties in identifying human individuals with progressive but asymptomatic autoimmune diabetes, after-onset approaches are favored for clinical trials.

In the NOD mouse, elimination of the majority of the autoreactive Τ-cells can be achieved by administration of anti-CD3 monoclonal antibodies (78). These studies offered a rationale for the launch of a multicenter clinical trial involving the administration of humanized anti-CD3 antibodies to type 1 diabetic patients, a clinical trial currently underway (79). The ability to recover a near-normal endocrine function in diabetic mice is even higher when down-modulation of autoimmunity is combined with treatments aimed at stimulating proliferation and regeneration (e.g., exendin-4) of the endocrine pancreas (63).

Transplantation of bone marrow cells from diabetic resistant mice into NOD mice before or after diabetes onset proved successful in abrogating autoimmunity, but still required sub-lethal irradiation of the recipient for the establishment of a mixed allogeneic chimerism (80,81). Once the pressure of autoimmunity is released, the endocrine pancreas was able to recover its functional properties slowly but progressively over time (80). Recovery of the islet Β-cell function is fostered by maintaining glycemia at near-normal ranges during the period after bone marrow transplantation. Because this result is usually achieved by concomitant allogeneic islet transplantation, it is possible that islet grafts provide a battery of trophic factors (such as glucagon-like peptides) critical to enhancing Β-cell function. Despite the promising results, protocols requiring irradiation of the recipients, even at low doses, are not eligible for clinical applications. On the other hand, substitution of the sub-lethal irradiation by administration of immuno-ablating compounds is not advisable because of the toxic effects on the Β-cell exerted by the majority of immunosuppressants (75).

A less dangerous yet still effective approach for autoimmune abrogation was recently used by Tian et al. (82). The authors transfected ex vivo the gene encoding a diabetes-resistant Asp-57–positive Β-chain (39,40) into the bone marrow cells isolated from the diabetic-prone NOD mouse (33). After lethal irradiation of the recipient, the cells were subsequently re-infused in the donor-become-recipient in the setting of auto-transplantation. The presence of bone marrow cells expressing the resistant MHC class II molecule, even in coexistence with NOD native diabetes-susceptible molecules, was effective in preventing insulitis and diabetes, suggesting the reestablishment of a correct negative selection of autoreactive Τ-cell clones in the thymus (4). In theory, other gene therapy protocols exist that could successfully be used and implemented in clinical trials aimed at relieving some of the patients’ problems. Although the proposition of controlling the expression of self-antigens in the thymus, either by optimizing antigen presentation or by augmenting the expression of self-antigens, such as insulin or other insufficiently represented Β-cell proteins, is technically possible, there is concern of negative clinical consequences that may derive from interfering with the physiological activities of the thymus, especially in young individuals. More promising could be the approach proposed by Tian et al. (82) once successfully applied to diabetic individuals (and not pre-diabetic subjects) and without the need for the lethal preconditioning Tian used. This type of intervention might be possible and successful only when implemented immediately after the onset of the disease (4).

When the need for an alternative approach to hormone injection becomes compelling, Β-cell replacement, achieved by either pancreas or islets of Langerhans allotransplantation, becomes the only available option. The grafted tissue is mainly obtained from cadaveric organ donors. Pancreas transplantation has shown more satisfactory metabolic control over time than islet transplantation (83). Concurrent pancreas and kidney transplantation is a procedure the American Diabetes Association suggests to consider for type 1 diabetic patients suffering from kidney failure (84). The risks consequent to whole organ surgery are unfortunately still high, and only selected centers can offer consolidated programs that warrant outstanding results. Because of the potential risks, pancreas transplant alone is only considered in select cases exhibiting severe life-threatening diabetic symptoms.

Islet β-cells are unique in their ability to sense changes in glucose and respond with insulin secretion, making them the only natural source of physiologically regulated insulin production and release. Isolated islets are the alternative to whole pancreas for transplantation, and this type of intervention is more favorable for the relatively simple and safer procedure required for graft implant into the recipient (85). The very encouraging results obtained by pioneer clinical trials culminating with the success of the Edmonton protocol (86) represent an impressive advance and have provided a selected group of patients with...
at least temporary relief from insulin injections and a possibly better lifestyle. Islet transplantation alone is currently recommended for type 1 diabetic patients with major difficulties related to achieving glycemic control and in particular the risk for hypoglycemia, so-called “brittle diabetes” (84). However, several problems limit a wider application of islet transplantation. Unfortunately, as for whole organs, islet transplantation requires the use of anti-rejection drugs for life. In type 1 diabetic islet graft recipients, immunosuppressors serve the two distinct purposes of preventing rejection and modulating autoimmunity. The transplanted islets could in fact not only be the target of rejection, but are also doomed to succumb to autoimmunity renovated attacks (87). General immunosuppression can indirectly act on both destructive mechanisms. Its efficacy, however, is not yet satisfactory and more importantly is hampered by the series of unwanted side effects that such pharmacological protocols entail. Several systemic side effects are very common after administration of immunosuppressors and include pneumonitis, suppression of bone marrow function, mouth ulcers, deteriorating renal function, diarrhea, tremor, hyperlipidemia, hypertension, weight loss, and finally a small but significant increase in the risk of malignancy (88). A number of immunosuppressants also have a direct negative effect on β-cell function and regeneration and can contribute to an increase in insulin resistance. Some clinicians argue that long-term use of maintenance immunosuppressive drugs will lead to worse outcomes than long-term insulin therapy (83,88). Successful tolerance induction to the transplanted insulin-producing cells in the absence of continuous immunosuppression is one of the strategies researchers are currently examining to resolve the problem of toxicity of the immunosuppression drugs (89). Tolerogenic protocols are based on the principle that the recipient immune system can be driven to accept or at least ignore the transplanted cells, thus allowing for their survival over time. Whether transplant tolerance is also effective in preventing autoimmune damage is not clear.

Organ availability and quality of the donor tissue are other problems that limit a more widespread use of the Edmonton protocol (86). The need for two to three donors for accumulating a sufficient islet mass for the diabetic recipient is certainly a limiting factor. The damage inflicted on the islet cells during islet isolation are such that not even one-third of the available islets of the donor organ pool can be successfully transplanted into the recipient’s liver. The negative impact of variables that apply to cadaveric human donors such as brain death, length of hospitalization, downtime, multi-drug treatment, and high glycemic peaks should also be prevented.

A possible solution to these persistent problems is offered by the use of animals instead of humans as islet donors (90). Physiologically, pig islets offer a very competitive product for β-cell replacement because of their morphological characteristics (Fig. 1), their ability to respond to glucose challenge, their cell composition, and the fact that pig differs from human insulin in only one amino acid. Xenografts entail more aggressive immune rejection than allografts but may offer putative resistance to the autoimmune attack (91). The immune rejection remains a serious and unresolved problem, despite the fact that new generations of genetically manipulated pigs were generated to reduce the immunological reactivity of humans to pig tissues. α 1-3 Galactosyltransferase (α-Gal) knockout pig tissues (pigs that, in contrast to the wild type, do not express the enzyme α-Gal on the surface of their cells) were proven able to elicit a less aggressive humoral immune response mediated by natural antibodies (anti-α-Gal antibodies) present in humans and old world monkeys and responsible, to a large extent, for hyperacute rejection (Fig. 2) (90). Modulation of xenorejection is likely to involve more epitopes than α-Gal alone, and it may require several generations of manipulated pigs to achieve clinical applicability. Before going to clinical tri-
The only group of type 2 diabetic patients to receive islet transplantation within the cohort of type 1 diabetic patients, with children perhaps the first to benefit from it.

**WOULD ISLET CELL THERAPY EVER BE RECOMMENDED TO TYPE 2 DIABETIC PATIENTS?**

The group of type 2 diabetic patients to receive islet transplants were also recipients of liver grafts. In this limited group of patients, hyperglycemia was mainly due to an incorrect functionality of the liver before transplantation, so improvements in the glycemic controls after double liver and islet transplantation could not be directly attributed to islet graft activity alone (92).

Type 2 diabetic patients include a very heterogeneous population and the treatments reflect such variability. Changes in environmental factors and lifestyles can contribute to improved glucose homeostasis and reduced insulin resistance. Oral hypoglycimants are also largely used. When insulin supplementation is required, or when other treatments are insufficient, exogenous cell supplementation may in theory help to increase in a more physiological way the functional β-cell mass. Type 2 diabetic patients that suffer from β-cell–specific defects might also benefit from the replacement of functional β-cells. Here again, a very careful evaluation of each patient, a clear identification of possibly interfering genetic backgrounds, and monitoring of the environmental factors and symptomatology that contribute to the development of diabetes will help in selecting appropriate treatments.

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