Gene Therapy

Diabetes

Diabetes mellitus is a complex metabolic condition affecting not only carbohydrate metabolism but also the use of protein and lipid. There are two main forms of diabetes, types 1 and 2, each with an apparently different pathogenesis and both resulting in hyperglycemia.

At least 90% of patients with diabetes have type 2 or non-insulin-dependent diabetes, in which a resistance to the action of insulin necessitates above-normal levels of insulin secretion. When either the resistance worsens or the insulin secretion cannot be maintained, the blood glucose rises. The cause of this form of diabetes is unknown, but it is usually associated with obesity and has an increased prevalence with age.

Most of the remaining patients with diabetes manifest a state of absolute insulin deficiency. The pathogenesis of type 1 or insulin-dependent diabetes is also incompletely understood, but there is strong evidence for a genetic predisposition, closely associated with the major histocompatibility complex genes and others, leading to an autoimmune destruction of pancreatic β cells. Both forms of diabetes are associated with early morbidity and premature death from secondary complications.

The Diabetes Control and Complications Trial established that most if not all of the microvascular and even macrovascular complications of type 1 diabetes, and by inference type 2 diabetes, can be delayed or prevented by normalizing blood glucose. Thus, prevention of these long-term sequelae is the aim of the currently recommended targets: normal preprandial blood glucose and postprandial rises to only 180 mg per dl or less with a hemoglobin A1c below 7.0%.

These are laudable goals and would be effective, but this level of blood glucose control is usually unattainable for the vast majority of patients with diabetes. Even in the DCCT, fully half of the highly motivated participants in an intensive trial failed to achieve the hemoglobin A1c goal. In clinical practice, as was true in the DCCT, people who achieve the stated blood glucose goals are at high risk for severe hypoglycemic episodes.

The goal of gene therapy in diabetes is therefore control of blood glucose in the normal range with a low risk of hypoglycemia and with minimum to no effort on the part of the patient. Although the principle of blood glucose control applies equally to both major forms of diabetes, the strategy for type 1 diabetes is quite clear: replacement of insulin. It is not clear at present that insulin alone would provide optimal therapy in type 2 diabetes.

Early attempts at insulin replacement by gene therapy have been successful in establishing the scientific principle that sufficient insulin can be produced by this strategy to re-establish normoglycemia. A retroviral vector was used to introduce the rat preproinsulin gene into the livers of normal rats after partial hepatectomy, and the rats were then made diabetic by injecting streptozotocin, a β cell toxin. Ketoacidosis was prevented in the insulin vector-treated rats, and weight was maintained over a 21-day follow-up period. In contrast, the control animals lost more than 25% of their weight within four days, and all died within six days.

Immunoreactive insulin was present in the serum of the treated rats, and serum glucagon levels were suppressed toward normal. Histological examination of the liver revealed accumulation of glycogen stores, which were depleted in the control rats. Non-fasting blood glucose levels remained in the 250 to 350 mg per dl range, and fasting blood sugars remained normal, with no evidence of hypoglycemia even after 24 hours of fasting.

The proinsulin synthesized by this construct could not be cleaved to normal insulin because the responsible proteases are not present in liver. This issue was addressed by engineering the proinsulin gene in such a way that mature insulin could be released by furin, an endogenous hepatic protease.

Infusion of 2×10^6 colony-forming units of retrovirus containing this engineered rat insulin gene construct resulted in the same absence of ketosis and normal weight gain, as well as blood glucose levels in the near-normal low 200 mg per dl range postprandially and no lower than 100 mg per dl after 24 hours of fasting. Higher viral doses were associated with normalization of glucose in the fed state, but hypoglycemia on fasting. The nephrotoxicity of streptozotocin limited the duration of these experiments to 21 days.

These preliminary experiments have served to highlight some of the problems that will need to be addressed if gene therapy is to replace insulin injection as the treatment of choice for type 1 diabetes. First, the use of the retroviral vector to deliver the insulin gene to the liver was effective but required partial hepatectomy to stimulate the cell division required for stable transduction. Such an invasive

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method would be unacceptable in human trials.

Secondly, the amount of insulin produced was dependent solely on the number of hepatocytes transduced, as insulin was produced continuously at a constant rate. While this approach could potentially be used to produce small amounts of insulin to prevent sudden death from ketoacidosis, attempts to provide enough insulin to prevent postprandial hyperglycemia would undoubtedly also result in fasting hypoglycemia.

Finally, after stable gene transduction, the production of insulin would be expected to continue as long as the hepatocytes survive. Thus, if the amount of insulin production had to be reduced, another partial hepatectomy would be required. These three areas, noninvasive insulin gene transduction, regulated insulin production, and a fail-safe mechanism short of hepatectomy, will need to be advanced before insulin gene therapy can replace insulin by injection or even pancreas transplants.

An ideal vector for insulin gene delivery would be nontoxic, targeted to the liver and other insulin-sensitive tissues, capable of transducing enough cells to ameliorate diabetes after peripheral injection, and immunologically silent. Viral-mediated gene transfer is ideal in diabetes because the current vectors are largely or completely taken up by the liver after systemic delivery. At present, however, none of the available viral vectors meet the criteria just listed for use in humans. Research is under way in a number of laboratories, and progress has been rapid, so a suitable vector system should be available in the near future.

Gene therapy for diabetes must also deal with the episodic nature of endogenous insulin secretion. In the fasting state, insulin secretion is low, but postprandially the levels in the portal circulation rise at least 100-fold within minutes, resulting in the exquisitely regulated disposal of ingested glucose. Duplication of all of the components of this mechanism may not be necessary to markedly improve the glucose excursions in patients with type 1 diabetes.

Production of insulin after gene therapy must rely on synthetic mechanisms in target cells because in diabetes, the normal storage of insulin in secretory granules of pancreatic β cells is absent. In the case of the liver, most secreted proteins are synthesized in hepatocytes, packaged as membrane-bound vesicles that move rapidly to the cell membrane, and secreted by exocytosis. The first problem, the production of mature insulin from its precursor, has been solved as already noted.

Hence the production of insulin after gene transduction into liver cells is dependent upon the specific promoter regions designed into the genetic construct. The challenge for insulin gene therapy will be to identify promoters whose activities are modified by the ambient levels of glucose or insulin or both. Several promoters are known to contain negative insulin response elements, and some of these have been shown to result in transgene regulation by insulin in cultured liver cells. It remains to be determined whether control of the transduced insulin gene by one or more of these control elements will be sufficient to meet the twin goals of avoiding postprandial hyperglycemia and fasting hypoglycemia.

The final concern that needs to be addressed before insulin gene therapy can be considered in human type 1 diabetes is the issue of overdosing. This proved to be important even during the retroviral transduction experiments, when rats given the highest dose of virus died of hypoglycemia even though only a minority of hepatocytes were transduced. In humans, an overdose of insulin gene therapy, producing hypoglycemia, might be ameliorated only by partial hepatectomy. A less drastic alternative must be identified.

Perhaps this problem will also be solved by finding a promoter that can effectively regulate transgene expression. Alternatively, multiple small doses of virus could be given until the optimum effect is achieved. Another approach could be to include a "suicide gene" in the same expression cassette as insulin. In the event of overexpression of insulin, the antiviral agent ganciclovir would cause death of some of the insulin-producing cells and restoration of normoglycemia.

Somatic cell gene therapy may prove to be an excellent method to help improve metabolic control in type 1 diabetes. However, because diabetes is no longer a life-threatening disease, significant advances over current technologies will be needed before gene therapy can be used. The rate of progress in this field has been rapid, and it is hoped that the problems detailed here will be solved in the near future.

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