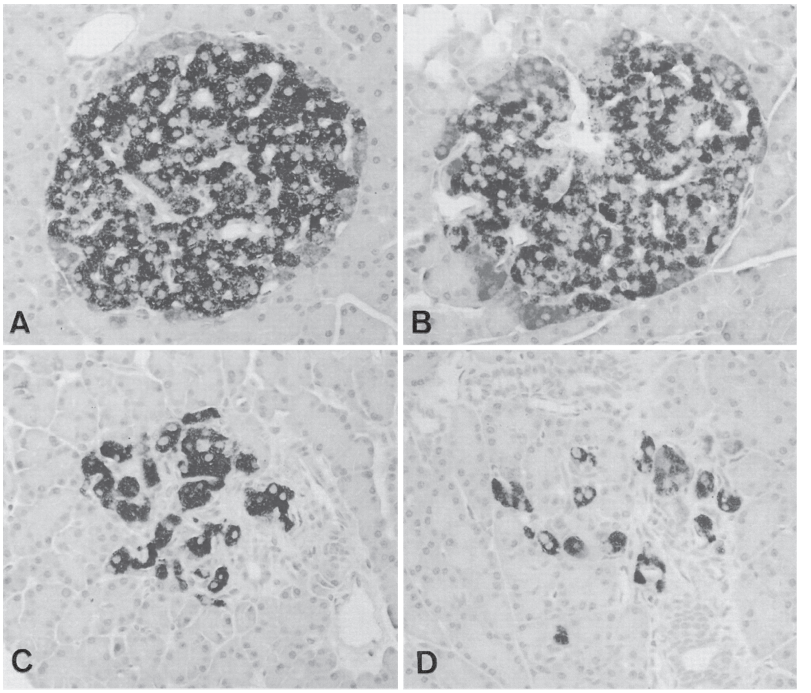
19

PANCREATIC REGENERATIVE ENGINEERING



Islet structures and distribution of insulin-positive cells (black) and glucagon-positive cells (red) in Wistar rat (A), sucrose-fed Wistar rat (B), Goto–Kakizaki (GK) rat (C), and sucrose-fed GK rat (D) at 12 weeks of age. Note that the GK rat is a spontaneously diabetic animal model of non-insulin-dependent diabetes mellitus, which is characterized by progressive loss of β cells in the pancreatic islets with fibrosis. There is marked islet fibrosis with β-cell depletion in GK and sucrose-fed GK rats, in which the latter showed more severe changes. Double immunostaining for insulin and glucagon. Magnification ×300 (A–D). (Reprinted with permission from Koyama M et al: Accelerated loss of islet β cells in sucrose-fed Goto-Kakizaki rats, a genetic model of non-insulin-dependent diabetes mellitus, *Am J Pathol* 153:537–45, copyright 1998.) See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

ANATOMY AND PHYSIOLOGY OF THE PANCREAS

Structure [19.1]

The pancreas is an organ located in the upper abdominal cavity, behind the stomach, and between the spleen and duodenum. The pancreas consists of two functional systems: the endocrine and exocrine systems. The *endocrine system* is composed of the islets of Langerhans, which produce and secret insulin, glucagon, and somatostatin into the blood (Fig. 19.1). In the human pancreas, there are more than 1 million of Langerhans islets. These islets contain several types of cell, including the α, β, and δ cells. The *exocrine system* is composed of secretary units known as *pancreatic acini*, which contain exocrine cells. These cells produce and secrete enzymes for the digestion of proteins, carbohydrates, and fats. The pancreatic acini are connected to small ductules, which converge to larger ducts and eventually to the pancreatic duct. The pancreatic duct system conducts pancreatic juice to the duodenum, where proteins, carbohydrates, and fats are digested.

Functions of the Pancreatic Endocrine System [19.1]

The endocrine α, β, and δ cells of the Langerhans islets produce three hormones, including glucagon, insulin (chapter 19 cover page), and somatostatin, respectively. *Insulin* is a hormone that participates in regulating the metabolism of carbohydrates, fats, and proteins. Insulin is initially produced in the form of preproinsulin, which is cleaved into

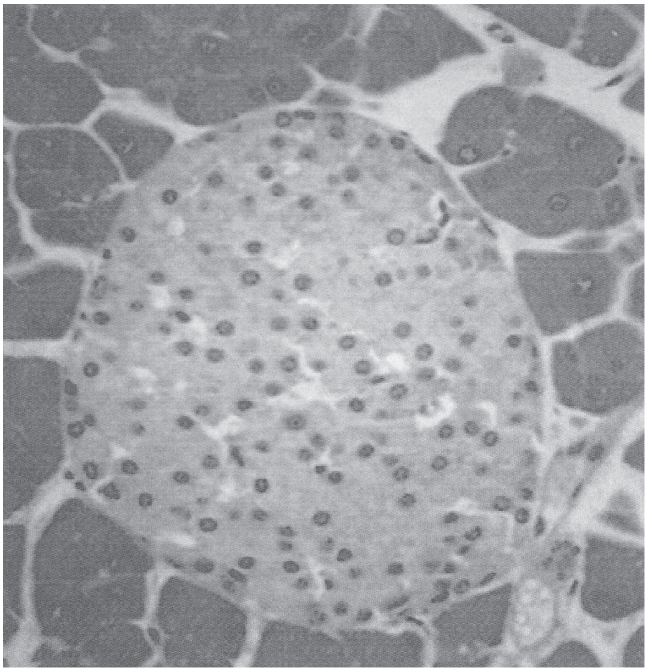


Figure 19.1. Histological micrograph of pancreatic islet from a wildtype mouse (C57BL/6J). Magnification ×200. (Reprinted with permission from Barlow SC et al: *Am J Pathol* 165:1849–52, copyright 2004.)

pro-insulin and then into insulin. Insulin is stored in the secretory granules. The release of insulin is triggered in response to an increase in the level of blood glucose after food ingestion. Once in the blood, insulin stimulates glucose uptake, metabolism, and storage by almost all cell types, thus reducing the level of blood glucose. There are several mechanisms for these processes. In the skeletal muscular system, insulin enhances glucose transport from the blood into the muscular cells. Excessive glucose is converted to glycogen for glucose storage. In addition, insulin stimulates the liver cells to take up glucose, which is converted to glycogen in the liver. This is a rapid process when the level of blood insulin is increased following food ingestion. When insulin is degraded and blood insulin is decreased, the stored glycogen is converted to glucose, an important mechanism for the maintenance of the blood glucose level. The lack of insulin results in a persistent increase in blood glucose, a pathological disorder known as diabetes mellitus (see page 822 of this chapter). After released into the blood, insulin can be degraded within about 15 min, a mechanism by which glucose metabolism can be effectively controlled.

Insulin plays a role in the regulation of fat metabolism. Insulin stimulates the synthesis of fatty acids from glucose. When the glycogen level reaches a critical level, excessive glucose is used to synthesize fatty acids and triglycerides in the liver cells. Insulin mediates the storage of fatty acids and triglycerides in adipocytes. In the absence of insulin, fatty acids are metabolized and used for energy production. Insulin also participates in the regulation of protein metabolism. Insulin stimulates protein synthesis and storage, which occur primarily following food ingestion. There are several mechanisms for these processes. Insulin enhances the transport of amino acids into the cell, stimulates mRNA transcription and protein translation, and inhibits protein degradation. Thus, the lack of insulin not only influences carbohydrate metabolism, but also fat and protein metabolism.

Glucagon is a hormone produced by the α-cells of the Langerhans islets. Its functions are opposite to those of the insulin. Glucagon is capable of degrading glycogen to produce glucose and enhancing gluconeogenesis from amino acids, thus increasing the level of blood glucose. Glucagon is activated when the blood glucose level reduces to a critical level, and suppressed when blood glucose is increased to a critical level. *Somatostatin* (SST or SMST) is a hormone produced by the δ cells of the Langerhans islets. It is first generated as a preproprotein (116 amino acids, about 13 kDa in molecular weight). The preproprotein is cleaved into two active forms of somatostatin: 14- and 28-amino acid peptides. The primary function of somatostatin is to inhibit the secretion of insulin and glucagon via interacting with the G-protein-coupled somatostatin receptors. This hormone is activated by an increase in blood glucose, amino acids, and fatty acids. Somatostatin also interacts with pituitary growth hormone and hormones produced by the gastrointestinal tracts and enhance the function of these hormones. Taken together, insulin, glucagon, and somatostatin coordinately control the level of blood glucose, amino acids, and fatty acids, ensuring appropriate metabolism of these nutrients.

Functions of the Pancreatic Exocrine System [19.1]

The acini of the pancreatic exocrine system produce a number of enzymes, including trypsin, chymotrypsin, polypeptidase, amylase, lipase, cholesterol esterase, phospholipase, and nuclease. Trypsin and chymotrypsin are responsible for the digestion of proteins into

peptides. Polypeptidase can digest peptides into amino acids. Pancreatic amylase hydrolyzes glycogens and starches into disaccharides and trisaccharides. Pancreatic lipase can break down fats into fatty acids and glycerides. Cholesterol esterase and phospholipase are responsible for the digestion of cholesterol and phospholipids, respectively. Deoxyribonuclease and ribonuclease can digest DNA and RNA, respectively. It is important to note that the exocrine enzymes are initially produced in inactive forms, which are activated by enzyme cleavage in the small intestine. For example, the inactive forms of trypsin and chymotrypsin are trypsinogen and chymotrypsinogen, respectively. These inactive proenzymes are produced in the pancreas and secreted into the intestine, where the proenzymes are cleaved by trypsin and enterokinase, resulting in the activation of these enzymes.

The epithelial cells of the pancreatic ductules and ducts can produce and secret sodium bicarbonate, which plays a critical role in neutralizing the acidic solution secreted by the stomach. The formation of sodium bicarbonate involves mechanisms of ion transport across the epithelial cells of the pancreatic ductules and ducts. The source of bicarbonate is carbon dioxide, which diffuses from the blood to the epithelial cells. Under the action of an enzyme called *carbonic anhydrase*, carbon dioxide reacts with water to form carbonic acid, which is further dissociated into bicarbonates and hydrogen ions. The bicarbonate ions are transported into the pancreatic ductules and ducts. The hydrogen ions are transported into the blood in exchange with sodium transport in the opposite direction. The sodium ions are secreted into the pancreatic ductules and ducts and react with bicarbonate to form sodium bicarbonate.

The secretion of pancreatic exocrine enzymes and sodium bicarbonate is regulated by several substances, including acetylcholine, cholecystokinin, and secretin. *Acetylcholine* is a neurotransmitter for the parasympathetic nervous system. The ingested foods, when entering the small intestine, can activate the parasympathetic nerves to release acetylcholine, which stimulates the pancreatic acinar cells to secret exocrine enzymes. Similarly, the ingested foods stimulate the release of *cholecystokinin* by the epithelial cells of the duodenum. This substance stimulates the acinar cells of the pancreas to secret exocrine enzymes. *Secretin* is released by the epithelial cells of the duodenum when acidic foods enter the duodenum. This substance stimulates the secretion of sodium bicarbonate by the pancreatic ductule cells.

PANCREATIC DISORDERS

Diabetes Mellitus

Pathogenesis, Pathology, and Clinical Features [19.2]. *Diabetes mellitus* is a metabolic disorder induced by decreased or abolished secretion of insulin from the pancreatic β cells and characterized by an increase in the level of blood glucose. Diabetes is also associated with abnormalities in the metabolism of glucose, fatty acids, and proteins as well as the formation of pathological lesions in blood vessels (arteriosclerosis). During the end stage, acidosis and diabetic coma may occur. Diabetes is a relatively common disorder. The prevalence of the disorder is about 1%. Diabetes is often associated with obesity, hypertension, hyperlipidemia, and atherosclerosis. However, the cause-and-effect relationship between these disorders remains poorly understood.

Diabetes is classified in to two types: primary and secondary diabetes. *Primary diabetes* is defined as diabetes that is not induced by other diseases and is further divided

into two subtypes: insulin-dependent and non-insulin-dependent diabetes. Primary diabetes is also defined on the basis of pathogenic mechanisms related to the involvement of immune reactions. Immune reaction-mediated diabetes is defined as type I diabetes, whereas non-immune-reaction-mediated diabetes is defined as type II diabetes. Type I diabetes may include insulin- and non-insulin-dependent diabetes. Type II diabetes is usually non-insulin-dependent. *Secondary diabetes* is defined as diabetes induced by other diseases and is further divided into several subtypes: diabetes due to pancreatic diseases, such as pancreatitis and cancers, chemical toxicity, hormonal abnormalities, and genetic mutation. Chronic pancreatitis is often associated with diabetes due to the involvement of the β cells. Certain drugs and chemicals may impair the function of the β cells and reduce the production and release of insulin. Hormonal abnormalities such as Cushing's syndrome, pheochromocytoma, and administration of steroid hormones, may cause malfunction of the β cells, leading to a reduction in insulin release. Several genetic disorders, including myotonic dystrophy, lipodystrophy, and ataxia–telangiectasia, are often associated with the impairment of the β cells, reducing the secretion of insulin.

The pathogenic mechanisms vary among different types of diabetes. Primary type I insulin-dependent diabetes is a disorder potentially induced by autoimmune reactions. In patients genetically susceptible to diabetes, viral infection activates the host immune system. Certain viruses may contain antigens that are similar in structure to the membrane components of the pancreatic β cells. Virus-activated T cells may infiltrate into the islets of Langerhans and attack the β cells that contain proteins similar to the viral antigens, resulting in β-cell destruction and insulin deficiency. The pathogenic mechanisms of non-insulin-dependent primary diabetes remains poorly understood. It has been thought that this type of diabetes may be a result of genetic disorders. Gene mutation may play a role in the initiation and development of non-insulin-dependent primary diabetes. In patients with this type of diabetes, abnormal insulin secretion is often found. In addition, cells are usually resistant to insulin, meaning that insulin is no longer effective in the regulation of glucose metabolism. A reduction in the density of insulin receptor and intracellular disorders of glucose metabolism may be responsible for the pathogenesis of non-insulin-dependent primary diabetes. In *secondary diabetes,* the destruction of the β cells due to pancreatic diseases as listed above is responsible for the deficiency of insulin and the pathogenesis of diabetes.

In diabetes, there are several common pathophysiological changes regardless the types and causes of the disorder. The β cells are often committed to apoptosis, a major cause for insulin deficiency (Fig. 19.2). Because of insulin deficiency, the level of blood glucose increases, a change known as *hyperglycemia*. When the blood glucose content exceeds a critical level (about 180 mg/dL), glucose is excreted from the kidneys. Because the presence of glucose increases the osmotic pressure in the renal tubules, which reduces tubular reabsorption and enhances urea formation, osmotic diuresis often occurs, resulting in increased urination. In severe cases, diabetes may induce two acute complications: ketoacidosis and hyperosmotic coma. Ketoacidosis is usually found in insulin-dependent diabetes and results from cessation of insulin administration. Because of insulin deficiency, the utilization of glucose is reduced. As a compensating mechanism, fatty acids are mobilized from fat-storage tissue (liver and adipose tissue) and used for energy production. The metabolism of fatty acids generates several acidic substances, including keto acids and acetoacetic acid, resulting in an increase in the serum concentration of hydrogen ions, a condition known as acidosis. The acidic environment induces impairment of cell functions. When the serum pH reduces to a critical level, the central nerve cells are injured,

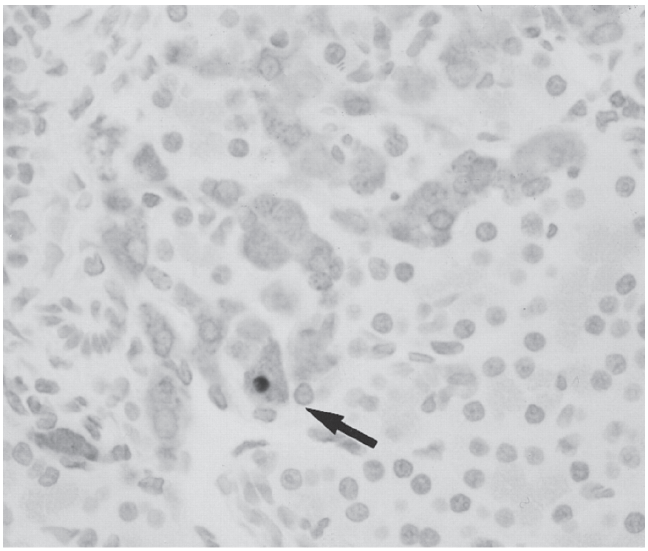


Figure 19.2. Apoptotic β cells (arrow) (black nucleus) in sucrose-fed Goto–Kakizaki (GK) rat, a spontaneously diabetic animal model of non-insulin-dependent diabetes mellitus, at 12 weeks of age detected by the TUNEL method. Apoptotic β cells were found only in sucrose-fed GK rats, not in GK and Wistar rats. Double staining is shown for β cells positive for insulin (red) and apoptosis (black). Magnification ×480. (Reprinted with permission from Koyama M et al: Accelerated loss, of islet beta cells in sucrose-fed Goto-Kakizaki rats, a genetic model of non-insulin-dependent diabetes mellitus, *Am J Pathol* 153:537–45, copyright 1998.)

resulting in acidosis coma. In addition, hyperglycemia is associated with an increase in the osmotic pressure in the serum. This osmotic change exerts a dehydration effect on the cells, mobilizing water from the interior to the exterior of the cells. Hyperosmotic coma occurs when the central nerve cells are injured due to overdehydration.

Long-term diabetes is associated with chronic complications in the vascular system. A major complication is arteriosclerosis. The incidence of arteriosclerosis in diabetics is much higher than that in the general population. Arteriosclerotic lesions are often found in the arteries of the heart, brain, kidney, and extremities of patients with diabetes. Pathological changes of the atherosclerotic lesions are similar to those described in Chapter 15. Clinical consequences of these changes include cardiac ischemia and infarction, stroke, renal ischemia, and extremity ulcers and gangrene. In addition to pathological lesions in large arteries, other blood vessels including small arteries, arterioles, and capillaries also undergo pathological changes in diabetes. A typical example is retinopathy. In this case, diabetes induces an increase in the permeability of the retinal capillaries, which is followed by gradual destruction and occlusion of the capillaries. The capillary lesions are associated with scattered hemorrhages in the retina. These pathological changes induce proliferative reactions, fibrosis, and scar formation, leading to retinal detachment and blindness. However, the pathogenic mechanisms of diabetic retinopathy remain poorly understood. Another example is nephropathy, one of the leading causes of death due to diabetes. In this disorder, diabetes induces thickening of the basement membrane of the glomerular blood vessels, and hyalinization and occlusion of glomerular arterioles. These

lesions are collectively defined as *glomerulosclerosis*. The consequences of this disorder are renal dysfunction and failure.

Experimental Models of Diabetes Mellitus [19.2]. Experimental diabetes can be established in rodents by intravenous injection of an antineoplastic biotic streptozocin (2-deoxy-2-[(methylnitrosoamino)carbonyl]amino-D-glucopyranose), which is derived from *Streptomyces achromogenes*. This substance induces the destruction of pancreatic β cells, inducing experimental diabetes. In the rat and mouse models, the substance can be injected into the femoral vein. One injection is sufficient for the induction of diabetes. Blood glucose can be measured with a glucose sensor at desired timepoints. An increase in the blood glucose level can be seen within 5 days. It is important to note that streptozocin is only effective in rodents and does not induce diabetes in large animals and humans.

Another model is pancreatectomy or the removal of the pancreas. To create a pancreatectomy model, an animal is anesthetized by peritoneal injection of sodium pentobarbital at a dose of 50 mg/kg body weight. The upper abdominal skin is sterilized with 75% alcohol, Betadine, and 75% alcohol again. The abdominal cavity is opened at a location in the upper middle area and the pancreas is identified. The pancreatic blood vessels are tied off with surgical sutures, the pancreas is removed, and the abdominal wound is closed. At scheduled times following the surgery, blood glucose level can be measured with a glucose sensor.

Conventional Treatment of Diabetes [19.3]. There are several strategies for the treatment of diabetes. These include dietary control, insulin administration, and managements of complications, if any. The most important treatment is to control diet and to prevent obesity. The total calories necessary for each patient should be estimated on the basis of accepted standards, which are about 40 kcal (1000 calories) per kg body weight per day for youths and about 35 kcal per kg body weight per day for adults. Insulin administration is necessary for all type I diabetics and is recommended for type II diabetics. Such a treatment significantly reduces or prevents the occurrence of diabetic complications such as arteriosclerosis, retinopathy, and nephropathy. Insulin can be administrated via muscular and subcutaneous injections or mechanical pump-mediated subcutaneous insulin infusion. The insulin infusion method provides a sustained injection of insulin at a constant rate, an effective approach for the achievement of a relatively stable concentration of blood glucose. Treatments for common diabetic complications, including arteriosclerosis, retinopathy, and nephropathy are discussed in Chapter 15, 20, and 23, respectively. In the case of gangrene, it is often necessary to carry out amputations.

Molecular Regenerative Engineering. As discussed above, the administration of insulin is effective in the treatment of diabetes. However, it is difficult to achieve a rate of insulin delivery that matches or simulates the physiological insulin profile. Often, blood insulin concentration overshoots the physiological level immediately following insulin injection, resulting in rapid development of hypoglycemia. Since insulin is rapidly degraded in the blood, hyperglycemia occurs when the concentration of blood insulin is below the physiological level. Even though the pump-mediated insulin infusion method provides insulin delivery at a constant rate, the insulin level is often insufficient for the metabolism of glucose immediately following food ingestion, but exceeds the physiological level in the fasting state. To date, a method that controls insulin release in response to the blood glucose level is not available.

Molecular regenerative engineering or therapy has been proven a potential approach to control glucose metabolism. Several strategies have been established for the molecular treatment of diabetes, including the enhancement of glucose uptake and storage, inhibition of glucose production, facilitation of insulin synthesis, promotion of the survival and proliferation of the pancreatic β cells, and suppression of autoimmune processes.

Enhancement of Glucose Uptake and Storage and Inhibition of Glucose Production [19.4]. Glucose is stored in the form of glycogen. Glycogen formation is a process mediated by enzymes. A critical enzyme is glucokinase, which catalyzes the phosphorylation of glucose in the presence of ATP, forming glucose-6-phosphate (see Table 19.1). Glucose-6-phosphate can be catalyzed to form glucose-1-phosphate, which is further converted to uridine diphosphate glucose. Uridine diphosphate glucose is the final form used for the synthesis of glycogen. Thus an increase in the glucokinase activity enhances glycogen synthesis and reduces the level of blood glucose. Mutations of the glucokinase gene have been shown to induce non-insulin-dependent diabetes mellitus (NIDDM), also known as type 2 maturity onset diabetes of the young (MODY2). The transfer of the glucokinase gene into the liver and skeletal muscles results in over-expression of the glucokinase gene and enhances glycogen synthesis. The enhancement of glycogen synthesis is associated with an increase in glucose uptake and a decrease in glucose production from the stored glycogen in the cells, thus lowering the blood glucose concentration. In general, genes encoding proteins that facilitate glycogen synthesis can all be used for the molecular treatment of diabetes.

Facilitation of Insulin Synthesis and Activation [19.5]. Insulin is produced in the pancreatic β cells by several processes. The translation of insulin mRNA generates preproinsulin (~12 kDa), which is cleaved in the β cells to form proinsulin (~9 kDa). A large fraction of proinsulin (~80%) is further cleaved in the β cells to form insulin (~6 kDa), while the remaining proinsulin is released into the blood. One of the molecular approaches for treating diabetes is to facilitate the expression of insulin gene in diabetes. The transfer of the insulin gene into the pancreatic β cells enhances the expression of preproinsulin, leading to an increase in the production of insulin. An important aspect in molecular treatment of diabetes is to enhance the responsiveness of the β cells for releasing insulin upon an increase in the blood glucose level. In experimental investigations, several

TABLE 19.1. Characteristics of Glucokinase*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Glucokinase	GK, GCK, GLK, hexokinase 4 (HK4)	466	52	Liver, skeletal muscle, pancreas	Phosphorylating glucose to produce glucose-6-phosphate and promoting the formation of glycogen

*Based on bibliography 19.4.

glucose-responsive gene promoters, including those from the glucose-6-phosphatase gene and L-pyruvate kinase gene (see list of proteins in Table 19.2), have been used for such a purpose. These investigations have demonstrated that the overexpression of these gene promoters by gene transfer enhances the responsiveness of the insulin gene upon the stimulation of increased blood glucose. However, there is always a lag of several hours in insulin release following the stimulation. This period is necessary for gene transcription and protein translation.

Another approach to enhance insulin activity is to activate proinsulin in the liver and other tissues. As discussed above, about 20% insulin exists in the form of proinsulin, which does not have insulin activity. The activation of the proinsulin may significantly increase the activity of insulin. In pancreatic β cells, the proinsulin is converted to insulin by proinsulin convertases. However, other types of cell are not able to conduct such a function because of the lack of these conversion enzymes. To solve such a problem, researchers have engineered the structure of the insulin gene by adding proteolytic target sites for proteases. An example of such proteases is furin, also known as *paired basic amino acid cleaving* enzyme and proprotein convertase subtilisin/kexin type 3 (794 amino acids and 87 kDa in molecular weight). Furin is expressed in the liver cells and can convert precursor proteins to their active forms by cleavage at their paired basic amino acid sites. When a target encoding site for furin is inserted into the insulin gene at an appropriate location and the modified insulin gene is transferred into the liver cells, the proinsulin proteins can be cleaved by furin to form insulin. This is a potential method that can be used to activate proinsulin, thus enhancing the total activity of insulin. In addition to proinsulin, furin can cleave other protein precursors such as proparathyroid hormone, transforming growth factor β1 precursor, proalbumin, pro-β-secretase, membrane type 1 matrix metalloproteinase, the β subunit of pronerve growth factor and von Willebrand factor.

Promotion of Survival and Prevention of Apoptosis of β-Cells [19.6]. The apoptosis of pancreatic β cells is a major cause for type I and type II diabetes. Thus it is essential to prevent apoptosis and promote the survival and proliferation of the β cells. Adult pancreatic β cells can regenerate through two mechanisms: β cell proliferation and differentiation of stem and progenitor cells into β-cells. The latter is referred to as β-*cell neogenesis*. β-cell proliferation and neogenesis are regulated by a number of growth factors, including insulin-like growth factor (IGF)1, growth hormone (GH), epithelial growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), nerve growth factor (NGF), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF). These growth factors also play a role in the prevention of cell apoptosis. The genes encoding these growth factors can be used for molecular treatment of diabetes by gene transfer. The characteristics of these growth factors are described in Chapter 15.

Among the growth factors listed above, the role of insulin-like growth factor 1 in the regulation of β-cell survival and proliferation have been investigated extensively. Insulin-like growth factor 1 interacts with its receptor, inducing activation of the receptor tyrosine kinase in the cytoplasmic domain. The receptor induces tyrosine phosphorylation of insulin receptor substrate (IRS) family members (primarily IRS2) and a Src family member Shc. The phosphorylated tyrosine residues serve as docking sites for the recruitment of downstream signaling molecules, including Grb2 and PI3 kinase. Grb2 is coupled to the Ras-MAPK signaling pathway. The Ras-MAPK and PI3 kinase signaling pathways

TABLE 19.2. Characteristics of Glucose-6-Phosphatase and L-Pyruvate Kinase*

Protein	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Glucose-6-phosphatase	G6PC, G6Pase, G-6-Pase	357	41	Liver, skeletal muscle, brain, kidney	Catalyzing the hydrolysis of D-glucose 6-phosphate to D-glucose and orthophosphate
L-Pyruvate kinase	Pyruvate kinase liver and RBC, pyruvate kinase type L, pyruvate kinase liver and blood cell	574	62	Liver, red blood cells	Catalyzing the formation of phohsphoenolpyruvate from pyruvate and ATP and causing chronic hereditary nonspherocytic hemolytic anemia (CNSHA) when mutated

*Based on bibliography 19.5.

are described in Chapter 5. The activation of these pathways promotes the transcription of mitogenic genes, prevents cell apoptosis, and enhances cell survival and proliferation. Investigations with transgenic and gene transfer models have demonstrated the role of insulin-like growth factor-1 in promoting the survival of the β cells (Fig. 19.3). The overexpression of the insulin-like growth factor 1 gene in a transgenic mouse model suppresses hyperglycemia when the mouse was administrated with streptozocin, a substance causing β-cell death and diabetes. The administration of the same dose of streptozocin to wild-type mice induced a significantly higher level of hyperglycemia (Fig. 19.4). The overexpression of the insulin-like growth factor 1 gene also extended the lifespan of the mice with streptozocin-induced diabetes (Fig. 19.4).

The gene of insulin-like growth factor 1 is a potential candidate for the molecular treatment of human diabetes. In addition, genes that encode signaling factors, such as

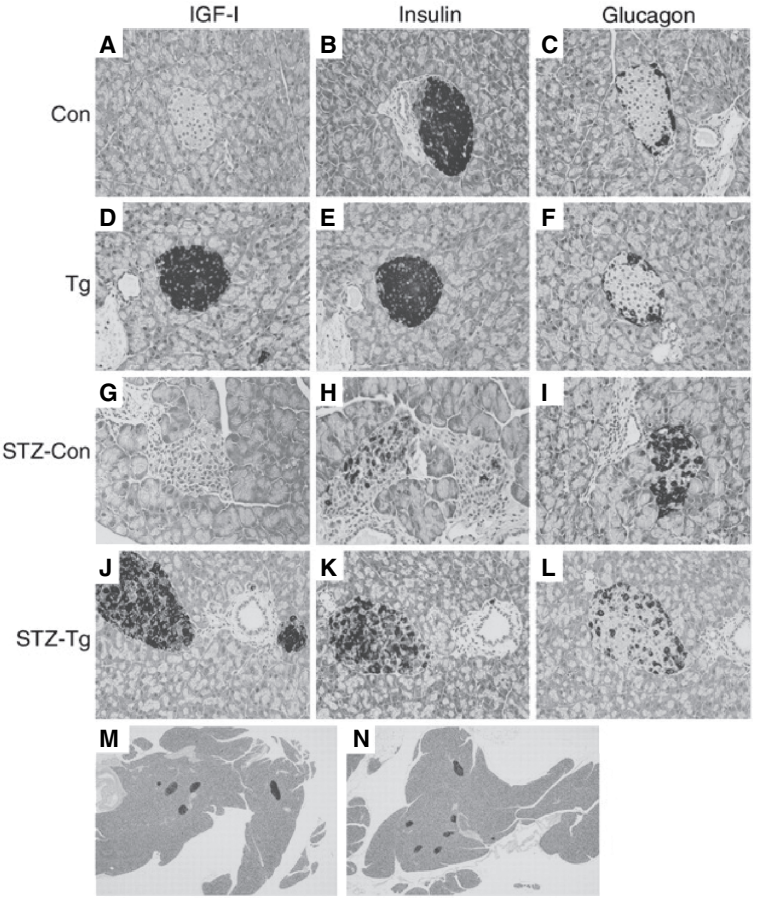


Figure 19.3. Immunohistochemical analysis of insulin growth factor I (IGF-I), glucagon, and insulin expression in pancreatic islets. IGF-I (A,D,G,J), insulin (B,E,H,K,M,N), and glucagon (C,F,I,L) staining of representative sections of pancreas before (A–F) and 3 months after (G–N) STZ treatment. Wildtype mice (Con): A–C, G–I (×400) and M (×40); C57BL/6–SJL transgenic mice (Tg, overexpressing IGF-I): D–F, J–L (×400) and N (×40). (Reprinted with permission from George M et al: *J Clin Invest* 109:1153–63, copyright 2002.)

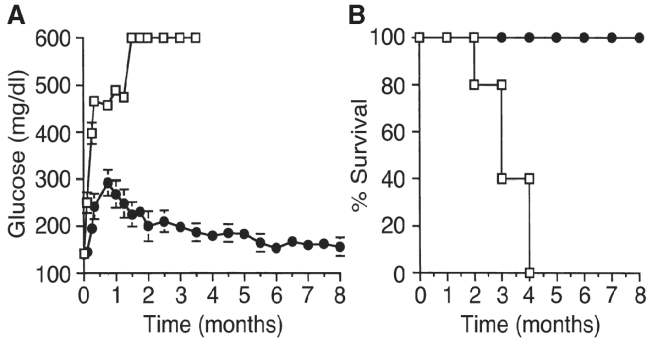


Figure 19.4. (A) Blood glucose levels in wildtype mice and C57BL/6–SJL transgenic mice, which overexpress insulin growth factor-I (IGF-I), after STZ treatment. Squares, wildtype mice ($n = 15$); circles, transgenic mice ($n = 15$). (B) Percent survival of control (squares; $n = 20$) and transgenic (circles; $n = 20$) mice after STZ treatment. Results are mean ± SEM of the indicated mice. (Reprinted with permission from George M et al: *J Clin Invest* 109:1153–63, copyright 2002.)

MAPK and protein kinase B (PKB; note that PBK is a molecule downstream to insulin receptor substrate 2), can be potentially used for molecular therapy.

There are several genes encoding proteins that regulate the differentiation of stem and progenitor cells to pancreatic β cells. One of such genes is the insulin promoter factor 1 gene (IPF1), also known as *pancreatic duodenal homeobox gene 1* (Pdx1) and somatostatin transcription factor 1 (STF1). This gene encodes the insulin promoter factor 1 protein (283 amino acids, 31-kDa), which is expressed in the pancreas, brain, and intestine. This protein promotes the differentiation of stem and progenitor cells to insulin-producing β cells, stimulates pancreatic development, and activates the transcription of the insulin and somatostatin genes. When this gene is transferred into the liver cells in animal models, its protein product stimulates the formation of β cells and the expression of insulin in these cells.

Another gene is the neurogenic differentiation factor 1 (NeuroD1; see Table 19.3) gene that encodes a protein for the regulation of β-cell differentiation. In humans and mice, the deficiency of this gene results in pathological changes found in diabetes. In experimental models of diabetes, the transfer of the NeuroD gene into the mouse liver induces the formation of pancreatic islets. The islet cells can produce insulin, glucagon, and somatostatin. As a result, the level of blood glucose is restored and diabetic changes are reduced. Fibroblast growth factor (FGF) has also been shown to regulate the development and survival of pancreatic β cells. The genes of insulin promoter factor 1, NeuroD, and FGF can be considered potential genes for the molecular treatment of human diabetes.

Suppression of Autoimmune Processes [19.7]. Autoimmune reactions, which are immune processes directed against host cells, play a critical role in the induction of type I diabetes. Thus, an important strategy in molecular treatment for diabetes is to suppress autoimmune reactions. Cell types that are directly involved in autoimmune reactions are antigen presenting dendritic cells and antigen-specific T cells. These cell types are the targets of molecular therapy for autoimmune disorders. Antiautoimmune cytokine genes can be prepared and transferred into these cells, reducing the cell immune activities. Potential antiautoimmune cytokines include transforming growth factor (TGF)β, interleukin (IL)4,

TABLE 19.3. Characteristics of Selected Proteins that Stimulate the Differentiation of Insulin-Producing β Cells*

Protein	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Insulin promoter factor 1	Pancreatic duodenal homeobox gene 1 (Pdx1) and somatostatin transcription factor 1 (STF1)	283	31	Pancreas, brain, intestine	Promoting the differentiation of insulin-producing β cells, stimulating pancreatic development, and activating the transcription of the insulin and somatostatin genes
Neurogenic differentiation factor 1	NeuroD1, NEUROD, β-cell E box transactivator 2	356	40	Pancreas, retina, placenta	Stimulating the differentiation of nerve cells and pancreatic β cells

*Based on bibliography 19.6.

and IL10, which suppress the activity of T cells. Genes encoding these factors can be transferred into T cells and antigen-presenting cells. The transferred cells can be transplanted into the host. Preliminary investigations have demonstrated that the transfer of these genes significantly prevents the damage of pancreatic islet cells and reduces diabetic changes in animal models of diabetes.

Cell and Tissue Regenerative Engineering. Diabetes is induced primarily by β-cell malfunction and apoptosis. There are about 10^9 β cells in the pancreatic islets of Langerhans. At a given time, certain β cells are committed to apoptosis. The apoptotic cells are replaced with new cells generated from progenitor cells or existing β cells (Fig. 19.5). Thus, the total number of β cells is maintained at a relatively constant level. Under the influence of genetic and environmental factors, such as viral infection, the rate of cell apoptosis may increase and exceed that of cell proliferation. When the majority of cells are destroyed, diabetes occurs. Thus, the principle of cell and tissue engineering for treating diabetes is to restore and maintain the β-cell population. In the section on molecular regenerative engineering, various methods have been discussed for the promotion of cell regeneration and prevention of cell apoptosis. In this section, strategies and methods are introduced for the restoration of β cells by cell transplantation.

There are several steps for the restoration of β cells, including the identification and collection of candidate cells, manipulation of collected cells, if necessary, packaging of cells into appropriate devices, and transplantation of prepared cells into a desired organ or tissue. There are several types of candidate cells for the treatment of diabetes, including autogenous β cells, autogenous stem and progenitor cells, embryonic and fetal stem cells,

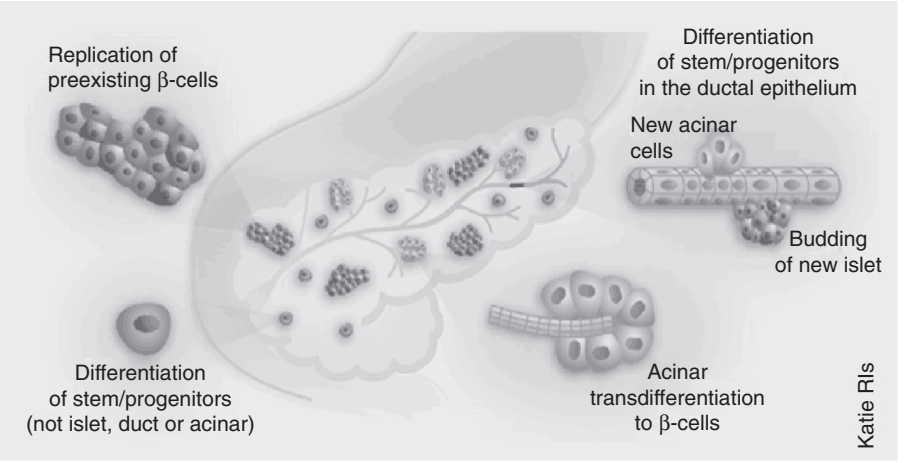


Figure 19.5. The pancreas as a source of new β cells. The pancreas itself is likely to be the main source of new insulin-producing β cells and of cells that can regenerate the acini and ducts. Several processes have been proposed: replication of preexisting β cells, possibly thorough epithelial–mesenchymal transition, including dedifferentiation, expansion, and redifferentiation; differentiation of progenitors within the ductal epithelium; transdifferentiation of acinar cells; and differentiation of pancreatic stem/progenitors that are not of β-cell, duct, or acinar origin. (Reprinted by permission from Macmillan Publishers Ltd.: Bonner-Weir S, Weir GC: New sources of pancreatic beta-cells, *Nature Biotechnol* 23:857–61, copyright 2005.)

allogenic β cells, allogenic stem and progenitor cells, xenogenic β cells, and xenogenic stem and progenitor cells. It is important to note that the cell transplantation therapy for diabetes is only in its infant stage. At present, cell transplantation may have not offered advantages over insulin injection. It is hoped, however, that with a better understanding of the differentiation control of the β cells the function of the Langerhans islets can be restored permanently with molecular and cellular regenerative approaches.

Candidate Cell Types

AUTOGENOUS PANCREATIC β CELLS [19.8]. Autogenous β cells derived from the host pancreas are an ideal cell type for the treatment of diabetes by cell transplantation (Fig. 19.5). However, a large number of cells are usually needed for cell transplantation. Diabetic patients rarely possess a sufficient number of functional β cells when the disease is identified. Even though the disease can be identified in the early stage, it is difficult to collect a large number of β cells. Whereas host β-cell regeneration and transplantation are not a suitable approach at present, it is possible to generate semi-autogenous β cells by transferring the host β-cell nuclei into donor oocytes. Such an approach may produce functional β cells with reduced immune rejection reactions. Further research is necessary to achieve such a goal.

ALLOGENIC β CELLS [19.8]. Allogenic β cells can be harvested from donor subjects, cultured for expansion, and used for cell transplantation. To successfully restore the function of the islets of Langerhans, a sufficient number of β cells are needed for each recipient. While allogenic cell transplantation is potentially an appropriate treatment for diabetes, three problems hinder the application of this approach: (1) there is a shortage of organ donors—in the United States, the incidence of type I diabetes is about 1 million per year, whereas, the number of suitable organ donors is only about 10,000 per year; (2) allogenic cell transplantation always induces acute immune rejection—patients with allogenic β-cell transplantation will have to receive lifetime immunosuppression therapy; and (3) transplanted β cells may undergo cell apoptosis even under immunosuppression therapy. It is usually difficult to maintain the survival of the transplanted cells. To overcome these difficulties, allogenic β cells can be transfected with oncogenes or growth factor genes, promoting cell survival and preventing cell apoptosis. Transformed β cells usually become immortal with enhanced cell proliferation. Such a manipulation has been shown to improve the survival rate of transplanted β cells. However, oncogene transformation may impair the physiological function of the β cells and introduce the risk of tumorigenesis to the cell recipient.

PANCREATIC STEM AND PROGENITOR CELLS [19.9]. The pancreas contains stem and progenitor cells, which can differentiate into the insulin-producing β cells. The epithelial cells of the pancreatic ductules and ducts are potential stem cells. In preliminary studies conducted in humans and mice, the pancreatic epithelial cells could be collected, cultured, and induced to form insulin-producing cells in cell culture models. These cells are suitable candidates for the cellular treatment of diabetes. However, a practical difficulty is that diabetic patients, when diabetes is diagnosed, do not have a sufficient number of functional stem and progenitor cells. It is often necessary to collect stem and progenitor cells from allogenic donors.

EMBRYONIC STEM CELLS [19.10]. Embryonic stem cells can be induced to differentiate into various specialized cells types, including the insulin-producing β cells. Thus, embryonic cells are candidate cells for the cellular treatment of diabetes. To use embryonic cells, it is necessary to carry out several steps: (1) collecting embryonic cells, (2) inducing the differentiation of stem cells to insulin-producing β cells, (3) identifying and selecting β cells, (4) engineering selected cells to express desired features such as enhanced survival and antiautoimmune capabilities, (5) expanding selected cells to a sufficient number for cell transplantation, and (6) transplanting the insulin-producing cells directly to a target tissue or packaging the cells into a desired device followed by device transplantation.

Embryonic stem cells can be collected from the embryonic blastocyst as described in Chapter 9. The collected stem cells can be cultured under pancreatic conditions to induce differentiation into insulin-producing β cells. Preliminary investigations have demonstrated the possibility of forming insulin-producing β cells from human and mouse embryonic stem cells, although the fraction of insulin-producing β cells is small. To identify insulin-producing cells, it is necessary to introduce a marker specific to these cells. The promoter of the insulin gene can be turned on by factors that stimulate insulin gene transcription and thus can be used as a specific marker. A green fluorescent protein (GFP) gene can be inserted into the insulin gene to form a recombinant gene so that the GFP gene can be driven by the insulin gene promoter. The recombinant gene can be transferred into embryonic stem cells. Any cells that express the green fluorescent protein are cells with activated insulin gene. These cells are considered insulin-producing β cells. The identified cells can be collected by fluorescence-activated cell sorting and further expanded in culture. The collected cells can be engineered by transferring genes for enhancing desired features. For example, the insulin-producing β cells can be transferred with oncogenes or growth factor genes to enhance their survival and proliferative capabilities. Certain cell membrane molecules may serve as antigens for autoimmune reactions. Such antigens can be identified and the genes of the antigens can be removed, resulting in a reduction in autoimmune responses. When the cell number reaches a sufficient level, the cells can be used for transplantation.

ADULT STEM CELLS [19.11]. There exist stem cells in various types of adult tissue and organ, including the bone marrow, liver, intestine, and the nerve system. These cells are responsible for the regeneration of adult cells when cell injury and death occur. While most adult stem cells are committed to the formation of specialized cells within a defined developmental system, certain types of adult stem cells are capable of differentiating into cells for different systems. A typical example is the bone marrow stromal cells. These cells have been shown to differentiate into various specialized cell types including muscular cells and neurons, depending on the local environment of a tissue or organ, in humans and rodents. The transplantation of bone marrow stem cells into the pancreas may induce differentiation of the stem cells into insulin-producing β cells.

XENOGENEIC β CELLS, STEM CELLS, AND PROGENITOR CELLS [19.11]. Xenogeneic β cells, stem cells, and progenitor cells are considered only when no other cell sources are available. The identification, collection, culture, manipulation, and transplantation of these cells are similar to those described above. A major concern is that, because of their xenogenic nature, these cells cause severe acute immune rejection reactions. It is necessary to establish devices for the isolation of the transplanted cells from the host system. This issue is discussed in the section on "transplantation of β-cell-protecting devices."

Prevention of Immune Reactions and β-Cell Injury [19.12]. While the approaches discussed above show potential for the treatment of diabetes, there is a common problem for most approaches: acute immune rejection. Furthermore, autoimmune reactions may occur as these reactions are the original cause of diabetes. Immunity-suppressing agents have long been used for suppressing immune responses. The requirement of lifelong administration of immune suppressing agents renders molecular and cellular engineering approaches less favorable compared to insulin injection. To overcome such a problem, selected cells for transplantation can be transfected with genes that encode immune suppressing cytokines, such as interleukin (IL)4, IL10, and transforming growth factor (TGF)β. These cytokines inhibit the function of T cells, which are responsible for immune rejection and autoimmune responses. Preliminary investigations have demonstrated the effectiveness of these suppressing cytokines. In addition, calls can be transfected with genes encoding protective antioxidant proteins, such as copper/zinc and manganese superoxide dismutases, catalase, and thioredoxin, and antiapoptotic proteins, such as Bcl2 and A20. Experimental investigations have demonstrated that the transfer of these genes protects insulin-producing β cells from injury and apoptosis.

Transplantation of β-Cell-Protecting Devices [19.13]. Because of the susceptibility of transplanted cells to immune attacks, several types of protective devices have been developed and used for β-cell transplantation. These devices include microcapsules and biohybrid pancreas-mimicking apparatuses with blood circulation. These devices are manufactured with semipermeable membranes, which prevent the entrance of immune cells into the device and protect the β cells from immune attack. Various polymeric materials can be used to fabricate semipermeable membranes with a desired pore size (see Chapter 12 for polymeric materials). Microcapsules can be used to enclose β cells and transplant into a desired tissue or cavity of the recipients. Since the capsules are usually small, allowing oxygen and nutrient diffusion into the enclosed cells, it is not necessary to introduce blood circulation into the capsules.

When a large pancreas-mimicking device is used, it is often necessary to establish a blood circulation system. A double-tube system can be fabricated with a semipermeable membrane. One of the tubes is used for packaging the β cells and the other is used for introducing bloodflow to the β cells (Fig. 19.6). The device can be implanted into the abdominal cavity of the recipients. The inlet and outlet ports of the blood circulatory system can be anastomosed to a selected artery and vein, such as the inferior mesentery

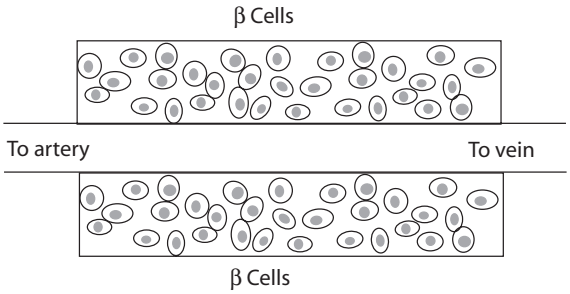


Figure 19.6. Schematic representation of an artificial pancreas containing functional β cells. Based on bibliography 19.13.

artery and vein. The blood circulation provides oxygen and nutrients to the cells, and insulin and other molecules from the β cells can diffuse across the semipermeable membrane into the blood compartment. Such a device can be potentially used for the transplantation of β cells into diabetic patients. A problem for such a device is blood coagulation and thrombosis. Blood coagulation occurs within the device, and thrombosis occurs at the anastomoses. It is necessary to administrate anticoagulants when such a device is transplanted.

Pancreatic Cancer

Pathogenesis, Pathology, and Clinical Features. Pancreatic cancer is one of the four most frequent types of cancer in humans, which include lung, colon, breast, and pancreatic cancers. Pancreatic cancer is often originated from the epithelial cells of the pancreatic ductules and ducts. Pancreatic cancer progresses rapidly. When pancreatic cancer is diagnosed, more than half of the patients are associated with cancer metastasis. As for other types of cancer, the pathogenesis of pancreatic cancer remains poorly understood. Epidemiologic studies have suggested that cigarette smoking, fat diets, and coffee intake may serve as risking factors for the development of pancreatic cancer. Pathological changes in pancreatic cancer are similar to those found in other types of cancer as described in Chapter 25. There are several unique clinical features for pancreatic cancer. These include rapid and extensive loss of body weight (due to the loss of digestion enzymes), severe upper abdominal pain, anorexia, nausea, vomiting, hyperglycemia (due to the destruction of the β cells) and jaundice (due to the compression of the bile duct by tumors in the head region of the pancreas).

Treatment of Pancreatic Cancer. Pancreatic cancer exhibits genetic, pathological, and clinical characteristics similar to those described in Chapter 25. Thus, similar therapeutic strategies can be used for the treatment of pancreatic cancer. In general, surgical removal of pancreatic cancer is the most effective treatment, provided that the cancer is identified in the early stage before the occurrence of metastasis. In the presence of metastasis, chemotherapy and radiotherapy are the methods of choice. Molecular therapy can be potentially used. The principles and methods of molecular therapy for cancers are discussed in Chapter 25.

BIBLIOGRAPHY

19.1. Anatomy and Physiology of the Pancreas

Guyton AC, Hall JE: *Textbook of Medical Physiology*, 11th ed, Saunders, Philadelphia, 2006.

McArdle WD, Katch FI, Katch VL: *Essentials of Exercise Physiology*, 3rd ed, Lippincott Williams & Wilkins, Baltimore, 2006.

Germann WJ, Stanfield CL (with contributors Niles MJ, Cannon JG): *Principles of Human Physiology*, 2nd ed, Pearson Benjamin Cummings, San Francisco, 2005.

Thibodeau GA, Patton KT: *Anatomy & Physiology*, 5th ed, Mosby, St Louis, 2003.

Boron WF, Boulpaep EL: *Medical Physiology: A Cellular and Molecular Approach*, Saunders, Philadelphia, 2003.

Ganong WF: *Review of Medical Physiology*, 21st ed, McGraw-Hill, New York, 2003.

19.2. Pathogenesis, Pathology, and Clinical Features of Diabetes

Schneider AS, Szanto PA: *Pathology*, 3rd ed, Lippincott Williams & Wilkins, Philadelphia, 2006.

McCance KL, Huether SE: *Pathophysiology: The Biologic Basis for Disease in Adults & Children*, 5th ed, Elsevier Mosby, St Louis, 2006.

Porth CM: *Pathophysiology: Concepts of Altered Health States*, 7th ed, Lippincott Williams & Wilkins, Philadelphia, 2005.

Frazier MS, Drzymkowski JW: *Essentials of Human Diseases and Conditions*, 3rd ed, Elsevier Saunders, St Louis, 2004.

Kahn SE: The relative contributions of insulin resistance and β-cell dysfunction to the pathophysiology of type 2 diabetes, *Diabetologia* 46:3–19, 2003.

Remuzzi G, Benigni A, Remuzzi A: Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes, *J Clin Invest* 116(2):288–96, 2006.

Kelkar P: Diabetic neuropathy, *Semin Neurol* 25(2):168–73, 2005.

Wolf G, Chen S, Ziyadeh FN: From the periphery of the glomerular capillary wall toward the center of disease: Podocyte injury comes of age in diabetic nephropathy, *Diabetes* 54(6):1626–34, 2005.

Van den Berghe G: How does blood glucose control with insulin save lives in intensive care? *J Clin Invest* 114(9):1187–95, 2004.

Donath MY, Halban PA: Decreased beta-cell mass in diabetes: Significance, mechanisms and therapeutic implications, *Diabetologia* 47(3):581–9, 2004.

Mandrup-Poulsen T: Beta cell death and protection, *Ann NY Acad Sci* 1005:32–42, 2003.

Wiernsperger NF, Bouskela E: Microcirculation in insulin resistance and diabetes: More than just a complication, *Diabetes Metab* 29(4 Pt 2):6S77–87, 2003.

Rorsman P, Renstrom E: Insulin granule dynamics in pancreatic beta cells, *Diabetologia* 46(8):1029–45, 2003.

Tsilibary EC: Microvascular basement membranes in diabetes mellitus, *J Pathol* 200(4):537–46, 2003.

Caramori ML, Mauer M: Diabetes and nephropathy, *Curr Opin Nephrol Hypertens* 12(3):273–82, 2003.

Sesti G: Apoptosis in the beta cells: cause or consequence of insulin secretion defect in diabetes? *Ann Med* 34(6):444–50, 2002.

Bloomgarden ZT: Developments in diabetes and insulin resistance, *Diabetes Care* 29(1):161–7, 2006.

Verges B: New insight into the pathophysiology of lipid abnormalities in type 2 diabetes, *Diabetes Metab* 31(5):429–39, 2005.

Liu SQ, Fung YC: Influence of STZ diabetes on zero-stress state of rat pulmonary and systemic arteries, *Diabetes* 41:136–146, 1992.

19.3. Conventional Treatment of Diabetes

Cryer PE: Banting lecture. Hypoglycemia: The limiting factor in the management of IDDM, *Diabetes* 43:1378–89, 1994.

Cryer PE: Hypoglycaemia: The limiting factor in the glycaemic management of type I and type II diabetes, *Diabetologia* 45:937–48, 2002.

Chan L, Fujimiya M, Kojima H: In vivo gene therapy for diabetes mellitus, *Trends Mol Med* 9:430–5, 2003.

McKinney JL, Cao H, Robinson JF, Metzger DL, Cummings E et al: Spectrum of HNF1A and GCK mutations in Canadian families with maturity-onset diabetes of the young (MODY), *Clin Invest Med* 27(3):135–41, 2004.

Johansen A, Ek J, Mortensen HB, Pedersen O, Hansen T: Half of clinically defined maturity-onset diabetes of the young patients in Denmark do not have mutations in HNF4A, GCK, and TCF1, *J Clin Endocr Metab* 90(8):4607–14, 2005.

Gloyn AL, Odili S, Zelent D, Buettger C, Castleden HA et al: Insights into the structure and regulation of glucokinase from a novel mutation (V62M), which causes maturity-onset diabetes of the young, *J Biol Chem* 280(14):14105–13, 2005.

Weedon MN, Frayling TM, Shields B, Knight B, Turner T et al: Genetic regulation of birth weight and fasting glucose by a common polymorphism in the islet cell promoter of the glucokinase gene, *Diabetes* 54(2):576–81, 2005.

Barrio R, Bellanne-Chantelot C, Moreno JC, Morel V, Calle H et al: Nine novel mutations in maturity-onset diabetes of the young (MODY) candidate genes in 22 Spanish families, *J Clin Endocr Metab* 87(6):2532–9, 2002.

19.4. Enhancement of Glucose Uptake and Storage and Inhibition of Glucose Production

Brocklehurst KJ, Davies RA, Agius L: Differences in regulatory properties between human and rat glucokinase regulatory protein, *Biochem J* 378(Pt 2):693–7, March 2004.

Farrelly D, Brown KS, Tieman A, Ren J, Lira SA et al: Mice mutant for glucokinase regulatory protein exhibit decreased liver glucokinase: A sequestration mechanism in metabolic regulation, *Proc Natl Acad Sci USA* 96:14511–6, 1999.

Grimsby J, Coffey JW, Dvorozniak MT, Magram J, Li G et al: Characterization of glucokinase regulatory protein-deficient mice, *J Biol Chem* 275:7826–31, 2000.

Hayward BE, Dunlop N, Intody S, Leek JP, Markham AF et al: Organization of the human glucokinase regulator gene GCKR, *Genomics* 49:137–42, 1998.

Vaxillaire M, Vionnet N, Vigouroux C, Sun F, Espinosa R III et al: Search for a third susceptibility gene for maturity-onset diabetes of the young: Studies with eleven candidate genes, *Diabetes* 43:389–95, 1994.

Bali D, Svetlanov A, Lee HW, Fusco-DeMane D, Leiser M et al: Animal model for maturity-onset diabetes of the young generated by disruption of the mouse glucokinase gene, *J Biol Chem* 270:21464–7, 1995.

Byrne MM, Sturis J, Clement K, Vionnet N, Pueyo ME et al: Insulin secretory abnormalities in subjects with hyperglycemia due to glucokinase mutations, *J Clin Invest* 93:1120–30, 1994.

Danial NN, Gramm CF, Scorrano L, Zhang CY, Krauss S et al: BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis, *Nature* 424:952–6, 2003.

Froguel P, Vaxillaire M, Sun F, Velho G, Zouali H et al: Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus, *Nature* 356:162–4, 1992.

Froguel P, Zouali H, Vionnet N, Velho G, Vaxillaire M et al: Familial hyperglycemia due to mutations in glucokinase: Definition of a subtype of diabetes mellitus, *New Eng J Med* 328:697–702, 1993.

Gloyn AL, Noordam K, Willemsen MAAP, Ellard S, Lam WWK et al: Insights into the biochemical and genetic basis of glucokinase activation from naturally occurring hypoglycemia mutations, *Diabetes* 52:2433–40, 2003.

Grimsby J, Sarabu R, Corbett WL, Haynes NE, Bizzarro FT et al: Allosteric activators of glucokinase: Potential role in diabetes therapy, *Science* 301:370–3, 2003.

Grupe A, Hultgren B, Ryan A, Ma YH, Bauer M et al: Transgenic knockouts reveal a critical requirement for pancreatic beta cell glucokinase in maintaining glucose homeostasis, *Cell* 83:69–78, 1995.

Matschinsky F, Liang Y, Kesavan P, Wang L, Froguel P et al: Glucokinase as pancreatic beta-cell glucose sensor and diabetes gene, *J Clin Invest* 92:2092–8, 1993.

Njolstad PR, Sovik O, Cuesta-Munoz A, Bjorkhaug L, Massa O et al: Neonatal diabetes mellitus due to complete glucokinase deficiency, *New Eng J Med* 344:1588–92, 2001.

Rowe RE, Wapelhorst B, Bell GI, Risch N, Spielman RS et al: Linkage and association between insulin-dependent diabetes mellitus (IDDM) susceptibility and markers near the glucokinase gene on chromosome 7, *Nature Genet* 10:240–5, 1995.

Sun F, Knebelmann B, Pueyo ME, Zouali H, Lesage S et al: Deletion of the donor splice site of intron 4 in the glucokinase gene causes maturity-onset diabetes of the young, *J Clin Invest* 92:1174–80, 1993.

Velho G, Petersen KF, Perseghin G, Hwang JH, Rothman DL et al: Impaired hepatic glycogen synthesis in glucokinase-deficient (MODY-2) subjects, *J Clin Invest* 98:1755–61, 1996.

Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI et al: Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus, *Nature* 356:721–2, 1992.

Ferre T et al: Correction of diabetic alterations by glucokinase, *Proc Natl Acad Sci USA* 93:7225–30, 1996.

O'Doherty RM et al: Metabolic impact of glucokinase overexpression in liver, *Diabetes* 48:2022–7, 1999.

Shiota M et al: Glucokinase gene locus transgenic mice are resistant to the development of obesity-induced type 2 diabetes, *Diabetes* 50:622–9, 2001.

Desai UJ et al: Phenotypic correction of diabetic mice by adenovirus-mediated glucokinase expression, *Diabetes* 50:2287–95, 2001.

Morral N et al: Adenovirus-mediated expression of glucokinase in the liver as an adjuvant treatment for type 1 diabetes, *Hum Gene Ther* 13:1561–70, 2002.

Morral N: Novel targets and therapeutic strategies for type 2 diabetes, *Trends Endocr Metab* 14:169–75, 2003.

Otaegui PJ et al: Expression of glucokinase in skeletal muscle: A new approach to counteract diabetic hyperglycemia, *Hum Gene Ther* 11:1543–52, 2000.

Slosberg ED et al: Treatment of type 2 diabetes by adenoviral-mediated overexpression of the glucokinase regulatory protein, *Diabetes* 50:1813–20, 2001.

Wu C et al: Increasing fructose 2,6-bisphosphate overcomes hepatic insulin resistance of type 2 diabetes, *Am J Physiol Endocr Metab* 282:E38–45, 2002.

O'Doherty RM et al: Activation of direct and indirect pathways of glycogen synthesis by hepatic overexpression of protein targeting to glycogen, *J Clin Invest* 105:479–88, 2000.

Newgard CB et al: Organizing glucose disposal. Emerging roles of the glycogen targeting subunits of protein phosphatase-1, *Diabetes* 49:1967–77, 2000.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

19.5. Facilitation of Insulin Synthesis and Activation

Blanchette F, Day R, Dong W, Laprise MH, Dubois CM: TGF-beta-1 regulates gene expression of its own converting enzyme furin, *J Clin Invest* 99:1974–83, 1997.

Dubois CM, Laprise MH, Blanchette F, Gentry LE, Leduc R: Processing of transforming growth factor beta-1 precursor by human furin convertase, *J Biol Chem* 270:10618–24, 1995.

Hendy GN, Bennett HPJ, Gibbs BF, Lazure C, Day R et al: Proparathyroid hormone is preferentially cleaved to parathyroid hormone by the prohormone convertase furin: A mass spectrometric study, *J Biol Chem* 270:9517–25, 1995.

Short DK et al: Adenovirus-mediated transfer of a modified human proinsulin gene reverses hyperglycemia in diabetic mice, *Am J Physiol* 275:E748–56, 1998.

Muzzin P et al: Hepatic insulin gene expression as treatment for type 1 diabetes mellitus in rats, *Mol Endocr* 11:833–7, 1997.

Auricchio A et al: Constitutive and regulated expression of processed insulin following in vivo hepatic gene transfer, *Gene Ther* 9:963–71, 2002.

19.6. Promotion of the Survival and Prevention of β-cell Apoptosis

Dickson LM, Rhodes CL: Pancreatic β-cell growth and survival in the onset of type 2 diabetes: A role for protein kinase B in the Akt? *Am J Physiol Endocr Metab* 287:E192–8, 2004.

Pick A et al: Role of apoptosis in failure of β-cell mass compensation for insulin resistance and β-cell defects in the male Zucker diabetic fatty rat, *Diabetes* 47:358–64, 1998.

Withers DJ et al: Disruption of IRS-2 causes type 2 diabetes in mice, *Nature* 391:900–4, 1998.

Bruning JC et al: Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles, *Cell* 88:561–72, 1997.

Bonner-Weir S: Islet growth and development in the adult, *J Mol Endocr* 24:297–302, 2000.

Kahn SE: Clinical review 135: The importance of β-cell failure in the development and progression of type 2 diabetes, *J Clin Endocr Metab* 86:4047–58, 2001.

Lingohr MK, Buettner R, Rhodes CJ: Pancreatic β-cell growth and survival—a role in obesity-linked type 2 diabetes? *Trends Mol Med* 8:375–84, 2002.

Nielsen JH et al: Regulation of β-cell mass by hormones and growth factors, *Diabetes* 50(Suppl 1):S25–9, 2001.

Argetsinger LS, Carter-Su C: Mechanism of signaling by growth hormone receptor, *Physiol Rev* 76:1089–107, 1996.

Benito M et al: IGF-I: A mitogen also involved in differentiation processes in mammalian cells, *Int J Biochem Cell Biol* 28:499–510, 1996.

Pearson G et al: Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions, *Endocr Rev* 22:153–83, 2001.

Chan TO et al: AKT/PKB and other D3 phosphoinositide-regulated kinases: Kinase activation by phosphoinositide-dependent phosphorylation, *Annu Rev Biochem* 68:965–1014, 1999.

Withers DJ et al: IRS-2 coordinates IGF-1 receptor-mediated β-cell development and peripheral insulin signaling, *Nat Genet* 23:32–40, 1999.

Lingohr MK et al: Activation of IRS-2 mediated signal transduction by IGF-1, but not TGF-β or EGF, augments pancreatic β-cell proliferation, *Diabetes* 51:966–76, 2002.

Tuttle RL et al: Regulation of pancreatic β-cell growth and survival by the serine/threonine protein kinase Akt1/PKBβ, *Nat Med* 7:1133–7, 2001.

Dickson LM et al: Differential activation of protein kinase B and p70(S6)K by glucose and insulin-like growth factor 1 in pancreatic β-cells (INS-1), *J Biol Chem* 276:21110–20, 2001.

Sesti G: Apoptosis in the beta cells: Cause or consequence of insulin secretion defect in diabetes? *Ann Med* 34(6):444–50, 2002.

NeuroD

Fajans SS, Bell GI, Polonsky KS: Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young, *New Eng J Med* 345:971–80, 2001.

Lee JE, Hollenberg SM, Snider L, Turner DL, Lipnick N et al: Conversion of Xenopus ectoderm into neurons by NeuroD, a basic helix-loop-helix protein, *Science* 268:836–44, 1995.

Malecki MT, Jhala US, Antonellis A, Fields L, Doria A et al: Mutations in NEUROD1 are associated with the development of type 2 diabetes mellitus, *Nature Genet* 23:323–8, 1999.

Naya FJ, Huang HP, Qiu Y, Mutoh H, DeMayo FJ et al: Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neurod-deficient mice, *Genes Dev* 11:2323–4, 1997.

Yan RT, Wang SZ: Requirement of neuroD for photoreceptor formation in the chick retina, *Invest Ophthalm Vis Sci* 45:48–58, 2004.

Insulin Promoter Factor 1

Cockburn BN, Bermano G, Boodram LLG, Teelucksingh S, Tsuchiya T et al: Insulin promoter factor-1 mutations and diabetes in Trinidad: Identification of a novel diabetes-associated mutation (E224K) in an Indo-Trinidadian family, *J Clin Endocr Metab* 89:971–8, 2004.

Hani EH, Stoffers DA, Chevre JC, Durand E, Stanojevic V et al: Defective mutations in the insulin promoter factor-1 (IPF-1) gene in late-onset type 2 diabetes mellitus, *J Clin Invest* 104:R41–8, 1999.

Hart AW, Baeza N, Apelqvist A, Edlund H: Attenuation of FGF signalling in mouse beta-cells leads to diabetes, *Nature* 408:864–8, 2000.

Jonnson J, Carlsson L, Edlund T, Edlund H: Insulin-promoter-factor 1 is required for pancreas development in mice, *Nature* 371:606–9, 1994.

Kim SK, Selleri L, Lee JS, Zhang AY, Gu X et al: Pbx1 inactivation disrupts pancreas development and in Ipf1-deficient mice promotes diabetes mellitus, *Nature Genet* 30:430–5, 2002.

Macfarlane WM, Frayling TM, Ellard S, Evans JC, Allen LIS et al: Missense mutations in the insulin promoter factor-1 gene predispose to type 2 diabetes, *J Clin Invest* 104:R33–9, 1999.

Sharma S, Jhala US, Johnson T, Ferreri K, Leonard J et al: Hormonal regulation of an islet-specific enhancer in the pancreatic homeobox gene STF-1, *M Cell Biol* 17:2598–604, 1997.

Stoffers DA, Stanojevic V, Habener JF: Insulin promoter factor-1 gene mutation linked to early-onset type 2 diabetes mellitus directs expression of a dominant negative isoprotein, *J Clin Invest* 102:232–41, 1998.

Ferber S et al: Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia, *Nat Med* 6:568–72, 2000.

Kojima H et al: NeuroD/betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice, *Nat Med* 9:596–603, 2003.

Zalzman M et al: Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells, *Proc Natl Acad Sci USA* 100:7253–8, 2003.

Newgard CB: While tinkering with the β-cell: metabolic regulatory mechanisms and new therapeutic strategies. American Diabetes Association Lilly Lecture, 2001, *Diabetes* 51:3141–50, 2002.

Bailey CJ et al: Prospects for insulin delivery by ex vivo somatic cell gene therapy, *J Mol Med* 77:244–9, 1999.

Levine F: Gene therapy for diabetes: strategies for β-cell modification and replacement, *Diabetes Metab Rev* 13:209–46, 1997.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

19.7. Suppression of Autoimmune Processes

Gallichman WS et al: Lentivirus-mediated transduction of islet grafts with interleukin 4 results in sustained gene expression and protection from insulitis, *Hum Gene Ther* 9:2717–26, 1998.

Moritani M et al: Abrogation of autoimmune diabetes in nonobese diabetic mice and protection against effector lymphocytes by transgenic TGF-beta1, *J Clin Invest* 102:499–506, 1998.

Goudy K et al: Adeno-associated virus vector-mediated IL-10 gene delivery prevents type 1 diabetes in NOD mice, *Proc Natl Acad Sci USA* 98:13913–8, 2001.

Tominaga Y et al: Administration of IL-4 prevents autoimmune diabetes, but enhances pancreatic insulitis in NOD mice, *Clin Immunol Immunopathol* 86:209–18, 1998.

Pennline KJ, Roque-Gaffney E, Monahan M: Recombinant human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse, *Clin Immunol Immunopathol* 71:169–75, 1994.

Koh JJ et al: Degradable polymeric carrier for the delivery of IL-10 plasmid DNA to prevent autoimmune insulitis of NOD mice, *Gene Ther* 7:2099–104, 2000.

Tarner IH, Slavin AJ, McBride J, Levicnik A, Smith R et al: Treatment of autoimmune disease by adoptive cellular gene therapy, *Ann NY Acad Sci* 998:512–9, 2003.

19.8. Pancreatic β Cells

Kubota C, Yama Kuchi H, Todoroki J et al. Six cloned calves produced from adult fibroblast cells after long-term culture, *Proc Natl Acad Sci USA* 97:990–5, 2000.

Shapiro AM et al: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen, *New Engl J Med* 343:230–8, 2000.

Efrat S et al: Conditional transformation of a pancreatic beta-cell line derived from transgenic mice expressing a tetracycline-regulated oncogene, *Proc Natl Acad Sci USA* 92:3576–80, 1995.

Fleischer N et al: Functional analysis of a conditionally-transformed pancreatic beta-cell line, *Diabetes* 47:1419–25, 1998.

Milo-Landesman D et al: Correction of hyperglycemia in diabetic mice transplanted with reversibly-immortalized pancreatic beta cells controlled by the tet-on regulatory system, *Cell Transplant* 10:645–50, 2001.

19.9. Pancreatic Stem and Progenitor Cells

Bonner-Weir S et al: In vitro cultivation of human islets from expanded ductal tissue, *Proc Natl Acad Sci USA* 97:7999–8004, 2000.

Ramiya VK et al: Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells, *Nat Med* 6:278–82, 2000.

19.10. Embryonic Stem Cells

Thomson JA et al: Embryonic stem cell lines derived from human blastocysts, *Science* 282:1145–7, 1998.

Soria B et al: Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice, *Diabetes* 49:157–62, 2000.

Assady S et al: Insulin production by human embryonic stem cells, *Diabetes* 50:1691–7, 2001.

Lumelsky N et al: Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets, *Science* 292:1389–94, 2001.

Halban PA et al: Gene and cell-replacement therapy in the treatment of type 1 diabetes: How high must the standards be set? *Diabetes* 50:2181–91, 2001.

Edlund H: Pancreas: How to get there from the gut? *Curr Opin Cell Biol* 11:663–8, 1999.

Shih DQ, Stoffel M: Dissecting the transcriptional network of pancreatic islets during development and differentiation, *Proc Natl Acad Sci USA* 98:14189–91, 2001.

Tiedge M et al: Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells, *Diabetes* 46:1733–42, 1997.

19.11. Adult Stem Cells

Colter DC et al: Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells, *Proc Natl Acad Sci USA* 98:7841–5, 2001.

Woodbury D et al: Adult rat and human bone marrow stromal cells differentiate into neurons, *J Neurosci Res* 61:364–70, 2000.

Ferber S et al: Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia, *Nat Med* 6:568–72, 2000.

19.12. Prevention of β-Cell Injury and Death

Gallichman WS et al: Lentivirus-mediated transduction of islet grafts with interleukin 4 results in sustained gene expression and protection from insulitis, *Hum Gene Ther* 9:2717–26, 1998.

Moritani M et al: Abrogation of autoimmune diabetes in nonobese diabetic mice and protection against effector lymphocytes by transgenic TGF-beta1, *J Clin Invest* 102:499–506, 1998.

Goudy K et al: Adeno-associated virus vector-mediated IL-10 gene delivery prevents type 1 diabetes in NOD mice, *Proc Natl Acad Sci USA* 98:13913–8, 2001.

Kubisch HM et al: Transgenic copper/zinc superoxide dismutase modulates susceptibility to type I diabetes, *Proc Natl Acad Sci USA* 91:9956–9, 1994.

Hohmeier HE et al: Stable expression of manganese superoxide dismutase (MnSOD) in insulinoma cells prevents IL-1 beta-induced cytotoxicity and reduces nitric oxide production, *J Clin Invest* 101:1811–20, 1998.

Benhamou PY et al: Adenovirus-mediated catalase gene transfer reduces oxidant stress in human, porcine, and rat pancreatic islets, *Diabetologia* 41:1093–100, 1998.

Hotta M et al: Pancreatic betacell-specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes, *J Exp Med* 188:1445–51, 1998.

Rabinovitch A et al: Transfection of human pancreatic islets with an anti-apoptotic gene (bcl-2) protects beta-cells from cytokine-induced destruction, *Diabetes* 48:1223–9, 1999.

Dupraz P et al: Lentivirus-mediated Bcl-2 expression in beta TC-tet cells improves resistance to hypoxia and cytokine-induced apoptosis while preserving in vitro and in vivo control of insulin secretion, *Gene Ther* 6:1160–9, 1999.

Grey ST et al: A20 inhibits cytokine-induced apoptosis and nuclear factor kappa B-dependent gene activation in islets, *J Exp Med* 190:1135–45, 1999.

Efrat S et al: Adenovirus early region 3 (E3) immunomodulatory genes decrease the incidence of autoimmune diabetes in nonobese diabetic (NOD) mice, *Diabetes* 50:980–4, 2001.

19.13. Transplantation of β-Cell-Protecting Devices

Sullivan SJ, Maki T, Borland KM, Mahoney MD, Solomon BA et al: Biohybrid artificial pancreas: Long-term implantation studies in diabetic, pancreatectomized dogs, *Science* 252:718–21, 1991.

Sun Y et al: Normalization of diabetes in spontaneously diabetic cynomolgus monkeys by xenografts of microencapsulated porcine islets without immunosuppression, *J Clin Invest* 98:1417–22, 1996.

Duvivier-Kali VF et al: Complete protection of islets against allorejection and autoimmunity by a simple barium–alginate membrane, *Diabetes* 50:1698–705, 2001.

Shapiro AMJ et al: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen, *New Engl J Med* 343:230–8, 2000.

Paty BW et al: Intrahepatic islet transplantation in type 1 diabetic patients does not restore hypoglycemic hormonal counterregulation or symptom recognition after insulin independence, *Diabetes* 51:3428–34, 2002.

Ryan EA et al: Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol, *Diabetes* 50:710–9, 2001.

Giannoukakis N, Pietropaolo M, Trucco M: Genes and engineered cells as drugs for type I and type II diabetes mellitus therapy and prevention, *Curr Opin Invest Drugs* 3(5):735–51, 2002.

Holland AM, Gonez LJ, Harrison LC: Progenitor cells in the adult pancreas, *Diabetes Metab Res Rev* 20(1):13–27, 2004.