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Development of the Pancreas and Response to Disease

The pancreas develops from the primitive gut, which is derived from endoderm. An interdependent series of signals is necessary to form the primitive gut prior to the first appearance of buds recognizable as the nascent pancreas. Continued complex interactions of gene products produce growth and differentiation first of the primitive tubules and subsequently of the islet cells, acinar cells, and mature ducts characteristic of the fully formed pancreas.

Essential elements, including blood vessels, lymphatics, and connective tissue, are contributed from the mesoderm-derived mesenchyme surrounding the developing pancreas. An extensive nervous system is produced in the pancreas by the entry of cells and nerve fibers from different parts of the developing general nervous system, derived from the ectodermal germ layer [1–4].

These events usually proceed properly, resulting in a pancreas that provides the necessary hormones and digestive enzymes throughout the life of the individual. On occasion, normal development fails, resulting in functional deficiencies. More often, however, a normal pancreas is produced, with pancreatic disease occurring considerably later. Pain, altered function, and threat to life can result. The changes that occur with pancreatic disease may sometimes be better understood on the background of pancreatic development. Moreover, it is possible that a better understanding of the mechanisms involved in pancreatic development will provide clues leading to a reversal of pancreatic disease. A common finding in pancreatic disease is a reversion of the elements derived from the embryonic endoderm to a more primitive state [5–7]. A significant goal is to learn how to reverse the regressive changes, restoring functional endocrine and exocrine cells in the diseased pancreas [8–10].

Early Development Depends on Interactions Between Major Embryonic Elements

In the early embryo, the notochord intervenes between and interacts directly with the neural tube and the endoderm, the latter representing the primitive gut. The dorsal aorta is paired and lies on either side (Fig. 1.1a). Mesenchyme lies adjacent to these developing elements. The paired dorsal aortae move toward the center and fuse with each other. The single aorta thus produced lies over the primitive gut, in the location previously occupied by the notochord (Fig. 1.1b). The vitelline veins develop within the mesenchyme ventral to the primitive gut. The right vitelline vein persists (Fig. 1.1b). The aorta and the vitelline vein interact directly with the primitive gut, playing an important role in the induction of the pancreatic primordia. The dorsal pancreatic primordium appears at the region of interaction with the aorta, and the ventral pancreatic primordium at the region of interaction with the vitelline vein (Fig. 1.1b).

The endoderm-derived epithelium of the dorsal and ventral pancreatic primordia proliferates and grows into the surrounding mesenchyme as primitive tubules that divide and sometimes reunite with each other (Fig. 1.2a). The primitive pancreatic tubules are initially solid. Lumens subsequently develop within them, eventually forming a continuous pathway from the secretory cells to the duodenum.

The cells making up the walls of the primitive ducts are morphologically similar at first, but with time begin changing into the varied elements of the mature pancreas [11]. Cells that will comprise the endocrine system of the pancreas differentiate from the primitive epithelial ductular cells, migrate into the mesenchyme, and aggregate into groups, producing islets of Langerhans (Fig. 1.2b). Examination of pancreas during fetal development reveals the importance of ductular cell proliferation in humans [12]. Differentiation of acinar cells from the primitive ductular cells in regions initiates the development of acini. Acinar cells remain within the original con-

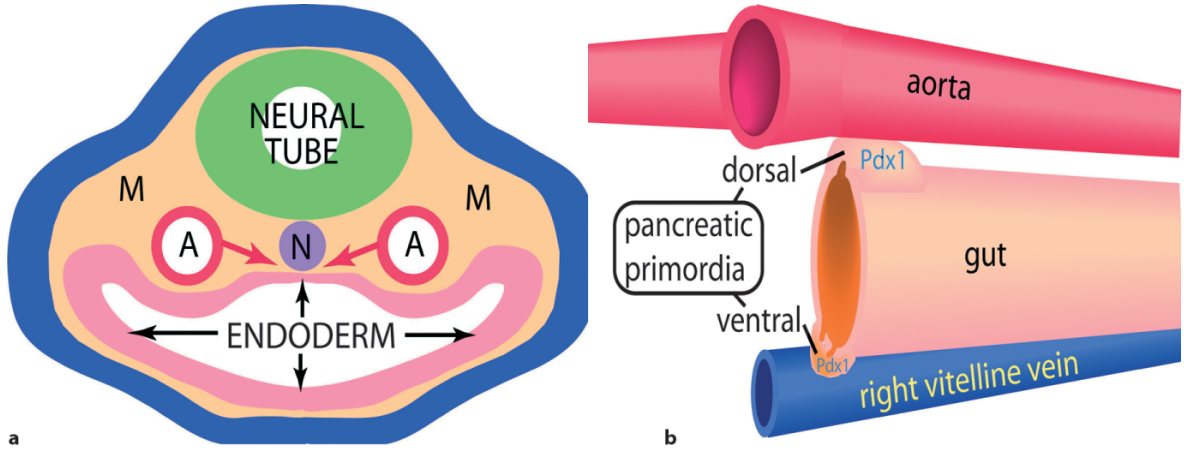

Figure 1.1

Embryonic processes leading to the induction of pancreatic primordia. **a** Cross section of an embryo showing the interaction between the notochord (*N*) and the neural tube and endoderm. Paired dorsal aortas (*A*) migrate between the notochord and endoderm and fuse. *M* Mesenchyme. **b** Induction of the pancreatic primordia (taken from Bockman [21]). The epithelial interaction between the aorta and gut produces the dorsal pancreatic bud. The originally paired aorta is single in the region of induction. Only the right vitelline vein is shown. The right vein persists to form the hepatic portal vein. Epithelial interaction between the right vitelline vein and the gut produces the ventral pancreatic primordium. *Pdx1* Homeobox gene Pdx1

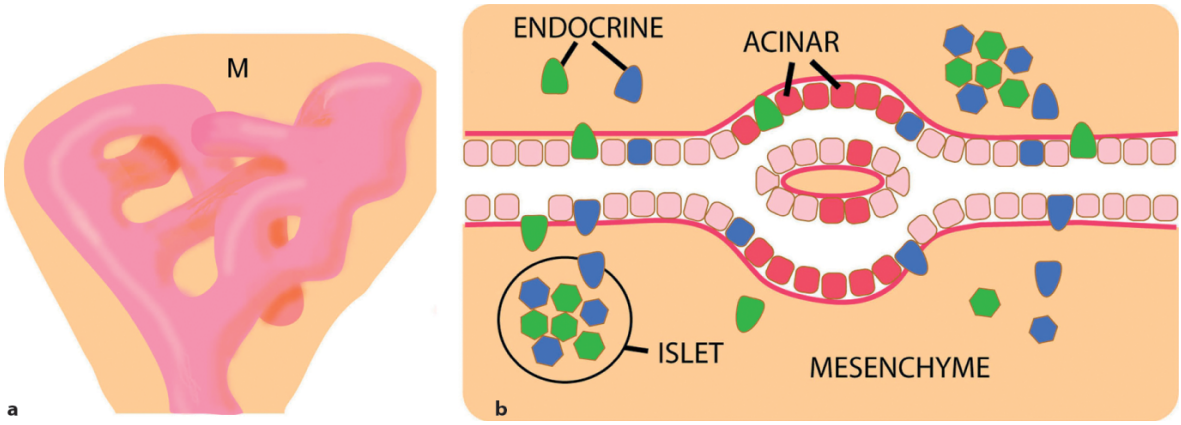

Figure 1.2

Development of pancreatic architecture and differentiation of cell types. **a** Pancreatic anlagen grow into the mesenchyme (*M*) as primitive tubules. Solid at first, the lumens develop within. The tubules branch, extend, and sometimes fuse with each other. **b** The cells forming tubules, which are derived from the endoderm, are uniform at first. Primitive ductular cells mature. Acinar cells differentiate from the primitive ductules, remaining in contact with each other, ductular cells, or centroacinar cells. Duct, ductular, and acinar cells are separated from the mesenchyme by a lamina propria (*red*). Other ductular cells differentiate, migrate into the mesenchyme, and assemble to form islets. The mature pancreas develops from these elements

fines of the primitive ductules, separated from the mesenchyme by a lamina propria (Fig. 1.2b). Differentiation of the primitive ductular cells also produces definitive ductular and ductal epithelial cells. Ductular and centroacinar cells maintain the rather nondescript morphology of primitive cells, while ductal cells and associated glands may take on the characteristics of mucous cells.

Precise Genetic Control is Necessary for Normal Pancreatic Development

The notochord, aorta, and mesenchyme all are important in producing the proliferation and differentiation inherent in achieving the fully mature and functional pancreas from small regions of cells in the primitive gut. Gene products causing stimulation and allowing responses among the involved elements are produced at the correct time and location. The products may be transient, and may rely on the presence of other, stimulative or permissive factors.

Many of the genes involved in the process of pancreatic development have been discovered relatively recently, in large as a result of animal experimentation. Nevertheless, it is likely that the same or equivalent key genes are active in human pancreatic development. Several good reviews reveal the extent and complexity of genetic controls necessary for pancreatic development and the markers convenient for assessing progress and interactions [1, 4, 8, 11].

At the time of the interaction between the notochord and the primitive gut, the notochord provides permissive signals to the endoderm [13]. The notochord secretes molecules of the transforming growth factor (TGFβ) family (activin- B) and the fibroblast growth factor (FGF) family (FGF-2). Pancreatic gene expression is activated by repressing Sonic hedgehog in the endoderm [14]. If Sonic hedgehog is not repressed, pancreatic development is inhibited. A bipotential population of precursor cells exists in the region from which the ventral pancreas originates. FGF from cardiac mesoderm induces the expression of Sonic hedgehog, inhibiting development of the pancreas, but allowing liver development [15]. The ventral pancreas develops from cells that are not affected by Sonic hedgehog expression.

A marker for induction of the pancreas is the homeobox gene Pdx1 (Fig. 1.1b). *Pdx1* expression is initiated at the point of contact between the aorta and vitelline vein, and the gut endoderm, and this interaction is necessary for appropriate pancreatic formation [16]. *Pdx1* is required for the outgrowth of gut endoderm and differentiation of the posterior foregut [17].

Mesenchyme, in addition to providing vessels and connective tissue, is essential for proper pancreatic development. Mesenchyme is required for exocrine development and seems to be necessary for the normal balance between exocrine and endocrine elements [18]. Migration of endocrine cells from ductules to form islets involves interaction with elements in the extracellular matrix.

Cells that are induced early to follow the endocrine pathway differentiate into the different definitive types: insulin-secreting β cells, glucagon-producing α cells, somatostatin (δ) cells, and PP cells, which make pancreatic polypeptide. Most of these will migrate from the duct wall to form islets in the mesenchyme. At intervals along the walls of primitive ducts and at their termination, cells proliferate and form primitive acini. Zymogen granules accumulate in the cells. Enzymes are identifiable by their immunocytochemistry. Continued development produces multiple arrangements. Acini become spheroid, elongate, or multilobed. Acinar cells may surround a lumen that follows a continuous, circular pathway. Cells of the primitive ducts show minimal morphological changes as they differentiate into definitive ductules; they are recognized mainly by their lack of zymogen granules, although distinguishing markers become detectable using immunochemical or genetic techniques. Acini that develop at the termination of primitive ducts end up with a ductule at one place, conducting secretions from each acinus. Acini that develop along the course of a primitive duct end up with a ductule that is continuous with it at two places; secretions from upstream will flow into it, secretions are added from that acinus, and the combined secretions flow out the other side. Centroacinar cells, which are differentiated from primitive ducts, are situated within acini and are in contact with acinar cells and/or ductular cells.

The Single Pancreas is Produced From Two Primordia

The precursors of the pancreas appear early in development as protrusions from the primitive gut at a stage when it is simple and quite small (Fig. 1.1b). Although the dorsal and ventral primordia are on opposite sides of the primitive gut, they are in fact very close to each other. In the ventral region, the ventral pancreatic primordium develops along with the primordia of the liver, gallbladder, and associated ducts (Fig. 1.3a). While the dorsal and ventral pancreatic primordia are expanding, the part of the primitive gut that gives rise to them is also growing and changing. The part of the primitive gut that becomes the duodenum grows more on one side than another, and rotates to the right. The result is that the ventral pancreas (primordium) comes to lie adjacent to and immediately posterior to the dorsal pancreas (primordium), and the ducts from the hepatic system and the ventral pancreas join the duodenum close to where

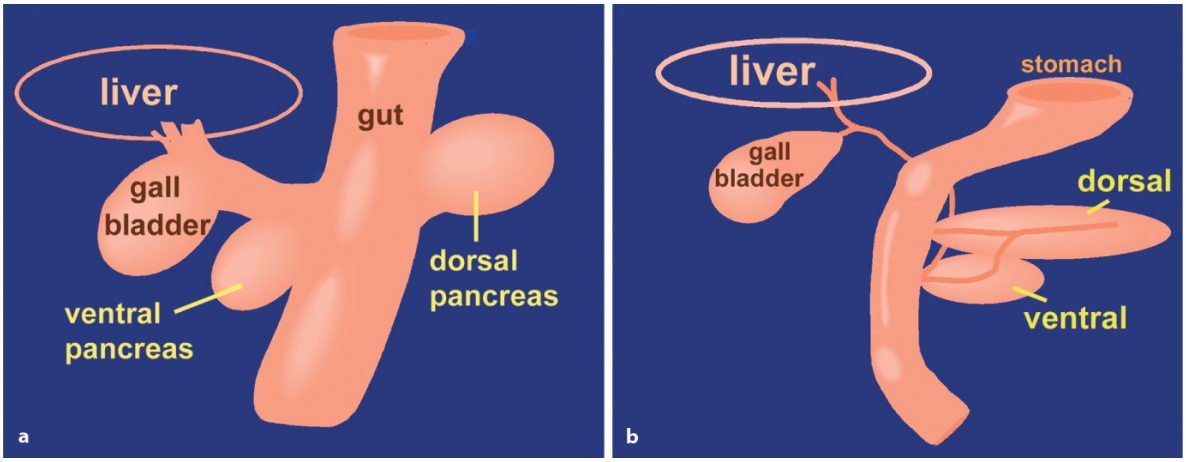

Figure 1.3

Early development of the pancreatic primordia. **a** The dorsal and ventral primordia grow and begin the differentiation of different cell types. The liver, gall bladder, and bile duct also develop from the ventral primordium. **b** Diagram of later development of the pancreatic primordia (from Bockman and Freeny [22]). The ventral pancreatic primordium swings around to the same side as the dorsal. The primordia fuse, as do their ducts. The duct from the liver and gall bladder (primitive bile duct) maintains a close association with the duct of the ventral pancreatic primordium

the duct of the dorsal pancreas joins the duodenum. The dorsal pancreas and ventral pancreas fuse. Their ducts anastomose (Fig. 1.3b). The main pancreatic duct is produced from the ventral pancreatic duct plus the distal part of the dorsal pancreatic duct. If the remainder of the dorsal pancreatic duct remains patent, it becomes the accessory pancreatic duct, emptying into the duodenum at the minor papilla. The bile duct empties into the duodenum along with the main pancreatic duct at the major papilla.

Blood Vessels Develop in the Mesenchyme

As development of the endodermally derived pancreatic epithelium continues, mesodermally derived cells in the surrounding mesenchyme differentiate into blood-vessel-forming cells. Fusion of the cells produces cysts and then elongated structures composed at first of a single layer of endothelial cells surrounding a lumen. These primitive vessels join with each other, forming a network that eventually connects with and is supplied from the aorta, as well as with the venous system draining through the hepatic portal vein. Branching and extension from the network produces the arteries, capillaries, and veins that permeate the definitive pancreas. Smooth muscle cells are derived from the mesenchyme to surround the arteries and veins. Connective tissue derived from the same source accumulates around the vessels, in greater amounts around the larger ones. The arteries that arise from the aorta and supply the mature pancreas are branches of the celiac trunk and superior mesenteric artery. It should be noted that the vessels supplying the pancreas do not originate at the duodenum and accompany the pancreatic ducts inward, but supply the organ from its periphery. This is also true for nerves.

Lymphatic vessels arise in a manner similar to that described for blood vessels. They form a network that lies in the extracellular matrix; however, their endothelial cells are not as tightly joined to each other as those of blood vessels, and they do not accumulate a coat as thick as blood vessels. Lymph nodes develop along their path, mainly external to the pancreas. The lymph carried from the pancreas eventually empties into the thoracic duct.

Nerves Connect to the Spinal Cord, Brain, and Enteric Nervous System

The nerve supply of the pancreas develops from the ectoderm and establishes itself through migration of cells as well as by extension of the nerve fibers from cells located well outside the pancreatic domain. Cells migrate from the neural crest (a population of nerve cell precursors that form dorsal to the embryonic spinal cord as its borders unite) into the developing pancreas to form intrinsic ganglia. Nerve fibers from

nerve cell bodies in the brain pass through the vagus nerve and synapse on intrinsic ganglia. This combination establishes parasympathetic flow to the pancreas, with fibers extending from intrinsic ganglia to end in the extracellular matrix adjacent to acini, providing a stimulus for secretion.

Another group of neural cell precursors migrate from the neural crest to form ganglia around the celiac trunk. Nerve fibers extend from cell bodies in the intermediolateral column of the spinal cord to synapse on these celiac ganglia. Nerve fibers from the celiac ganglia enter the pancreas around arteries and provide the sympathetic stimulation for the pancreas.

A sensory nerve supply is provided through the vagus nerve by fibers extending from pancreas to nerve cell bodies in the nodose ganglion and from there into the brain. A sensory nerve supply also is provided by nerve fibers extending from pancreas through splanchnic nerves to nerve cell bodies in dorsal root ganglia and from there into the spinal cord.

In the mature normal pancreas, neural regulation of secretion and blood flow proceeds without conscious awareness. Sensation, usually pain, is a consequence of pancreatic disease.

Reorganization of the Pancreas is Associated with Disease

In the absence of disease, once the pancreas has reached its definitive state it will be maintained. So long as its blood supply and control are adequate, secretions are free to enter the duodenum, and there is no inflammation, the endocrine and exocrine divisions of the pancreas will usually function silently and efficiently. Exceptions include genetically induced diseases that involve the inability of cells to function properly. Regardless of initiating causes, known or unknown, changes in the structure and function of the pancreas are accompanied by changes in gene expression. Common changes are inadequately controlled growth of cells and loss by apoptosis or necrosis. An additional type of change is for cells to change to another type, losing normal markers and gaining markers not characteristic of their fully differentiated phenotype, frequently assuming a different morphology. Many of these changes seem to represent reversion to a previous differentiative stage.

As has already been described, primitive tubules or primitive ducts represent an early stage of differentiation of the pancreas, and several cell types stem from them. A common change associated with several pancreatic diseases is the appearance of accumulations of tubules or ductule-like structures. These have been referred to as tubular complexes.

Tubular complexes are found in the pancreas of patients with acute and chronic pancreatitis, pancreatic cancer, and cystic fibrosis. They occur with occlusion of ducts and may be produced experimentally by ligation of the pancreatic ducts. Experimental pancreatic cancer has been shown to originate through tubular complexes [6].

Tubular complexes can originate from acini [5, 19]. Similar tubules can originate from islets of Langerhans under certain conditions. Tubular complexes in the pancreas of diabetes-prone rats possess cells that display the stem cell marker nestin, *Pdx-1*, and mixed duct/endocrine and duct/acinar markers [7]. In both cases, seemingly terminally differentiated cells revert to an earlier differentiative type. In most pancreatic diseases, regressive changes are continuous, and there is little chance for reconstitution of a fully functional organ. A hope for assisting reconstitution lies in acquiring an understanding of the controlling factors that are necessary to initiate and sustain differentiation toward the mature cell type (i.e., acinar cell or endocrine cell).

When the human fetal pancreas was transplanted beneath the renal capsule of immunodeficient mice, a shift in cell types with minimal apoptosis, the presence of intermediate cell types, and an increase in endocrine cells led to the hypothesis that exocrine cells transdifferentiate into duct cells, and these eventually develop into endocrine cells [20]. Jamal et al. [10] have demonstrated a phenotypic switch of human islets to duct-like structures with markers of duct epithelium and progenitor cells; treatment of the duct-like structures with islet neogenesis-associated protein induced their reversion to islet-like structures.

The development of the pancreas from interactions between embryonic structures is quite clear and well documented. The nature of the signaling that initiates and controls these interactions is being revealed through the study of genes and their products as they occur during the developmental process. It is becoming more obvious that one of the changes that occurs with diseases of the pancreas is reversion from the definitive state to a more primitive one. Transdifferentiation of acinar and islet cells to earlier differentiative types is well established. Application of the knowledge of differentiation to the understanding of transdifferentiation may hold the key to learning how to reinitiate the formation of mature pancreatic elements from the products of pancreatic disease.

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