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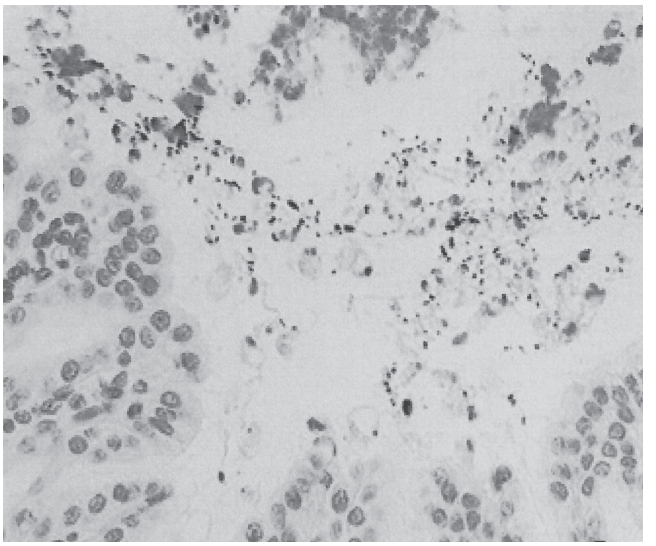
GASTROINTESTINAL REGENERATIVE ENGINEERING



Histological micrograph showing the distribution of *Helicobacter pylori* (*H. pylori*), a type of bacteria that cause peptic ulcers in mammalian animals and humans, in the stomach of Mongolian gerbil. The *Helicobacter pylori* bacteria were detected by immunohistochemistry and appears black in color. (Reprinted with permission from Ikeno T et al: *Am J Pathol* 154:951–60, copyright 1999.) See color insert.

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

ANATOMY AND PHYSIOLOGY OF THE GASTROINTESTINAL SYSTEM

Structure [18.1]

The gastrointestinal system includes the esophagus, stomach, small intestine, and large intestine. The primary functions of the gastrointestinal system are food ingestion, digestion, and absorption, as well as waste product elimination. The *esophagus* is a tubular organ that is extended from the pharynx to the stomach. The function of the esophagus is food transport from the mouth to the stomach. The esophagus is composed of four distinct tissue layers: mucosa, submucosa, muscular layer, and adventitia. The *mucosa* consists of stratified epithelial cells and a subendothelial connective tissue layer. The *submucosa* is a connective tissue layer, composed of fibroblasts, extracellular matrix (collagen fibers, elastic fibers, and proteoglycans), blood vessels, nerves, and small glands. The gland cells can secret mucus, which serves as a lubricant for food ingestion. The *muscular layer* is composed of striated muscular cells in the upper esophagus and smooth muscle cells near the stomach. There are two layers of muscular cells: the inner layer with circumferentially aligned muscular cells and the outer layer with longitudinally aligned muscular cells. In addition, the muscular layer contains extracellular matrix and nerves. The nerves control the movement of the esophagus. The *adventitia* is a thin layer composed of fibroblasts, extracellular matrix, and a squamous epithelium on the outer surface. The epithelium is also known as visceral peritoneum in gastrointestinal organs.

The stomach is located in the upper left abdominal cavity and is responsible for the primary digestion of ingested foods. As other digestive tracts, the stomach is composed of four layers: mucosa, submucosa, muscular layer, and adventitia. The structure of these layers is similar to that of the esophagus with several exceptions. First, the inner surface of the stomach is lined with a monolayer of columnar epithelial cells, instead of stratified cells. The epithelial cells of the stomach are specially differentiated cells. Some of these cells line the stomach surface and produce mucus. Others form gastric glands, which produce hydrochloric acid, pepsinogen, and mucus. The acidic environment (pH 1–3) helps to digest foods. Pepsinogen is the precursor of the enzyme pepsin, which cleaves proteins into peptides for further digestion. Pepsinogen can be converted into pepsin when it is released into the stomach under the action of hydrochloride acid and pepsin. Second, the muscular layer contains three sublayers of smooth muscle cells: oblique, circumferential, and longitudinal muscular sublayers. The reinforced muscular structure enhances gastric movement and sufficient food digestion. The stomach undergoes constant movements, which help to mix and digest foods.

The small intestine is a tubular organ that is located in the abdominal cavity and is extended from the stomach to the large intestine. The small intestine is composed of three segments: the duodenum, jejunum, and ileum. At the tissue level, the small intestine consists of the four standard layers: the mucosa, submucosa, muscular layer, and adventitia. The mucosa and partial submucosa form numerous folds about 1 mm in length, also known as villi, on the surface of the small intestine. The villi are covered with columnar epithelial cells. These cells form cell membrane projections called *microvilli*. The formation of villi and microvilli increases the total surface area of the small intestine, which facilitates nutrient absorption. There are epithelial goblet cells and submucosa gland cells. These cells produce mucus. The muscular layer of the small intestine contains circumferential and longitudinal smooth muscle cells. These cells conduct constant movements to move and mix ingested foods. The structure is similar among the duodenum, jejunum, and

ileum. The functions of the small intestine are food digestion and absorption. A number of digestive enzymes, including those for digesting proteins, polysaccharides, and lipids, are secreted from the pancreas into the small intestine. Enzymes for protein digestion include trypsin, chymotrypsin, and carboxypeptidase. Polysaccharides are digested by amylases, and lipids are digested by lipases. In addition, the pancreas secrets deoxyribonucleases and ribonucleases, which break down DNA and RNA, respectively.

The large intestine is a digestive tract that is extended from the ileum to the anus. The large intestine is usually divided into several segments, including the colon, rectum, and anal canal. The general structure of the large intestine is similar to that of the other digestive tracts. The large intestine possesses a large number of goblet cells which secret mucus. The primary function of the large intestine is to remove food wastes. Water is absorbed in the large intestine. However, little nutrient absorption takes place in the large intestine. The large intestine undergoes constant movements, which help to move the food remains.

Nutrient Digestion and Absorption [18.1]

The ingested foods contain three major types of nutrient: protein, carbohydrate, and lipid. All these nutrients are digested in the stomach and small intestine, and absorbed in the small intestine. Proteins from ingested meats and plants are first digested in the stomach by pepsin, which cleaves proteins into polypeptides. Remaining proteins and digested polypeptides are moved into the small intestine, where they are further digested into tripeptides, dipeptides, and amino acids by peptidases. Amino acids and short peptides are absorbed into the epithelial cells by an energy-consuming process involving sodium cotransport. The absorbed short peptides are digested into amino acids in the epithelial cells. The amino acids are transported into the blood of the portal vein and used for protein synthesis in different types of cell.

Ingested foods contains polysaccharides (starches and glycogen), disaccharides (sucrose and lactose), and monosaccharides (glucose). Polysaccharides are digested by salivary and pancreatic amylases into disaccharides and monosaccharides in the stomach and small intestine. Disaccharides are further digested into monosaccharides at the epithelial surface. Monosaccharides are absorbed into the epithelial cells of the small intestine by an active transport mechanism involving the co-transport of sodium and released into the intestinal capillaries.

Lipids include cholesterol, triglycerides, phospholipids, and steroids. Ingested lipids are emulsified in the small intestine by bile compounds produced by the liver and are digested by pancreatic lipases. Digested dietary lipids form small particles known as *micelles*. The lipid molecules diffuse into the epithelial cells according to the gradient across the cell membrane. Within the epithelial cell, lipids form chylomicrons (80–500 nm in diameter) by conjugating with apoproteins. The chylomicrons are transported into the blood and are further digested by bloodborne lipases, releasing chylomicron remnants and free fatty acids (triglycerides). The chylomicron remnants are taken up by hepatocytes in the liver through receptor-mediated endocytosis, digested in the lysosomes of the hepatocytes. Free cholesterol molecules are released from the chylomicron remnants and stored in the hepatocytes as cholesteryl esters. The cholesterol molecules are either excreted into the bile or used to form very-low-density-lipoproteins (VLDL, diameter 30–80 nm) in the hepatocytes. The VLDL molecules are released into the blood and digested by lipoprotein lipases, resulting in the formation of intermediate-density-lipoproteins (IDL) after releas-

ing a fraction of fatty acids. The IDL is digested by lipoprotein lipases into low-density-lipoproteins (LDL), which usually circulate in the blood for 1–2 days and constitute the major reserve of plasma cholesterol (60–70% of the total cholesterol pool). The cholesterol molecules of LDL can be taken up by cells and used for the construction of cell membranes. The promotion of cell intake of cholesterol reduces the plasma LDL and cholesterol level, a beneficial process for reducing the risk of atherogenesis. With the release of the majority of cholesterol molecules, LDL is converted to high-density-lipoproteins (HDL), which consist of apoproteins and residual cholesterol molecules and can form complexes with cholesterols and fatty acids, contributing to the clearance of plasma lipids.

GASTROINTESTINAL DISORDERS

Peptic Ulcer

Pathogenesis, Pathology, and Clinical Features [18.2]. *Peptic ulcer* is a disorder that occurs primarily in the duodenum and stomach and is characterized by the presence of round or oval ulcerative lesions with various sizes. The lesions include injury and detachment of epithelial cells, necrosis and fibrosis of mucosa and submucosa, and infiltration of inflammatory cells. These pathological changes are possibly induced by the corrosive effect of acid and pepsin. *Duodenal ulcer* is often found in the proximal segment of duodenum near the stomach. About 10% of the human population is affected by duodenal ulcers some times during the lifespan. The incidence of duodenal ulcer is higher than that of gastric ulcer. Duodenal ulcer is a chronic disorder with a high rate of recurrence and is often found in patients about 50 years old. A large fraction of patients experience reoccurring duodenal ulcers within a period of 2–3 years. *Gastric ulcer* occurs in the stomach and exhibit similar pathological changes as found in the duodenal ulcer. This type of ulcer is often found in patients about 60 years old.

The pathogenesis of duodenal and gastric ulcers is related to several factors:

1. A type of bacteria known as *Helicobacter pylori* (*H. pylori*) can cause inflammatory reactions and peptic ulcers in the stomach of mammalian animals and humans (Chapter 18 opening figure).
2. Acid and pepsin secreted by the stomach epithelial cells may exert a corrosive effect on the duodenal and gastric epithelial cells. Some ulcer patients are associated with increased secretion of acid and pepsin.
3. A reduction in the mucosal resistance to the corrosion of acid and pepsin contributes to the development of duodenal and gastric ulcers.
4. An increase in the release of gastrin, a molecule that stimulates the secretion of hydrochloric acid in the stomach, is another potential factor that contributes to the development of duodenal and gastric ulcers. Even though the level of gastrin may be normal, the gastric epithelial cells in ulcer patients often exhibit increased responsiveness to gastrin stimulation, enhancing the secretion of hydrochloric acid. Fourth, hereditary factors may also play a role in the development of duodenal and gastric ulcers. Ulcer patients often have a familial history of ulcer. In addition, cigarette smoking is associated with increased incidence of duodenal and gastric ulcer, although smoking does not alter acid secretion. The mechanisms of smoking-related ulcers remain poorly understood.

Patients with duodenal and gastric ulcers often experience epigastric burning pain. When a blood vessel is corroded and damaged, hemorrhage may occur. The severity of hemorrhage is dependent on the size of the damaged blood vessel. In general, ulcers do not significantly influence food digestion and absorption, since ulcers are usually small.

Experimental Models of Gastrointestinal Ulcers [18.3]. Gastric and duodenal ulcers can be induced by application of acid and pepsin to the stomach or duodenum of animal models. Here, the duodenum is used to demonstrate the experimental procedures. An animal can be anesthetized by peritoneal injection of sodium pentobarbital at a dose of 50 mg/kg body weight. The upper abdominal skin can be sterilized with 75% alcohol, Betadine (povidone-iodine), and 75% alcohol again. The abdominal cavity can be opened at a location in the upper middle area and the duodenum is identified. A segment of the duodenum can be isolated by applying a pair of intestinal clamps. A mixture of hydrochloric acid and pepsin can be injected into and kept in the duodenum for a desired period. The concentration of acid and pepsin and the treatment time should be determined by conducting a series of experiments with graded concentrations and different treatment times. The clamps can be released and the abdominal wounds closed after the treatment. At scheduled times following the surgery, specimens can be collected from the duodenum and used for pathological examinations.

Conventional Treatment [18.2]. There are several general approaches for the treatment of gastrointestinal ulcers (see Table 18.1 for a list of therapeatic proteins used to treat peptic ulcer). These include the administration of acid-reducing agents, protective coating agents, and diet control. Commonly used acid-reducing agents include antacids, anticholinergic agents, antagonists to histamine H2 receptor, and prostaglandins. The administration of antacid agents, such as sodium bicarbonate and calcium carbonate, results in direct neutralization of the acidic content in the stomach and duodenum, suppressing ulcer formation and progression. Acetylcholine is a neurotransmitter that promotes the production and secretion of gastric acid. A treatment with anticholinergic agents, such as atropine and atropine derivatives, can reduce the secretion of gastric acid. The H2 receptor of histamine mediates histamine-induced secretion of gastric acid. Antagonists of the histamine H2 receptor, such as cimetidine, suppress the activity of the receptor and reduce the secretion of gastric acid. Prostaglandins exert an inhibitory effect on gastric acid secretion in the stomach and enhance the capability of gastric mucosal resistance to acid corrosion. Stomach mucosal coating agents, such as sucralfate (polyaluminum hydroxide salt of sucrose sulfate), can adhere to the mucosal surface and protect the mucosa from acid and pepsin corrosion. In addition, the control of acidic diet intake reduces the progression of gastrointestinal ulcers. In the case of severe hemorrhage, it is necessary to remove the ulcerative tissue via surgery.

Molecular Regenerative Engineering. The principle of molecular engineering or therapy for peptic ulcer is to promote tissue recovery from ulcerative injury. Although the reduction of acid secretion in the stomach is the most important approach for treating peptic ulcer, there are no proteins or genes available for such a purpose. Several genes, including the serum response factor, platelet-derived growth factor, vascular endothelial growth factor, and angiopoietin-1 genes, have been identified and used in animal models for enhancing recovery from peptic ulcer-induced injury. These genes encode mitogenic factors that stimulate cell proliferation and migration, thus enhancing the recovery from

TABLE 18.1. Characteristics of Selected Therapeutic Proteins for Peptic Ulcer*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Serum response factor	SRF, c-fos serum response-element-binding transcription factor	508	52	Ubiquitous	A downstream target of the mitogen-activated protein kinase pathway, binding directly to the serum response element (SRE) in promoter region of immediate–early genes (e.g., c-fos), and stimulating cell proliferation and differentiation
Angiopoietin-1	ANG1, ANGPT1	498	58	Blood vessels (endothelial cells), heart, lung, placenta, bone	Regulating the early development of the heart, and promoting vascular development and angiogenesis

*Based on bibliography 18.4 and 18.7.

ulcerative injury. A selected gene can be transferred into the stomach and small intestine by catheter-mediated luminal instillation.

Serum Response Factor (SRF) [18.4]. Serum response factor is a transcriptional factor that mediates the transcription of immediate early genes (see Table 18.1 for the characteristics of SRF). Proteins encoded by the immediate early genes participate in the regulation of mitogenic responses, including cell proliferation and migration. When the SRF gene is transferred into the stomach of animal models with gastric ulcer, the expression level of SRF increases, which is associated with enhanced proliferation and migration of epithelial and smooth muscle cells in the ulcerative tissue. These activities enhance the recovery from gastric ulcer compared to a control ulcer model without SRF gene transfer. These observations suggest that the SRF gene may serve as a potential candidate gene for the molecular therapy of human peptic ulcer.

Platelet-Derived Growth Factor (PDGF) [18.5]. Platelet-derived growth factor is a protein that stimulates mitogenic activities, including cell proliferation and migration (see page 600 for the characteristics of PDGF). In particular, this growth factor enhances the survival and proliferation of smooth muscle cells. Experimental investigations have demonstrated that the overexpression of the PDGF gene by gene transfer in rat models of duodenal ulcer enhances cell proliferation in ulcerative tissue and facilitates the healing process of ulcer (Fig. 18.1).

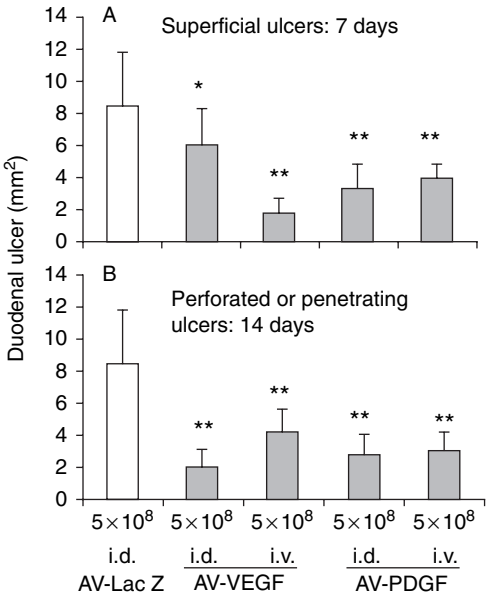


Figure 18.1. The size of duodenal ulcers at 7 and 14 days after transfection with adenoviral vector (AV) containing the vascular endothelial growth factor (VEGF) gene or the platelet-derived growth factor (PDGF) gene compared with a control vector containing the Lac Z gene (β-galactosidase gene). *, $P < 0.05$; **, $P < 0.01$. $n = 6–12$. (Reprinted with permission from Deng X et al: Gene therapy with adenoviral plasmids or naked DNA of vascular endothelial growth factor and platelet-derived growth factor accelerates healing of duodenal ulcer in rats, *J Pharmacol Exp Ther* 311:982–8, copyright 2004.)

Vascular Endothelial Growth Factor (VEGF) [18.6]. As platelet-derived growth factor, VEGF stimulates cell proliferation and migration (see page 600 for the characteristics of VEGF). This growth factor has been shown to particularly regulate the development, survival, and proliferation of the vascular endothelial cells. In experimental models of peptic ulcer, this growth factor enhances angiogenesis as well as the proliferation and migration gastrointestinal epithelial cells. Thus, the overexpression of the VEGF gene may potentially improve the healing of peptic ulcer (Fig. 18.1).

Angiopoietin-1 [18.7]. Angiopoietin-1 is a protein that regulates angiogenesis (see Table 18.1 for the characteristics of angiopoietin-1). Local delivery of angiopoietin-1 expression vectors results in up-regulation of the angiopoietin-1 gene and an increase in angiogenic activities. Since angiogenesis is required for the regeneration of ulcerative tissue, the overexpression of the angiopoietin-1 gene can enhance ulcer healing.

Gastrointestinal Cancers

Pathogenesis, Pathology, and Clinical Features [18.8]. *Gastrointestinal cancers* are malignant tumors found in the mucosal, submucosal, and muscular layers of the gastrointestinal tracts. Gastrointestinal cancers are divided into several types, including carcinoma, leiomyosarcoma, and lymphoma based on the cellular origin of the tumor. As cancers in other organs, these cancers are highly metastatic.

Gastrointestinal carcinoma is a form of cancer originated from the mucosal epithelial cells. This form of cancer is more common in men than in women. It is often found in people about 60 years old or older. The etiology of gastrointestinal carcinoma remains poorly understood. Hereditary and dietary factors may play a role in the development of the disease. In addition, the presence of several disorders, such as atrophic gastritis and intestinal metaplasia, may enhance the development of gastrointestinal carcinoma. Pathological examinations usually reveal several forms of carcinoma, including ulcerative, superficial, and infiltrative carcinomas. Ulcerative carcinomas are cancers that appear similar to ulcers at the surface. Superficial carcinomas are those found within the epithelial layer. Infiltrative carcinomas are those that invade the deep layers. At the cellular level, carcinoma cells are characterized by the enlargement of cell nuclei and an increase in cell density. During the end-stage, carcinoma cell metastasis or infiltration into deeper layers can be found. A cell proliferation assay often demonstrates an increase in the rate of cell proliferation.

Gastrointestinal leiomyosarcoma is a form of cancer originated from the submucosal smooth muscle cells. This type of cancer is not as common as gastrointestinal carcinoma and accounts for about 3% of total gastrointestinal cancers. The tumor is usually spherical in shape with necrosis at the center. *Gastrointestinal lymphoma* is a type of cancer originated from the lymphoid tissue. This type of cancer accounts for about 5% of gastrointestinal cancers. The etiological and pathological features of gastrointestinal leiomyosarcoma and lymphoma are similar to those of gastrointestinal carcinomas.

Conventional Treatment. Several conventional approaches, including surgical removal, chemotherapy, and radiotherapy, have been developed and used for the treatment of cancer. These approaches will be discussed in Chapter 25 in detail.

Molecular Therapy [18.9]. A number of molecular strategies have been developed and used for the treatment of cancers. These include the up-regulation of tumor suppressor

genes, correction of mutant tumor suppressor genes, enhancement of anti-cancer immune responses, activation of tumor suppressor drugs, introduction of oncolytic viruses, and application of antisense oligodeoxynucleotides. Since different types of cancers exhibit common pathogenic mechanisms and features, these therapeutic approaches can be applied to all types of cancers. These approaches will be discussed in detail in Chapter 25.

Tissue Regenerative Engineering. *Gastrointestinal tissue engineering* is to repair or reconstruct malfunctioned esophagus, stomach, or intestines with cell-containing tissue constructs. Cancer is a major disorder that requires tissue and organ repair or reconstruction. Several other disorders, including inflammatory bowel disease, intestinal infarction, and short bowel syndrome, may also require intestinal repair or reconstruction. Since the esophagus, stomach, and intestines possess distinct functions, disorders in these organs should be treated with different approaches. The function of the esophagus is to conduct foods from the mouth to the stomach. A simple structural reconstruction may be sufficient to restore the function of a disordered esophagus. Other gastrointestinal functions such as nutrient absorption and transport may not be a critical issue for esophagus tissue engineering. Since the length of the esophagus is limited (no excessive esophagus available), it is necessary to replace a malfunctioned esophagus with a substitute (see following sections for details).

Compared to the esophagus, the stomach and intestines are responsible not only for food conduction, but also for food digestion and absorption (absorption occurring primarily in the small intestine). Thus in stomach and intestinal tissue engineering, these functional aspects should be taken into account. Another difference from esophagus engineering is that it is not necessary to replace the injured regions of the stomach and intestines when the lesions are small and do not involve the entire organ. The reason is that the stomach and intestines possess a large capacity of reserve that is not used under physiological conditions. A malfunctioned region of the stomach or intestines can be simply removed with the remaining organ reanastomosed. In general, the removal of 50% of the stomach or the intestine may not significantly influence food digestion and absorption. When the lesion involves a large area or the entire organ, which impairs the digestion and absorption of nutrients, fluids, and electrolytes, engineering replacement of the stomach or intestine is necessary. However, it remains difficult to construct a gastrointestinal substitute with the natural functions of molecular absorption and transport. The construction of such a substitute is a critical issue in gastrointestinal tissue regenerative engineering.

Several approaches have been established and used for the construction of gastrointestinal substitutes. These include organ transplantation, substitution with autogenous pedicle with blood supply, expansion of existing intestinal tracts, intestinal regeneration with the peritoneal membrane, substitution based on biodegradable and nonbiodegradable polymeric materials, substitution based on allogenic intestinal submucosa, and substitution based on extracellular matrix components.

Gastrointestinal Transplantation [18.10]. Organ transplantation is an effective approach for the replacement of malfunctioned esophagus, stomach, and intestines. A fresh, viable, allogenic organ can be harvested and used to substitute for a host equivalent. The transplant can usually maintain the functions such as food digestion and nutrient absorption. However, allogenic cells induce immune reactions, resulting in acute transplant rejection. A common approach to reduce immune reactions is to administrate immunosuppressing agents. Patients with organ transplantation usually need to take immune suppressing

agents for the entire lifespan. While these agents inhibit immune rejection responses, they also suppress normal immune functions, resulting in increased susceptibility to infectious diseases.

Gastrointestinal Reconstruction Based on Autogenous Pedicles [18.11]. Given the difficulties in the maintenance of allogenic gastrointestinal transplants, scientists have established approaches for gastrointestinal regeneration based on autogenous connective and muscular tissue pedicles with blood supply. Abdominal wall pedicles with functional blood vessels can be prepared and used for such a purpose. A layer of the abdominal wall can be isolated at one end. The other end remains connected to the abdominal wall, ensuring sufficient blood supply. The free end of the pedicle can be tailored into a tubular structure and anastomosed to a malfunctioned gastrointestinal organ. In experimental models of intestinal reconstruction, the host intestinal mucosa can extend to the graft surface, forming a mucosa-like structure, known as neomucosa, within 1–2 months following the reconstruction surgery. Examinations by optical and electron microscopy have demonstrated that the neomucosa is similar in structure to the natural mucosa. Furthermore, the neomucosa exhibits certain intestinal functions such as absorption of glucose and electrolytes. These observations suggest that connective and muscular tissues can be transformed into intestinal tissue in an appropriate intestinal environment. Autogenous pedicle-based gastrointestinal reconstruction represents one of the most effective approaches for intestinal regeneration.

Expansion of Intestines [18.12]. Gastrointestinal cells, especially the mucosal epithelial cells, undergo constant proliferation, a mechanism for the replacement of apoptotic cells and for tissue expansion. Intestinal tissue expansion occurs in response to an increased demand for nutrient absorption when the absorptive surface is reduced due to various disorders, such as cancers and inflammatory bowel disease. Based on such a feature, scientists have developed an approach for intestinal self-regeneration or expansion. A segment of intact intestine can be removed and split longitudinally into two equal parts. Each part can be constructed into a tubular structure. Both tubes can be anastomosed to the intestine in a series so that the length can be doubled. The narrowed lumen can be expanded in the radial direction through adaptation to luminal inflation and enhanced wall tension. It is the nature of biological systems that tissues and organs can grow in response to mechanical stretch. This adaptive mechanism has also been found in the cardiovascular and pulmonary systems. Intestinal expansion is an effective approach for the regeneration of intestines. The regenerated intestine can be used for the repair or replacement of a malfunctioned intestine.

Regeneration with Peritoneum [18.13]. Peritoneum is an epithelium-covered connective tissue membrane found on the surface of the abdominal organs. A loose peritoneal membrane can be harvested and used to construct tubular structures. When these tubular structures are anastomosed into the intestine, host mucosal cells can migrate to the graft, forming neomucosa. The neomucosa is similar in structure to the host mucosa and exhibits intestinal functions, such as absorption of fluids and electrolytes. However, the source of the peritoneum is limited. It is difficult to collect sufficient peritoneal membrane for the replacement of a large area of malfunctioned intestine.

Gastrointestinal Reconstruction Based on Polymeric Materials [18.14]. While autogenous tissues are ideal materials for the regeneration of malfunctioned gastrointestinal

tracts, it is often difficult to collect sufficient amount of tissue for large lesions. To resolve such a problem, scientists have been searching for synthetic materials that can be used for gastrointestinal reconstruction. In early studies, nonbiodegradable polymers, such as Dacron and polytetrafluoroethylene (PTFE), have been used to repair malfunctioned gastrointestinal tracts. When grafted into the host esophagus or intestine, the polymeric materials can serve as a bridge for the generation of neomucosa. These investigations suggest that polymeric materials can be used as gastrointestinal conduits. However, polymeric materials cannot be integrated into the host systems and the regenerated tissue does not assemble the native gastrointestinal system.

Biodegradable polymeric materials have also been used for gastrointestinal reconstruction. Several types of such materials, including polyglycolic acid and polylactic acid, have been used in animal models of esophagus, stomach, and intestinal reconstruction. These polymers can be absorbed gradually in the host system. The rate of degradation can be controlled by altering the composition of the materials. Biodegradable polymers are often used to construct scaffolds for cell seeding and growth. It has been demonstrated that, with controlled degradation of the polymeric materials, the gastrointestinal cells are able to regenerate a gastrointestinal substitute with the native structure of the system. To enhance the regenerative process, disintegrated intestinal tissues can be seeded in the polymeric scaffold. Since intestinal tissues contain epithelial progenitor cells, the seeding of intestinal tissues facilitate intestinal regeneration. Preliminary investigations based on animal models have demonstrated that regenerated gastrointestinal tissues in biodegradable polymer scaffolds are similar in structure to the native gastrointestinal tissues and exhibit physiological functions such as absorption of fluids and electrolytes.

Extracellular Matrix-Based Gastrointestinal Reconstruction [18.15]. Extracellular matrix is a structure constituted with collagen fibers, elastic fibers, and proteoglycans and serves as a frame for the attachment, support, organization, and communication of cells and for the formation and assembly of tissues. The submucosa of the gastrointestinal tracts is an extracellular matrix-rich structure. Thus, extracellular matrix is an ideal structure for constructing gastrointestinal scaffolds, which can be used for tissue regeneration. Since collagen fibers provide structural support and mechanical strength to tissues and organs, collagen-rich matrix is often used for tissue reconstruction. Collagen-rich matrix materials can be collected from soft connective tissues, such as the dermis and the submucosa of intestines. The collected matrix specimens can be used to reconstruct the gastrointestinal tracts.

Allogenic decellularized extracellular matrix components, such as collagen, have long been used for constructing scaffolds for tissue regeneration. It is usually necessary to remove cellular components, because these components cause host immune reactions, resulting in acute transplant rejection. Unlike cellular components, allogenic matrix components exhibit low immunogenicity and do not cause acute immune rejection responses. Several methods can be used for extracting collagen-matrix, including treatment of connective tissues with alkalines, acids, and detergents, which lyse and remove cells, leaving a collagen-rich matrix. Such a matrix can be used to construct a scaffold with desired shape and dimensions and to seed gastrointestinal cells or stem cells for tissue regeneration. Collagen-based scaffolds usually stimulate cell proliferation and migration, enhancing tissue regeneration.

Experimental investigations based on animal models have demonstrated that collagen-based matrix scaffolds can be used for the regeneration of esophagus and small intestines.

When grafted into a host esophagus or intestine, host cells are able to migrate into the matrix scaffold, forming an esophagus- or intestine-like structure, respectively. The matrix scaffold can be gradually integrated into the host system. These preliminary investigations have demonstrated that extracellular matrix is a suitable material for the regeneration of gastrointestinal tracts.

Experimental Models of Gastrointestinal Reconstruction [18.16]. Malfunctioned esophagus, stomach, and intestines can be reconstructed in animal models by using allogenic tissue equivalents, extracellular matrix specimens, autogenous connective or muscular tissue pedicles. An animal can be anesthetized by peritoneal injection of sodium pentobarbital at a dose of 50 mg/kg body weight. To reconstruct a segment of the small intestine, the abdominal skin is sterilized, the abdominal cavity is opened, and the small intestine is identified. A segment of the small intestine can be isolated by applying a pair of intestinal clamps and removed between the clamps. An intestinal substitute with diameter similar to that of the host intestine can be anastomosed to the host intestine at the two ends by using continuous stitches. The two clamps are then released and the anastomotic sites are inspected for leakage. Leaking sites, if any, can be sealed by additional suture stitches. The abdominal wound can be closed by using continuous suture stitches for the muscular layer and disrupted suture stitches or surgical staples for the skin.

Inflammatory Bowel Disease

Pathogenesis, Pathology, and Clinical Features [18.17]. *Inflammatory bowel disease* is a gastrointestinal disorder characterized by the presence of continuous and uniform inflammatory reactions, ulcerative lesions, and hemorrhage in the mucosa of the esophagus, stomach, small intestine, and/or large intestine. The etiology of this disorder remains poorly understood. The incidence of the disease is 2–6 per 100,000 in the United States. The disease is often found in the population at the age of 15–35.

The pathogenesis of inflammatory bowel disease is related to several factors, including genetics, infection, and immune responses. Patients with inflammatory bowel disease usually show a familial history of the disease, suggesting a hereditary predisposition to the disease. Bacterial and viral infection induces acute inflammatory reactions in the gastrointestinal tracts. These inflammatory reactions may contribute to the pathogenesis of inflammatory bowel disease. However, bacteria or viruses specific to inflammatory bowel disease remain unidentified. Inflammatory bowel disease is sometime associated with autoimmune diseases, such as arthritis and pericholangitis. Antiinflammation agents, such as corticosteroids, often reduce the symptoms of the disease. Patients with inflammatory bowel disease may generate antibodies against bacterial antigens, which are similar to certain cellular components of the hosts. These observations suggest an autoimmune mechanism for the development of inflammatory bowel disease.

Inflammatory bowel disease is associated a number of pathological changes. These include the loss of epithelial cells, ulcerative lesions, leukocyte infiltration into the mucosa and submucosa, submucosal edema, hemorrhage, the presence of red blood cells in the submucosa, submucosal fibrosis, and thickening of the intestinal wall. Submucosal fibrosis can induce regional distortion of the intestine. In severe cases, inflammatory and fibrous changes can result in intestinal obstruction. These changes may significantly influence nutrient absorption in the small intestine, resulting in nutritional deficiency. Intestinal

perforation may occur as a result of altered structure and mechanical stiffness of the intestinal wall. Severe hemorrhage may occur occasionally.

Conventional Treatment [18.17]. There are two primary conventional treatments for inflammatory bowel disease: control of inflammation and surgical removal of malfunctioned intestine. Inflammatory reactions can be controlled by using hormones, including glucocorticoids and adrenocorticotropic hormone (ACTH). Glucocorticoids are produced in the cortex of the adrenal gland. ACTH is produced in the anterior pituitary and stimulates adrenal cortex to produce glucocorticoids. These hormones can be used to effectively suppress inflammatory reactions in inflammatory bowel disease. Nutritional treatment is necessary if there is a nutrient loss due to impaired absorption in the small intestine. Immunosuppressive agents, such as azathioprine may be used to suppress autoimmune reactions, which potentially contribute to the development of inflammatory bowel disease. In severe cases, such as those with potential perforation of intestinal wall and severe hemorrhage, it is necessary to remove the malfunctioned intestine by surgery.

Molecular Therapy. Inflammatory bowel disease is a disorder with chronic inflammation and possible autoimmune reactions. Molecular therapeutic approaches have been developed to suppress these activities. Two genes have been targeted for the potential treatment of inflammatory bowel disease: interleukin (IL)10 and IL18.

Interleukin-10 [18.18]. Interleukin (IL)10 is a cytokine that inhibits the release of proinflammatory cytokines and suppresses inflammatory reactions. Genetically induced deficiency of interleukin-10 is associated with accelerated inflammatory activities, which simulate inflammatory reactions found in inflammatory bowel disease. A treatment with the interleukin-10 protein reduces the degree of intestinal inflammation (Fig. 18.2). The delivery of the interleukin-10 gene into animal models of inflammatory bowel disease results in local upregulation of interleukin-10 expression, leading to a reduction in inflammatory reactions, such as leukocyte infiltration, edema, and fibrosis. These observations suggest that the interleukin-10 gene is a potential candidate gene for the molecular therapy of inflammatory bowel disease.

Interleukin-18 [18.19]. Interleukin (IL)18, also known as *interferon gamma inducing factor* (IGIF) and interleukin-1γ (IL1γ), is a cytokine of 193 amino acid residues and about 22 kDa in molecular weight. This cytokine is expressed in the gastrointestinal tract, lung, liver, kidney, and skeletal muscle. Interleukin-18 can induce interferon-γ production in T cells and accelerate inflammatory responses. The expression of interleukin-18 is upregulated in inflammatory bowel disease, contributing to the pathogenesis of the disease. A strategy for the treatment of inflammatory bowel disease is to suppress the expression of the IL18 gene. Local delivery of antisense oligodeoxynucleotides specific to the interleukin-18 mRNA is a potential method for such a purpose. Another approach is to transfer gene vectors that encode interleukin-18 antisense oligodeoxynucleotides. This approach has been used to suppress the translation of interleukin-18 in intestinal cells and reduce inflammatory reactions in experimental models. Alternatively, small interfering RNA (siRNA) specific to interleukin-18 can be delivered to target cells to degrade Interleukin-18 mRNA and thus reduce the translation of interleukin-18.

Tissue Regenerative Engineering. When inflammatory bowel disease involves a large fraction of the intestine, it is necessary to remove the malfunctioned intestinal segment

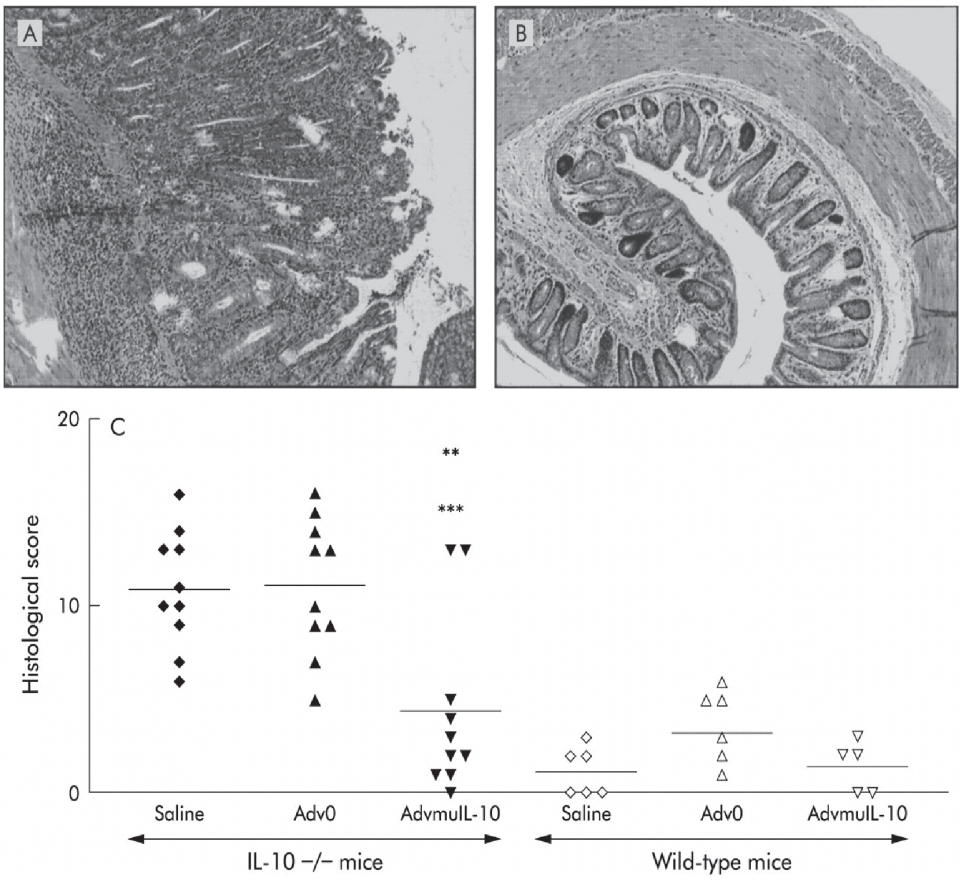


Figure 18.2. Local adenoviral vector encoding murine interleukin-10 (AdvmuIL10) therapy reduced histological colitis scores in IL10−/− mice with established disease. Four weeks after therapy, intestinal specimens were harvested for observation. Representative samples from an IL10−/− mouse treated with empty cassette adenoviral vector (Adv0) and AdvmuIL10 are shown in panels A and B, respectively. Panel C shows the histological colitis scores for different groups. (Reprinted with permission from Lindsay JO et al: *Gut* 52:363–9, copyright 2003.)

and reconstruct the intestine. There are two approaches for intestinal reconstruction: intestinal transplantation and substitution with engineered tissue substitutes. These approaches have been discussed on page 806 of this chapter.

Intestinal Ischemia and Infarction [18.20]

Pathogenesis, Pathology, and Clinical Features. *Intestinal ischemia and infarction* are disorders due to the reduction and obstruction of blood supply to the intestine, respectively. Common causes are arterial thrombosis and embolism. Arterial thrombosis occurs in response to arterial injury and bloodflow reduction due to low cardiac output. Arterial embolism is often found in patients with atrial fibrillation, artificial cardiac valves, and valvular heart diseases. Acute intestinal ischemia or infarction is associated with severe abdominal pain, vomiting, anorexia, and diarrhea. Pathological examinations often exhibit necrotic changes, including edema, cell death, hemorrhage, and tissue degradation.

Treatment of Intestinal Ischemia and Infarction. The treatment of intestinal ischemia and infarction is dependent on the nature and stage of the disorder as well as the condition of the involved intestine. In embolus-induced ischemia, embolectomy (surgical removal of emboli) is sufficient to restore the function of the intestine when the involved intestine is still viable. When infarction occurs in association with intestinal necrosis, intestinal resection is usually required. For intestinal ischemia and infarction due to severe arterial thrombosis, it is usually difficult to remove the thrombi. Instead, arterial bypass surgery may be carried out to reconstruct the occluded artery if the involved intestine is viable. For infarcted intestines, it is necessary to remove the involved intestinal segment. Intestinal reconstruction is usually necessary when a large fraction of intestine (>50%) is removed. Intestinal transplantation and substitution with engineered tissue equivalents are options for the treatment. These approaches have been discussed on page 806 of this chapter.

Molecular therapy can be applied to ischemic or partially occluded intestinal arteries. Since these vascular disorders are primarily induced by thrombosis and atherosclerosis, molecular therapies established for treating atherosclerosis can be used for treating intestinal ischemic disorders (see Chapter 15).

Short Bowel Syndrome [18.21]

Pathogenesis, Pathology, and Clinical Features. *Short bowel syndrome* is an intestinal disorder characterized by inadequate absorption of nutrients, fluids, and electrolytes resulting from massive surgical resection of the small intestine. Intestinal resection is often necessary for the following diseases: metastatic intestinal cancers, large area of inflammatory bowel disease, and large areas of intestinal infarction. These disorders result in an inadequate absorptive surface area. When more than half of the small intestine is removed, insufficient absorption of nutrients, fluids, and electrolytes often occurs.

Treatment of Short Bowel Syndrome. Several conventional approaches have been established and used for treating short bowel syndrome. These include: (1) supply of low fat diets but with high carbohydrates and proteins; (2) administration of vitamins and mineral supplements; (3) control of the motility of the intestine by using smooth muscle relaxants, allowing a sufficient amount of time for the absorption of nutrients, fluids, and electrolytes; and (4) parenteral hyperalimentation via the jugular vein and superior vena cava in severe cases. While these treatments are effective for a short-term relief of the symptoms, nutritional disorders often occur after long-term treatments because of insufficient nutrient absorption. An alternative approach for the treatment of short bowel syndrome is to reconstruct the disordered intestine with functional intestinal substitutes. This aspect has been discussed on page 806 of this chapter.

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