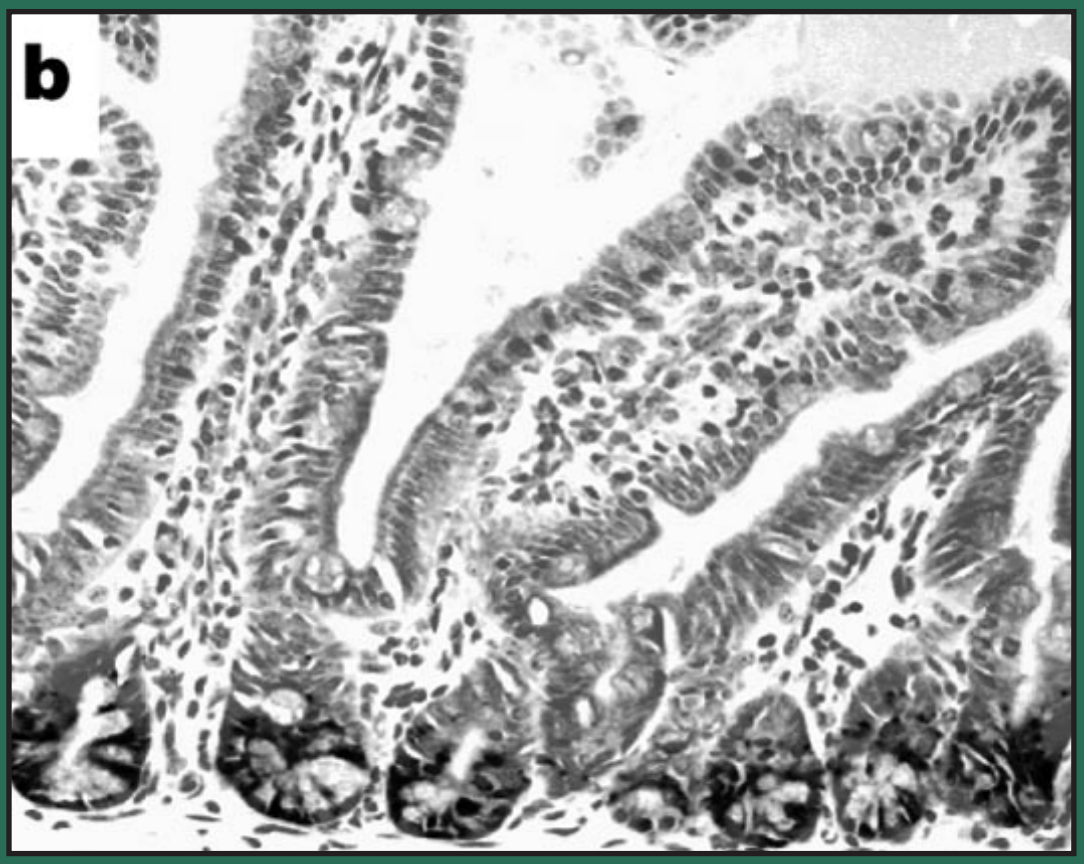

Photomicrograph showing expression of the *Lgr5-lacZ* reporter gene in the base of small intestinal crypts in adult mice. Through this type of research, scientists have been able to identify stem cells within the adult intestine that are capable of forming the cell types needed to continually renew this organ throughout life.

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Research on the Basic Biology of the Digestive System

SUMMARY OF RESEARCH GOALS

The Commission proposes multiple research goals to achieve the overarching mission of understanding the basic biologic underpinnings of the structurally and functionally complex digestive system. Developing new technologies to isolate, characterize, cultivate, and manipulate stem cells of the digestive system may provide new approaches to understand the pathogenesis and develop new therapies for digestive diseases. Uncovering the mechanisms that control development and differentiation of the digestive tract before birth and in neonatal life could generate new insights for regenerative therapies to treat digestive cancers and other diseases, as well as provide new insights into disease pathogenesis. Studying the fundamental mechanisms of digestion could point to new strategies for treating disorders of nutrient and fluid absorption, secretion, and metabolism. The enteric nervous system links the digestive system and the brain and controls motility within the gastrointestinal (GI) tract. Research on the function and organization of the enteric nervous system will enable a better understanding of gut motility in digestive health and disease. The intestinal microflora are essential to normal digestive function; studying the composition and activity of commensal organisms in healthy individuals could reveal important links between alterations in the microflora and human disease. Finally, the mucosal immune system is a critical component of the body's defenses against disease. Understanding the mechanisms by which this system operates could lead to new vaccination strategies or other approaches to prevent or treat infectious diseases that affect the digestive system.

INTRODUCTION AND BACKGROUND

Understanding the basic biologic functions of the digestive system, which includes the GI tract, pancreas, biliary system, and liver, is of fundamental importance to the diagnosis and treatment of all diseases related to these organ systems. This research plan of the National Commission on Digestive Diseases therefore begins with an overview of several areas of biology that have broad and overarching relevance for diseases covered in subsequent chapters of this research plan. These overarching biologic pathways include those related to the following topics that have seen enormous growth in scientific knowledge, poising them to lead to future therapies. They include: development (e.g., cancer and normal/abnormal development of cells and tissues of all organ systems); growth and integrative physiology (e.g., multi-organ diseases); digestion and metabolism (e.g., intestinal failure, lipid disorders, and pancreatic insufficiency); nutrient and fluid absorption and secretion (e.g., diarrhea and malabsorption); neurophysiology and motility (e.g., irritable bowel syndrome [IBS], obesity, and metabolic syndrome); microbiology and the microbiome (e.g., infectious diseases); and mucosal immunology (e.g., inflammatory bowel diseases [IBD] and vaccines against enteric pathogens). To accomplish the goals of this chapter, three themes of common importance to these areas of investigation need to be addressed: the identification, characterization and manipulation of intestinal epithelial stem cells; development of techniques to characterize and manipulate the human intestinal microflora; and delineation of and methods to target inflammatory pathways of the intestines, stomach, pancreas, and hepatobiliary system.

Development: Congenital malformations, IBD, certain malabsorption syndromes, epithelial metaplasia, disorders of motility, and bowel cancers are all related to development of the embryonic gut. GI development has long been a priority for the NIH, which supported visionary research leading to improved understanding of endoderm specification, patterning, stem cell kinetics, and crypt-villus organization. This field is now in a position to dissect the molecular basis of GI development, which holds enormous promise for understanding pathophysiology and therapy.

Growth and integrative physiology: The digestive system is a complex collection of organs that facilitates the intake, processing, and absorption of food and water while maintaining an effective barrier from the external environment. It is highly flexible and responds to varying nutritional and disease states by altering its structure and function. Understanding the mechanisms that lead to gut growth and remodeling in health and disease is, therefore, critical for development of therapeutics to treat a wide variety of digestive problems, including intestinal failure and obesity. Maintenance of digestive health requires integration of gut physiology with the functions of other tissues and systems, including hormones, the brain and peripheral nervous systems, and the immune system.

Digestion and metabolism: In the last several years, increased understanding of many pathways involved in intestinal nutrient absorption has resulted from our expanding knowledge of the hierarchy of membrane transporters with distinct substrate specificity, as well as a refined understanding of their subcellular itineraries and regulation. Researchers have identified intracellular acceptor molecules and nuclear hormone receptors that participate in metabolic channeling, as well as nutrient sensing, and that signal through both import and export pumps. Finally, the signaling dialogue between the host and the microbiome has been

explored. Together, these advances have yielded enormous implications for understanding and enhancing the relationship between nutrition and digestive health.

Nutrient and fluid absorption and secretion: Using both functional and genetic approaches, many membrane transport proteins mediating intestinal salt, solute, and water transport have been defined over the past 10 years. While there will be a continuing need to identify the proteins mediating and regulating absorption and secretion, the real challenge of the next decade is to leverage this information about the building blocks of fluid and nutrient movement into an integrated view of epithelial function. Given the centrality of the epithelial cell to a wide range of digestive diseases and responses to disease, such information has broad and enabling implications.

Neurophysiology and motility: In recent years, much knowledge has been gained about neural-hormonal control of gut functions and energy homeostasis. Unraveling the complexity of signaling between diverse cells in the enteric nervous system (ENS) provides the cellular and molecular basis for understanding many disorders in which the ENS plays a role. Characterization of the neurobiology of brain-gut interactions provides the necessary conceptual framework for developing new treatments of functional GI diseases and motility disorders. Understanding the mechanisms governing nutrient sensing and peptide secretion by enteroendocrine cells allows investigators to exploit these pathways in the development of new agents to combat obesity and diabetes. A better understanding of the molecular mechanisms leading to disease and age-related apoptotic cell death provides hope for preventive and/or regenerative therapy. Finally, the revelation that neural crest stem cells persist in the adult gut and undergo changes in self-renewal suggests that neuron replacement therapy can become a reality.

Microbiology and microbial-host interactions: The microbial population that normally inhabits the human GI tract, especially the colon, is one of the densest microbial populations known (approximately 10^{12} per gram of intestinal contents), accounting for at least 30 percent of the volume of colon contents. This highly complex population, containing thousands of bacterial species, is acquired shortly after birth and persists in the colon throughout life. The colonic microflora have a major impact on human health that includes a role in human nutrition, stimulation of mucosal cell turnover, suppression of intestinal pathogens such as *Clostridium difficile*, and as a reservoir for antibiotic resistance genes. Members of the microflora are also significant causes of post-surgical infections and infections in cancer patients. Less clearly established are a number of suspected, but still unproven, links to such conditions as IBD, colon cancer, and obesity. Despite its importance, little is known about either the composition or functions of this vast microbial community within the human intestine.

Mucosal immunology: The mucosal immune system encompasses a constellation of specialized cells and structures that enable the function of the immune system at the site of its greatest exposure to the microbial environment: the mucosal tissues of the intestines and their affiliated structures. Understanding the many unique functions of this system will lead to a better understanding of mucosal infections, including HIV infection, may permit the development of mucosal vaccines, and may lead to better treatments for immunologic diseases of the mucosa, such as IBD and celiac disease.

RECENT RESEARCH ADVANCES

Digestive System Development

Development of the crypt-villus axis

The critical roles of the Wnt, Notch, Hedgehog, BMP, Lgr5, and FGF signaling pathways have been elucidated in intestinal crypt homeostasis and in distinguishing the functions of crypt progenitors and the intestinal epithelial stem cell from those of differentiated villus epithelial cells. These advances provide important insights into the genetic basis of colorectal cancer—the second leading cause of U.S. cancer deaths—and to epithelial stem cell homeostatic mechanisms, as well as to normal development and inflammation. It is increasingly clear that Wnt signaling maintains proliferative capacity and lack of differentiation in crypts; its absence permits differentiation in villi; and constitutive Wnt activity is responsible in large part for dysregulated cell proliferation in colorectal and other GI tumors.

Mechanisms that pattern the undifferentiated embryonic gut tube into individual digestive organs

The GI tract and its evaginated derivatives (liver, pancreas, and biliary system) are a paradigm for inductive tissue interactions in development and, in particular, epithelial-mesenchymal interactions in organogenesis. Recent studies help define how undifferentiated endoderm is specified during embryogenesis in response to extraneous signals and the activities of tissue-restricted transcription factors and indicate how these activities combine to confer tissue- and organ-specific properties. The identity of some tissue-restricted transcription factors that regulate gut development is known, though many others remain to be discovered. There is also growing, though still limited, understanding of chromatin states that distinguish the precursors of some embryonic digestive organs. In parallel, cancer biologists are gaining a better understanding of epithelial-stromal (mesenchymal) interactions in neoplasia; thus, the principles of developmental interactions in digestive organs are likely to extend into the realm of tumor biology.

Growth and Integrative Physiology

Molecular events underlying intestinal growth and adaptation

Significant progress has been made in understanding changes in gene expression and cell signaling pathways that are induced by the loss of intestinal mass, such as occurs during bowel resection and other gut injuries and during the adaptive response that follows injury. Maintenance of intestinal homeostasis during development and adult life requires a proper balance among cell proliferation, apoptosis, and differentiation and involves interactions between epithelial and other cell types in the intestinal wall, including mesenchymal cells, such as fibroblasts. Understanding the molecular pathways that mediate normal intestinal growth and the response to injury and how extrinsic stimuli, such as nutrients, affect their activity is crucial for development of interventions to maintain intestinal mass and function. In particular, studies to elucidate the stem cell niche response following gut resection or injury (e.g., from ischemia, radiation, or trauma) may provide novel therapeutic targets to enhance gut mass and function.

Regulation of intestinal growth: roles of nutrients, trophic factors, and neurohumoral signaling

Evidence from animal models and human subjects with short bowel syndrome suggests that intestinal adaptive growth is regulated by several key hormonal mediators, including

glucagon-like peptide-2 (GLP-2), insulin-like growth factor-1 (IGF-1), epidermal growth factor, and growth hormone. However, their mechanisms are still poorly understood, including the involvement of other cell types besides enterocytes in the intestinal growth-stimulating effects.

Integration of brain-gut signaling, metabolism, and mucosal biology in the regulation of body mass

Considerable progress has been made in understanding how the presence of nutrient stimuli in the gut lumen is sensed by endocrine cells and nerves. This information is crucial in the normal digestive processes that occur in the gut and may be altered in disease. The presence of luminal nutrients is also important in the short-term regulation of food intake. Evidence from rodent models and human studies suggests that two GI hormones, CCK and PYY, are involved in the regulation of food intake via activation of neural substrates in the brain-gut axis. Moreover, long-term changes in the macronutrient content of the diet can alter the sensitivity of the brain-gut axis and may lead to changes in body mass.

Role of the nervous system in GI inflammation

Research has provided new insights into neuroimmune relationships that may facilitate translation of basic science into therapeutic applications, particularly with regard to GI inflammatory diseases. One example is the cholinergic anti-inflammatory pathway, which modulates release of pro-inflammatory mediators in models of colitis, ischemia-reperfusion, postoperative ileus, and pancreatitis. Neuronal signaling pathways in the gut are affected by inflammation; studies suggest that changes in function of the mucosal serotonin transporter and other neural pathways may underlie the altered motility, secretion, and sensation seen in inflammatory gut disorders.

Digestion and Metabolism

Diversity in genetic pathways for absorption of cholesterol and other sterols

An enterocyte membrane transporter (NPC1L1) specific for intestinal cholesterol uptake has been identified. Research has also advanced the understanding of the sensing and discrimination of subtle structural differences between cholesterol and plant sterols (sitosterol) and identification of sterol efflux pumps (ABCG5/G8) that minimize entry of plant-derived cholesterol mimics into the systemic circulation through selective export into the lumen. The basolateral cholesterol efflux-pump, ABCA1, has been identified, and its importance in the production of plasma HDL has been defined. Collectively, these advances have greatly expanded our understanding of the complexities of whole body cholesterol homeostasis in health and disease.

Hierarchy of ligands and receptors for intracellular signaling or metabolic compartmentalization of nutrients

The role of nuclear hormone receptors (FXR, LXR, PPARs) and other transporter/acceptor proteins (FABPs/FATPs) in energy sensing and in the maintenance of weight has been uncovered. In addition, new information concerning the metabolic compartmentalization of fatty acids (DGAT1/DGAT2) and mono-glycerides (MGAT1/MGAT2) has provided novel targets for obesity treatment. Finally, the development of innovative systems to probe the dialogue between the host and luminal bacteria has expanded understanding of the relevance of the luminal bacterial environment to digestive and absorptive functions.

Novel functions for genes involved in intestinal lipid absorption

Conserved pathways in intestinal and hepatic lipid absorption have been implicated in lipid antigen presentation and innate immunity. In particular, emerging data strongly suggest that the microsomal triglyceride transfer protein is responsible not only for lipidation of the export protein apoB, but also for lipid antigen presentation by the major histocompatibility molecule CD1d. In addition, research indicates that apolipoproteins involved in lipid export (apoE) also participate in lipid antigen presentation by CD1 molecules. Also noteworthy is the discovery that the peptide transporter, hPEPT1, may play a role in bacterial peptide presentation.

Nutrient and Fluid Absorption and Secretion

Blurring of classical boundaries of absorptive and secretory cell types

The intestinal epithelium is composed of a variety of cell types that are intermingled in a single layer that serves as a primary barrier separating the gut lumen from the body. Studies of intestinal epithelial development have revealed new pathways that regulate the balance among these different cell types, most notably Wnt signaling in regulating epithelial proliferation, as well as Math-1 and Gfi in regulating the census of secretory cell types. The molecular pathway for intestinal epithelial differentiation to absorptive cell types has yet to be established. With the constellation of absorptive and secretory proteins being defined, there is also increased interest in distinguishing gradations of function. Recent studies have revealed fluid and sodium absorptive functions in the structures of the colonic crypt that challenge our understanding of how the intestinal epithelium works in health and disease.

Epithelial barrier function

Researchers are investigating the molecular basis for the regulation of barrier function. Elucidation of the function of claudins, occludin, and other tight junction proteins is progressing rapidly. The coordinated action of TNF-α on barrier function and absorptive transporters, without effect on chloride secretion, is a new and important facet of intestinal regulation. Specialized mechanisms by which intestinal epithelial cells actively contribute to body defense through secretion of antibacterial peptides and repair of a disrupted epithelial layer are being defined. Complementary to understanding the barrier is research on the molecular basis of cellular water transport. Cloning of water channel (aquaporin) molecules in 1991 opened the door for advances in understanding cell and tissue fluid transport and led to the awarding of the 2003 Nobel Prize in Chemistry. Research on water channels sets the stage for understanding the pathways regulating barrier function versus fluid transport function.

Gut factors and epigenetic regulation of food intake and energy metabolism

The discovery of non-mutational regulation of gene expression that can be passed through generations has ushered in an era of new understanding about disease predisposition. For example, studies have shown that the offspring of European women undergoing starvation during World War II are predisposed to a higher incidence of diabetes. These observations validate a need for more studies to evaluate this mode of regulation. The study of nutrient absorption is now integrating data on the regulation of food intake (satiety). Ghrelin is a recently discovered peptide hormone that displays strong growth hormone-releasing activity and has a stimulatory effect on food intake and digestive function while reducing energy expenditure. Research on ghrelin has

led to new insights into how this hormone produced by the stomach connects the endocrine control of nutritional homeostasis through the brain-gut interactions.

Characterization of iron absorption

It is widely recognized that iron homeostasis in the body is almost exclusively regulated at the level of intestinal iron absorption because, while tissues such as the liver can readily store iron, the ability to excrete excess iron is poor. However, research on iron overload and iron deficiency syndromes has uncovered proteins originating from the liver and intestine that play a key role in the regulation of iron absorption in the intestine. Hepcidin and hephaestin were the first proteins to be ascribed a role in iron absorption. Other proteins that underlie genetic disorders of iron transport, including HFE and hemojuvelin, might also be involved. Exploiting these discoveries will require a more integrated approach to understanding intestinal absorptive function than we can currently pursue, as well as a stronger understanding of the cell types mediating absorption and their response to stimuli.

Neurophysiology and Motility

Diversity in the structure and functional organization of the enteric nervous system

In addition to nerve and smooth muscle cells, normal functioning of the ENS requires participation of the interstitial cells of Cajal (ICC), glial cells, and enteroendocrine cells. It would not be possible to generate the motor program stored in the ENS without patterned electrical activity and synaptic connectivity provided by the ICC. These findings have implications in human physiology and pathophysiology as abnormal networks of ICC have been reported in patients with a variety of motility disorders. Glial cells play an important role in synaptic transmission plasticity and immune protection. The neurotrophic factors that are produced by glial cells have strong anti-apoptotic effects on colonic epithelial cells, which may be responsible for their protective actions during mucosal inflammation. Understanding the cellular elements within the ENS will be critical to understanding its physiology and pathophysiology.

Brain-gut interactions in the pathogenesis of functional bowel disorders

Progress on functional GI disorders, such as IBS, can be attributed to a better characterization of the neurobiology of brain-gut interactions. The corticotropin-releasing factor (CRF) signaling pathway is the best described brain-gut circuit closely related to the pathogenesis of IBS. A CRF receptor antagonist directed at normalizing a sensitized CRF system holds great promise for the treatment of IBS and cyclical vomiting syndrome. Another major advance is the ability to image the living human brain, making it possible to investigate the role of genetic factors and receptor physiology on the pathophysiology of symptoms and, thus, to provide a more precise endpoint to evaluate therapeutic interventions. Finally, characterization of the 5-hydroxytryptamine (5HT, also known as serotonin) receptor signaling system in the ENS may be important for the pathogenesis of a subset of IBS and represents an important target for new therapeutic approaches.

Stem cell technology for neuron regenerative therapy

Neural crest stem cells have been shown to persist in the adult gut and to undergo changes in self-renewal. Cell-intrinsic differences

between stem cells from different regions regulate the generation of neural diversity. These exciting revelations suggest a new mechanism for regeneration of intestinal neurons after injury or disease. Further efforts should be directed toward identifying molecules and pathways that promote neural proliferation and differentiation in the gut and/or guide the growth of enteric axons to their targets.

GI tract in the regulation of satiety and energy homeostasis

Recognition of the pivotal role of gut hormones in glucose homeostasis has opened up new therapeutic options for the treatment of obesity and type 2 diabetes mellitus. Most importantly, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) promote insulin biosynthesis and islet beta cell survival. GLP-1 also inhibits glucagon secretion and gastric emptying and induces satiety. The multiple actions of these gut hormones have been exploited to develop novel therapeutics in the treatment of diabetes and disorders of energy homeostasis. Rational manipulation of the neuroendocrine pathways regulating appetite may be used to treat obesity. Another exciting finding is that gut microflora may contribute to the pathophysiology of morbid obesity through differing capacities to harvest energy from the diet, which suggests that the gut microbiome may be a biomarker, a mediator, and/or a new therapeutic target for people suffering from obesity.

Microbiology and Microbial-Host Interactions

Genomic analysis of the microbial population of the human colon

Testing hypotheses about the effects of the colonic microflora on human health has been hampered by the need to rely on cultivation-based methods for characterizing the microflora, an approach that is both time-consuming and unreliable. A newly available molecular approach, the amplification and sequencing of bacterial 16S rDNA and possibly other highly conserved genes, makes it possible to characterize the intestinal microflora more quickly and accurately. Researchers can now assess the species composition and contributions of the microflora to causation or exacerbation of such health problems as IBD, colon cancer, and obesity.

Moving from species composition to microflora function

Taking a "census" of the bacteria that are present in the colon at any particular time is important, but the species identity of a microbe does not usually reveal its metabolic potential. The metabolic potential of a microbial population can now be assessed by genome sequence analysis of cultivated members of the microflora and metagenomic analysis of the entire bacterial population. In addition, a better understanding of important bacterial products such as enzymes, toxins, and hormone-like compounds will make it possible to assess the role of bacteria in intestinal diseases at the molecular level.

Composition and functions of colonic end-product users: the archaea and sulfate-reducing bacteria

Although research on the human colonic microflora has focused on the numerically predominant populations and clinically significant minor populations, such as the *Enterobacteriaceae* and *Enterococci*, further studies have demonstrated the importance of a minor population that consists of fermentation end-product users, such as the methanogenic archaea and sulfate-reducing bacteria. For their carbon and energy needs, these microbes use hydrogen and carbon dioxide from the

fermentation of dietary polysaccharides and sulfate from fermentation of host-produced polysaccharides, including mucins and mucopolysaccharides. Such characteristics may increase the efficiency of the colonic fermentation of polysaccharides, and their products, such as methane and sulfide, are likely to have significant effects on the human body.

Horizontal gene transfer among human colonic bacteria

It is now known that bacteria in the colon interact genetically and metabolically with each other. Bacteria, unlike humans and other mammals, do not experience species limitations and are, therefore, able to transfer DNA across species, genus, and phylum lines. These transfers can involve antibiotic resistance and toxin genes that might contribute to intestinal disorders, but the extent or types of genes transferred is unknown.

Mucosal Immunology

Role of the intestinal microflora in the maintenance of mucosal immune homeostasis

The mucosal immune system is unique in that it lies in close proximity to an enormous consortium of commensal organisms that play multiple roles in maintaining gut homeostasis, including the prevention of colonization by pathogens and the promotion of epithelial cell repair following damage. These organisms are separated from mucosal lymphoid elements by a single layer of epithelium and overlying mucus that prevents wholesale entry of the bacteria. Nevertheless, it has been shown that commensal organisms do enter the mucosa via Peyer's patches and are taken up by dendritic cells (DC) in these lymphoid structures. An additional mode of entry of commensal organisms is via DC in the lamina propria, which extend processes between epithelial cells and take up organisms. This process is enhanced in epithelium exposed to Toll-like receptor (TLR) ligands. Such limited commensal uptake leads to the production of IgA antibodies, which function to reduce further bacterial uptake by coating organisms and preventing colonization. It also leads to the induction of regulatory T cells that control T cell responses to commensal antigens in the mucosal lumen and, thus, prevents the mucosal microflora from inducing inflammation.

Role of epithelial cells in mucosal host defense and inflammation

Intestinal epithelial cells produce chemokines and cytokines that initiate innate immune cell responses and, thus, set up a first line of defense against the intrusion of harmful organisms into the mucosa. Many of these responses are induced by TLRs and nucleotide oligomerization domain-LRR receptors interacting with microbial components. Epithelial cells also produce substances such as thymic stromal lymphopoietin (TSLP) that influence DC function and, thereby, determine the nature of T cell differentiation that occurs in relation to mucosal antigenic stimulation. Additionally, epithelial cells transport IgA via the polyimmunoglobulin receptor and IgG via the neonatal Fc receptor; in doing so, they carry antibacterial agents to the luminal surface and/or move immunoglobulin/antigen complexes in a bi-directional manner across the epithelium. Finally, epithelial cells produce antibacterial substances, including defensins (cryptins) and lectins that regulate the bacterial population in intestinal crypts and contribute to the development of IBD.

The role of antigen-presenting cells and other leukocytes in the mucosal immune system

Researchers are defining the biology of leukocytes and their role in innate (neutrophils,

eosinophils, and macrophages) and adaptive (DC and lymphocytes) immunity in numerous infectious, allergic, and inflammatory diseases of the gut. The DC is a key cellular player in the mucosal immune response; as such, it plays a role in mucosal host defense and in the pathogenesis of IBD. Studies of the function of the mucosal DC revealed that these cells are, as a population, unique and contain several sub-populations with unique functional properties, such as directing the differentiation of B cells into IgA-producing cells through the elaboration of B cell differentiation factors, including BAFF and APRIL. Evidence has emerged that mucosal DC may be uniquely involved in the induction of regulatory T cells in the mucosa via the production of TGF-β, in addition to the induction of Th17-producing cells via production of IL-6 and TGF-β. Thus, DC control the balance of effector cells and regulatory cells at mucosal sites.

Trafficking of mucosal cells to the mucosal immune system

Advances have been made in understanding how and why the mucosal immune system is unified by a cell circulation system that ensures that cells generated within the inductive areas of the system—the Peyer's patches and other lymphoid follicles—"home" back to the effector areas, the GI lamina propria and other "diffuse" mucosal areas in other organs. Early studies focused on the role of integrin/integrin receptors, particularly that of the $\alpha_4\beta_7$/MAdCAM-1 combination, in gut homing. Newer work has established that regional expression of epithelial chemokines in the small and large intestines is indispensable in the homing process. A major finding is that retinoic acid (vitamin A) acting through its receptor (RAR) induces IgA plasmablasts (T cells) to express homing receptors for the gut with regulatory capacity. It is now apparent that the traffic of cells in, around, and outside of the mucosal immune system is highly choreographed.

Mucosal unresponsiveness (oral tolerance) and regulatory T cell development

Researchers have improved our understanding of oral tolerance and the possible harnessing of its underlying mechanisms to the therapy of mucosal inflammation. A significant step came with the demonstration that, while oral tolerance could be due to exposure of mucosal cells to high doses of antigen in the absence of adequate T cell co-stimulation, it is more characteristically due to exposure of mucosal cells to low doses of antigen and the induction of regulatory T cells. Further work has established that the most important type of regulatory cell mediating oral tolerance is the "natural" regulatory T cell that develops in the thymus and is defined by its expression of surface markers, such as CD25, and a transcription factor known as FoxP3. Another regulatory cell that can develop in the mucosa and that may also mediate oral tolerance is the Tr1 regulatory cell, which lacks high-level FoxP3 expression and develops in relation to exogenous rather than self antigens. Factors that determine whether a mucosal antigenic stimulus will result in a positive immune effector response important for host defense or a negative regulatory T cell response important for maintenance of an unresponsive state and prevention of mucosal inflammation are still poorly understood, but may be due to the effects of TLR ligands.

Mucosal vaccination

Researchers have expanded our knowledge of the factors controlling the generation of both humoral (IgA and IgG) and cellular effector responses in the mucosal immune system. The follicle- and T cell-centered view of IgA-producing B cell differentiation has had to make room for a second pathway of IgA-producing B cell development, since it is clear that IgA-producing B cells develop in relation to exposure to components of the

commensal microflora in the absence of T cells, CD40 ligand, or mucosal follicles. This pathway of IgA-producing B cell development could be viewed as a more "innate" pathway, given evidence that it occurs in response to innate receptors, such as TLR receptors, and may be triggered by T cell-independent non-protein antigens. Extensive study of the mucosal adjuvant, cholera toxin, has provided important insights into the function of mucosal adjuvants. Thus, the picture that emerges is that mucosal adjuvants induce mucosal immunization, rather than tolerance, because they activate DC to express surface molecules and cytokines that activate effector T cells, rather than regulatory cells.

GOALS FOR RESEARCH [7]

DEVELOPMENT

Research Goal 1.1: Develop new technologies to isolate, characterize, cultivate, and manipulate stem cells of the digestive system for research and therapeutic applications.

Investigations into the origins and biology of intestinal stem cells (ISCs), mesenchymal stem cells (MSCs), and, to a lesser extent, neural stem cells (NSCs) have evolved to a point where it is possible to envisage new therapies based on stem cell biology. The identification of Lgr5, for example, as a specific functional marker for ISCs and a detailed elucidation of the regional factors that manage the ISC niche, such as those deriving from Notch and Wnt/β-catenin signaling pathways, open the possibility for the development of cell culture systems and, consequently, organ engineering with clinically relevant applications. Similarly, the recent illumination of the ways in which MSCs can be differentiated into a variety of intestinal cell types, such as neurons and endothelium, together with their unique biological properties, has important ramifications. MSCs are particularly appealing because of the relative ease by which they can be purified, manipulated, and administered for the treatment of diseases as diverse as intestinal ischemia and graft-versus-host disease. Developing methods to identify, isolate, and use ISCs for a wide variety of clinically relevant tissue engineering applications would also have enormous implications for tissue repair and organ transplantation. The advances that have been made in MSC and ISC biology need to be extended into a better understanding of NSCs, which hold similar promise for neurologically derived diseases of the gut. Finally, new stem cell technologies are needed to test hypotheses concerning the role of stem cells in cancer.

Objectives:

- Develop new markers to identify different stem cell populations of the digestive system.
- Develop new methods for isolation of stem cell populations.
- Develop new methods for cultivation of stem cell populations.
- Understand molecular pathways necessary for lineage differentiation of stem cells.
- Devise assays for characterization of specific stem cells and lineages.
- Devise animal models for development of potential therapeutic applications.

[7] *Research Goals are numbered for ease of reference only; the numbers do not indicate prioritization of scientific topics.*

Research Goal 1.2: Understand how particular cell and tissue niches are generated and maintained in the embryonic pancreas, liver, biliary tree, and digestive tract.

An important challenge in development and homeostasis of all tissues is to understand how a limited number of signaling pathways are able to generate enormous diversity. This question is starting to yield insights in the GI tract, where the roles of the Wnt, Notch, Hedgehog, BMP, Lgr5, and FGF pathways, among others, are being defined. Recent advances help delineate how these widely expressed signaling pathways act in concert to generate tissue- and organ-specific structures and functions, and they establish the GI tract as an exceptional model system in which to study developmental mechanisms.

Objectives:

- Apply diverse model systems to investigate aspects of gut development that are best approached through biochemical, genetic, and developmental studies in *Drosophila*, chicken, and zebrafish.
- Develop tools that permit accurately targeted genetic studies in the gut of various animal models, particularly a stable repertoire of transgenic animals that faithfully express Cre recombinase, green fluorescence protein or beta-galactosidase reporter genes, or toxigenes, ideally in inducible forms.
- Exploit the identification of intestinal epithelial stem cell markers to understand pathways governing the development of the diverse epithelial cell types that populate the intestines, stomach, pancreas, and biliary system.

Research Goal 1.3: Exploit the advanced understanding of Wnt-APC-β–catenin signaling in human epithelial function to develop new, effective treatment strategies for colorectal cancer. (See also Goal 4.11.)

The Wnt signaling pathway distinguishes the functions of crypt progenitors from those of differentiated villus epithelial cells, and its dysregulation underlies development of colorectal cancer, the second leading cause of U.S. cancer deaths. Wnt signaling maintains proliferative capacity and lack of differentiation in crypts, its absence permits differentiation in villi, and constitutive Wnt activity is responsible in large part for dysregulated cell proliferation in colorectal and some other GI tumors.

Objectives:

- Identify small molecules that interfere with distal steps in the Wnt signaling pathway for potential therapeutic use in cancer.
- Identify other signals that impinge on β–catenin stability and activity in colorectal epithelial cells and might serve as alternative targets for pharmacologic therapy.

Research Goal 1.4: Delineate specific signaling pathways, transcriptional regulation, and other interactions that mediate critical patterning events in gut endoderm, which generate and maintain its distinctive major derivatives (GI tract, liver, and pancreas).

Development and homeostasis of the developing and adult digestive system represent the outcome of interactions of cells with one another and with their surrounding matrix. Signals that emanate from these interactions drive tissue- and cell-specific transcriptional programs, and their disturbance is likely responsible for many disorders that are currently treated empirically. Improved understanding of essential signaling mechanisms will enable rational, targeted therapy.

Objectives:

- Delineate the relative contributions of specific signaling pathways and transcriptional regulators

in gut development and learn how the intersection between extrinsic and cell-intrinsic signals drives development.
- Distinguish factors whose functions are restricted to the developmental period from those that continue to influence critical activities in adult organs.

Research Goal 1.5: Translate advances from laboratory research in gut development to identify disease mechanisms and therapeutic targets for diverse GI disorders (e.g., congenital disorders, cancer).

In the long term, knowledge gained from basic research must be translated into a clear understanding of how specific developmental and homeostatic pathways are affected in individual GI disorders. Such understanding will lead to strategies for rational intervention to prevent development or progression of disease.

Objectives:
- Recognize the specific molecular defects associated with particular congenital diseases or disorders and with tissue metaplasia and cancer, especially Barrett's esophagus, gastric intestinal metaplasia, intestine-type gastric cancer, pancreatic *in situ* neoplasia and adenocarcinoma, and non-infectious hepatic disorders.
- Integrate molecular databases (e.g., gene expression, chromatin-immunoprecipitation, cis-element analyses) with functional studies (e.g., siRNA, genetically engineered animal models) to identify new pathways and to better appreciate underlying regulatory circuits.

GROWTH AND INTEGRATIVE PHYSIOLOGY

Research Goal 1.6: Define the physiologic basis for intestinal growth and adaptation and alterations with aging. (See also Goals 6.1 and 9.1.)

Maintenance of intestinal homeostasis during development and adult life requires a proper balance among cell proliferation, apoptosis, and differentiation and involves interactions between epithelial and other cell types, including mesenchymal cells such as fibroblasts. Understanding the molecular pathways that mediate normal intestinal growth and the response to injury and how extrinsic stimuli affect their activity is crucial for development of interventions to maintain intestinal mass and functional capacity. Intestinal adaptive growth is regulated by hormonal mediators, including GLP-2, IGF-1, and epidermal growth factor. Understanding the mechanisms by which these and other hormones affect gut growth is essential for developing therapies for individuals with intestinal failure, including patients who require parenteral nutrition.

Objectives:
- Determine downstream mediators of growth factor signaling that affect enterocyte proliferation and apoptosis, including the neural pathways that regulate hormone action.
- Understand cross-talk and synergism among intestinotrophic peptides, growth factors, nutrients, and other growth-promoting molecules using animal models.
- Characterize the molecular basis of stromal-epithelial interactions in gut injury and repair to identify potential therapeutic targets using transgenic models, microarrays, and proteomics.
- Develop strategies that leverage intestinotrophic mediators in the treatment of short bowel syndrome, IBD, intestinal damage induced by cancer chemotherapy, and ischemic injury.
- Develop novel methods of tissue engineering utilizing knowledge of the stem cell and its niche to create functional neomucosa.

Research Goal 1.7: Define the physiologic basis for energy balance, appetite, and satiety and their roles in obesity. (See also Goal 1.10.)

Disorders of energy balance, including obesity, diabetes, and the metabolic syndrome, are increasing in prevalence among adults and children, imposing severe personal and economic costs on individuals and society. Experimental and empirical evidence, including patient outcomes after bariatric surgery, point to neuroendocrine communication between the gut and the brain as a crucial element in understanding the pathogenesis of these disorders. Integration of the function of the brain-gut axis with other sites that regulate energy balance, including liver, pancreas, and adipose tissue, is essential to understanding the pathophysiologic basis of metabolic disorders. Understanding how the presence of nutrients in the gut lumen is sensed by endocrine cells and nerves is critical for treatment of diseases in which the signaling pathways are compromised and for the development of therapeutic strategies for the regulation of food intake and body mass. Some gut hormones (CCK and PYY) regulate food intake via activation of neural substrates in the gut-brain axis. Long-term changes in the macronutrient content of the diet can alter the sensitivity of the gut-brain axis and may lead to lasting changes in body mass.

Objectives:

- Assess the localization, expression, and regulation of gut peptide receptors and ligands influencing food intake within the gut.
- Integrate physiology with peptidomic, proteomic, metabolomic, or other technologies to identify adipokines secreted by fat cells that influence gut function and determine how signals originating from the gut affect adipose tissue biology, metabolism, and the brain-gut axis.
- Assess the synergistic or inhibitory interactions between pre- and postprandially released gut peptides influencing food intake and metabolism and examine the modulation of these interactions by dietary status and composition.
- Understand the mechanisms by which bariatric surgery leads to changes in body mass.
- Develop therapeutic interventions to mimic the effects of bariatric surgery on body mass.
- Develop effective, peripherally active substances for control of food intake and body weight, such as gut hormone-based therapies to target appetite circuits.
- Develop new assessment tools, technologies, and biomarkers to measure activity, nutrient intake, and energy balance.
- Define genetic risk alleles in order to discover critical pathways involved in obesity and satiety.
- Determine the mechanisms by which different macronutrients alter appetite and satiety.
- Understand the interrelationship of basic behavioral factors and brain-gut-nutrient axes in maintaining or changing body mass.

Research Goal 1.8: Define the physiology of neuroimmune pathways involved in inflammation.

Interactions between the nervous and immune systems play important roles in normal and disease states in the GI tract. Research has provided new insights into neuroimmune relationships that may facilitate translation of basic science into new therapies, particularly with regard to inflammatory diseases. An example is the cholinergic anti-inflammatory pathway, which modulates release of pro-inflammatory mediators in models of colitis, ischemia-reperfusion, postoperative ileus, and pancreatitis. Neuronal signaling pathways in the gut are also affected by inflammation. For example, pro-inflammatory cytokines can alter expression and function of the mucosal serotonin transporter (SERT), which affects neurohumoral signaling via serotonergic pathways. Changes in function of SERT and other neural pathways may therefore underlie the altered motility, secretion, and sensation seen in these inflammatory gut disorders. Better understanding of neuroimmune crosstalk in GI inflammatory disease is warranted.

Objectives:

- Address mechanisms responsible for neuroimmune protective and injurious states by combining expertise in neuroanatomy/neurophysiology, immunology/inflammation, trauma, nutrition, and gastroenterology.
- Understand the cause and effect relationships between inflammation and altered neural function and the functional implications of inflammation-induced changes in neural signaling.
- Understand the role of nutrition in animal models, including lipid-based diets, in neurally mediated anti-inflammatory pathways.
- Use animal models of GI inflammatory conditions to manipulate neural signaling through pharmacological, electrical, or nutritional interventions; identify mechanisms of response and effects on morbidity/mortality.
- Develop therapeutics (drugs or devices) that are based on neuroimmune pathways targeted to GI disease (e.g., IBD) and pathologies that have GI effects (e.g., shock, ischemia-reperfusion injury).

DIGESTION AND METABOLISM

Research Goal 1.9: Develop a comprehensive profile of intestinal genes that regulate mammalian absorptive functions.

Intestinal digestion, absorption, and metabolism reflect the integrated functions of many pathways, most of which are incompletely understood. For example, the single candidate cholesterol transporter, NPC1L1, accounts for most, but not all, cholesterol transport functions of the mammalian enterocyte, and there is an unanticipated redundancy with other pumps and membrane receptors. Newer murine models are needed using double, triple, and other combinatorial transgenic approaches (e.g., humanized knock-ins), coupled with targeted knock-out and knock-down technology.

Objectives:

- Extend studies of candidate genes to examine selected absorptive and metabolic pathways (e.g., cholesterol, bile acid, micronutrients) from human populations using humanized knock-ins of informative polymorphisms.
- Develop targeted approaches to obesity, hyperlipidemia, and diabetes through testing candidate small molecule inhibitors of gene function using mice and other models.
- Integrate advances in developmental biology to understand regional differentiation of intestinal absorptive functions (e.g., ileal bile acid transporter, duodenal iron absorption) and possible plasticity.
- Develop selective siRNA and other tractable knock-down methodologies for widespread use in digestive/absorptive pathway interrogation.
- Understand the dialogue between host and luminal bacteria and the signaling pathways involved.

Research Goal 1.10: Identify critical pathways in murine and other *in vivo* models to develop targets for treatment of obesity and other disorders of nutrient absorption and metabolism. (See also Goal 1.7.)

A major challenge is to transition from hypothesis-directed, mechanistic studies of known pathways into a reverse-genetic paradigm by which the etiology and complications of complex metabolic disorders, such as diabetes and obesity, can be modeled. A complete understanding of the major pathways that mediate macro- and micronutrient absorption should focus on a reverse genetics approach to transgenesis, using information from the human HapMap project to direct the study of nutrient absorption and metabolism.

Objectives:

- Design targeted therapeutics based on informative pathways that predict development of obesity, hyperlipidemia, and diabetes.
- Identify serum and tissue biomarkers that predict alterations in pathways identified above.
- Recognize the specific molecular defects associated with nutrient malabsorption, including obesity, and defective or inappropriately increased intestinal nutrient delivery.
- Define mechanisms by which metabolic pathways interface with immune function.

NUTRIENT AND FLUID ABSORPTION AND SECRETION

Research Goal 1.11: Define pathways that regulate barrier function and transport function. (See also Goal 9.2.)

Researchers are defining the molecular components of the epithelial barrier and understanding more about their regulation. The interplay between barrier function and net transport in the gut is becoming evident. Barrier and transport dysfunction is common in multiple intestinal disorders, and research in this area will improve understanding of health and disease and provide new therapeutic targets.

Objectives:

- Examine the structure and function of proteins composing tight junctions, adherens junctions, and other elements mediating the epithelial barrier.
- Identify membrane transport proteins and intracellular chaperones of micronutrient and metal ion absorption (e.g., iron, calcium, magnesium).
- Expand use of non-mammalian models to studies of gut absorptive and secretory functions (e.g., zebrafish, *C. elegans*, *Drosophila*).

Research Goal 1.12: Define molecular pathways leading to differentiated absorptive and secretory functions. (See also Goal 9.2.)

The commonalities between intestinal development and tissue remodeling after injury are striking. Both require re-establishing equilibrium among the cell types that are required for transport functions that are essential to life. Some of the essential molecular pathways promoting secretory cell types are known, but the routes to production of absorptive cell types are much less clear.

Objectives:

- Integrate information on the role of cellular and protein diversity in creating efficient absorptive and secretory function in healthy human and mouse tissues.
- Understand epithelial development and remodeling in response to injury, especially related to signals and pathways required to create a balanced population of absorptive and secretory cells.
- Apply and develop mouse models that allow fine genetic mapping of qualitative trait loci related to complex traits and multifactorial genetic disorders of absorption and secretion.
- Develop a proteome fingerprint of cell types important to gut absorptive and secretory functions and define the functional meaning of these profiles.

Research Goal 1.13: Develop means to measure and manipulate epithelial function.

As we develop greater understanding about epithelial cell function and adaptation to different conditions, either experimental or disease-based, it is important to take advantage of advanced models and techniques to translate key findings to the human condition and to benefit from broad, interdisciplinary approaches.

Objectives:

- Develop advanced mutant mouse models (e.g., tissue-specific, knock-in, inducible mutations, humanized models, superior gene transfer methods for GI tissues) to study human proteins that mediate or regulate nutrient and fluid absorption and secretion.
- Understand the molecular and functional adaptation of individual epithelial cells of the intestine to challenge (e.g., surgery, inflammation, diabetes, obesity, or experimental manipulation).
- Develop a conceptual basis and technical approaches for direct translation between human and animal studies of barrier, absorptive, and secretory processes in living tissues (e.g., imaging, molecular diagnostics, and therapeutics).
- Foster interdisciplinary teams among clinical research, basic biomedical research, engineering, and computational fields.

NEUROPHYSIOLOGY AND MOTILITY

Research Goal 1.14: Define the basic cellular and molecular mechanisms responsible for neural activation, integration, and regulation in the ENS.

The ENS regulates motor patterns in the GI tract. This division of the autonomic nervous system has been mapped extensively in a few animal models, but important mechanistic questions remain about the organization and function of enteric neurons and glial cells. Additional studies characterizing neural reflexes, neural plasticity, growth and development, and stem cell biology are needed to allow therapeutic control of enteric neural function.

Objectives:

- Understand the ionic and cellular regulatory mechanisms responsible for enteric nerve cell activation, synaptic transmission, integration, and motor pattern development.
- Characterize the molecular phenotypes of the different classes of enteric neurons (i.e., sensory, interneurons, and motor neurons). Develop unique biomarkers to allow evaluation of the state of specific classes of enteric neurons during the development of GI motor disorders.
- Determine how enteric neurons function as a network to generate motor patterns, respond to luminal contents, produce stereotypical gut reflexes, generate sensory signals, and adapt to conditions like inflammation or stress.
- Understand the development of the ENS and how adult neural stem cells might facilitate repair of a defective ENS resulting from developmental defects or pathophysiologic damage.
- Understand the role of glial cells in maintaining the structure and integrity of enteric ganglia and in regulating the functions and health of enteric neurons.
- Understand how inflammatory cells and mediators influence neural activation and integration.

Research Goal 1.15: Understand the structure, function, and regulatory mechanisms responsible for motility in the GI tract.

The ENS exerts control over smooth muscle cells to develop patterns of contractile responses, such as peristalsis, segmentation, tonic contraction, retroperistalsis, and others. ENS control is superimposed upon spontaneous activity of the musculature (so-called "myogenic" activity of smooth muscle tissues) and contributions from a variety of additional regulatory systems. A more complete understanding is needed of the interactions of the ENS with smooth muscle cells and the mechanisms responsible for regulation of contractile behavior in order to develop targeted therapies to improve motor function.

Objectives:

- Understand the molecular signaling pathways responsible for generation of tonic and phasic contractions in GI muscles and how these pathways are altered in pathophysiologic conditions.
- Clearly define the role and mechanisms of electro-mechanical and pharmaco-mechanical coupling in generating tone and phasic contractions in GI muscles and the effects of sex hormones, inflammatory factors, and aging on these mechanisms.
- Determine the molecular basis for electrical coupling between GI smooth muscle cells and between smooth muscle cells and interstitial cells of Cajal (ICC) and the consequences of a breakdown in electrical coupling on contractile behavior.
- Determine the mechanisms and the role of calcium sensitization in response to neurotransmitters, hormones, and paracrine substances in GI contractile behavior and whether this mechanism is altered by inflammation, sex hormones, or aging.
- Determine the basis for spontaneous electrical activity in smooth muscle cells and tissues and the mechanisms for propagation of electrical activity in the generation of motor behavior of GI organs.
- Understand stretch-dependent mechanisms that regulate the excitability of GI smooth muscles and contribute to patterns such as receptive relaxation, peristalsis, and gut stasis.
- Determine the effects of inflammatory mediators on the structure, function, and phenotype of GI smooth muscle cells and ICC.

Research Goal 1.16: Develop research tools to investigate the structure and functional organization of the ENS.

The multiple constituents of the ENS are characterized by a dynamic cross-talk between the enteric neurons, glial cells, ICC, smooth muscle cells, and enteroendocrine cells. Hence, functional and structural studies need to be performed with tools that allow dynamic visualization of the activity of the relevant cells *in situ*. Characterization of receptors, channels, and signal transduction systems unique to different cell types and how they interact with luminal events are critical to understanding how information is processed in the coordination of motility, secretion, and absorption.

Objectives:

- Investigate molecular and electrophysiological characteristics of various ENS cellular components, which may be targets for new developments to treat motility disorders.
- Develop tools to visualize the state of activity of relevant cells in live tissues, organs, and systems.
- Characterize alterations in gut-based 5HT and corticotropin-releasing factor (CRF) in IBS and motility disorders, including genetic polymorphisms affecting ligands, receptors, transporters, enzymes, and/or signaling systems.
- Identify molecules and pathways that promote proliferation and differentiation of enteric neurons and/or molecules responsible for directing enteric axons to their targets.
- Define the molecular basis for chemo- and mechano-receptors in the gut to sense ingested nutrient environment and gain better understanding about interactions between nutrient and microbe-sensing mechanisms in the gut.

Research Goal 1.17: Characterize the neuromuscular phenotypes of human GI tissues.

Much has been learned in recent decades about the organization of the ENS and cellular mechanisms involved in generating normal gut motility patterns. Most information has come from studies of laboratory animals. It is important to translate this information into studies of human GI muscles to

determine the neuromuscular phenotypes driving normal human GI motility. Although access to human samples is limited, surgery for GI conditions like cancer or obesity presents an opportunity for studying human physiology.

Objectives:

- Understand the excitability and contractility mechanisms in human GI muscles. Translate knowledge obtained from animal models to human physiology.
- Understand the structure and function of the human ENS. Determine which animal models best simulate the integration and cellular phenotypes of human enteric neurons.
- Characterize motor innervation of the human muscularis and identify the major neurotransmitters, the cells that are innervated by motor neurons, and the mechanisms of post-junctional neural responses.
- Define the receptors and signaling pathways that are involved in neural, hormonal, and paracrine regulation of human GI muscle function.
- Develop methods of organ or cell culture that preserve the phenotypes of human muscle cell components. Determine methods to culture smooth muscle cells, ICC, enteric neurons, and other cellular components without dramatic changes in the native phenotype.

Research Goal 1.18: Integrate cellular events in ENS with whole system physiology and translate findings to pathophysiologic conditions.

Normal functioning of the GI tract requires different components of the ENS to operate in unison, emphasizing the importance of identifying relationships in an organismal context. Advances in neurobiology of brain-gut interaction, together with availability of new neuroimaging modalities, greatly enhance our ability to study functional GI disorders and search for new therapeutic targets. Recognition of the GI tract's crucial role in satiety signaling and control of energy homeostasis, body weight, and various metabolic systems provides a new framework to study disorders of energy homeostasis. Pathophysiologic models coupled with genomic analysis offer new opportunities to discover molecular mechanisms responsible for age-related neuron degeneration and provide avenues to reconstitute the ENS networks in diseased organs.

Objectives:

- Identify distinct brain circuits responsible for various gut functions and pain perception and characterize the signaling systems and receptors within these neural circuits using PET ligand imaging in rodent models and humans with IBS and functional dyspepsia.
- Develop contemporary techniques for probing genetic and proteomic changes that occur with age. Establish the mechanism that maintains the integrity of the ENS and its capacity to respond to altered function or "plasticity" in adulthood and old age.
- Investigate the cellular and molecular mechanisms of neural and endocrine bi-directional communication between the gut and the central nervous system (CNS) for regulation of weight and metabolic function and the associated neurohumoral events.
- Develop suitable animal models to mimic diseases of the ENS.

Research Goal 1.19: Translate knowledge of the ENS in digestive health and disease into diagnostics and therapies for human disease.

Research on the pathophysiology of human motility disorders needs to be aggressively translated into the treatment of human diseases, given the progress in research on functional GI disorders. This includes advances in neuroimaging modalities to study brain-gut interactions, efforts to unravel the complexity of energy homeostasis systems, an understanding of some of the key neural circuits in the ENS,

and knowledge of developmental biology and organogenesis, which allows for manipulation of neural stem cells in the bowel wall for replacement therapy.

Objectives:

- Determine specific gene profiles in tissues that suffer ICC or neuron loss or in tissues in the process of losing these elements and develop a molecular test to detect these pathologic changes.
- Develop neural imaging techniques to correlate individual circuits identified with symptom production in IBS patients and establish correlation with distinct genotypes through such approaches as a genome-wide search for polymorphisms and haplotypes.
- Develop neuron replacement therapy to guide the growth of enteric axons to their targets as a therapy for neural degenerative disease of the ENS.
- Characterize the molecular, cellular, and behavioral mechanisms that link changes of stored body fat to adaptive adjustments of feeding behaviors by defining the diverse blood-borne and affective neural signals that transmit information regarding nutrient status and energy stores to the brain where it is integrated with cognitive, visual, olfactory, and taste cues.

MICROBIOLOGY AND MICROBIAL-HOST INTERACTIONS

Research Goal 1.20: Determine the biologic activities of the microflora in healthy humans. (See also Goals 5.3 and 9.3.)

The normal intestinal microflora consists of a total population that is at least 10^{12} microorganisms per gram of intestinal content. This microflora achieves a mass that is equivalent to or exceeds the size of several human visceral organs, but very little is known about it. Yet, the microflora are considered to be key to the biologic basis of numerous intestinal and non-intestinal conditions, including the maintenance of physiologic homeostasis. As such, the normal intestinal microflora likely function as a separate, yet integrated, organ within humans. A major impediment to understanding this biomass is the lack of adequate methods to interrogate the composition of the intestinal microflora and its function. Evidence now exists that the normal intestinal microflora can directly influence and regulate the structure and function of the normal intestine. The specific properties and mechanisms by which the intestinal microflora accomplish this are unknown, but essential for both co-opting these mechanisms for the development of new therapeutics and for understanding disease processes.

Objectives:

- Undertake a metagenomic analysis of the microflora of healthy people and determine the extent of person-to-person or diet-related variation.
- Determine whether members of the major bacterial populations can transfer DNA to mammalian cells and test this hypothesis with *in vitro* and *in vivo* models.
- Develop a "humanized" mouse model of the microflora in which germ-free mice are colonized with the human microbiome.
- Obtain genome sequences of the gram-positive anaerobic bacteria that account for over two-thirds of the colonic microflora, but about which virtually nothing is known, for use in interpreting the metagenomic data and guiding biochemical studies of the activities of these bacteria.
- Take a census of the methanogenic archaea and sulfate-reducing bacteria found in the colons of healthy people.
- Determine whether bacterial enzymes, toxins, or hormone-like compounds affect intestinal epithelial and non-epithelial cells.
- Examine genome sequences from colonic bacteria to identify possible gene transfer events using advanced computational methods for detecting such events.
- Understand the establishment of the microflora in the neonate and the influence of breast feeding compared to commercial infant formula feeding.

Research Goal 1.21: Determine the mechanisms of host-microbial interactions that are necessary to maintain health and contribute to pathological processes in disease. (See also Goals 3.1, 5.3, and 9.3.)

For the most part, mammals live in harmony with their enteric microbes and derive health benefits that are becoming increasingly recognized. This relationship relies on a continuous exchange of molecular and metabolic signals that are essential for keeping a balance that is mutually beneficial to host and microbe. A major challenge is to better understand these complex interactions, as they are the underpinnings of both health and disease. Perturbations in host-microbial interactions can arise from numerous causes, including processes that affect human responses to microbes as well as changes in the composition or behavior of the commensal microbes themselves. These disturbances may cause or be a contributor to many diseases, including IBD, infectious gastrointestinal diseases, metabolic syndrome/obesity, IBS, colon cancer, acid-peptic diseases, and autoimmune disorders. The insights gained through further studies of host-microbial interactions will promote the discovery of new ways to maintain health, as well as to treat and prevent numerous common diseases.

Objectives:

- Determine the interrelationship of digestion, microbes, and nutrients in normal health and digestive diseases.
- Define the properties of the microflora that are associated with the maintenance and repair of the epithelial barrier.
- Determine the mechanism by which the normal intestinal microflora provides resistance against infectious pathogen invasion.
- Determine why commensal organisms generally elicit protective and anti-inflammatory responses in normal individuals, whereas pathogens elicit inflammation.
- Develop approaches to manipulating the commensal microflora subpopulations to prevent or reverse infection and inflammation.
- Design probiotics and prebiotics for maintenance or restoration of healthy microflora.
- Determine whether the composition of the microflora or microbial gene expression has a role in conditions such as IBD, IBS, colon cancer, or obesity.
- Determine whether bacterial toxins or hormone-like compounds are involved in inflammatory intestinal diseases.
- Define the role of the normal microflora in maintenance of the mucosal immune system.

MUCOSAL IMMUNOLOGY

Research Goal 1.22: Determine the role of epithelial cells in mucosal host defense and inflammation.

Epithelial cells are not passive participants in the mucosal immune response but, on the contrary, play active and perhaps key roles in the shaping and/or initiation of that response.

Objectives:

- Identify factors that regulate the expression of innate immune receptors (e.g., TLR) in epithelial cells and the effect of stimulation of these receptors on epithelial barrier function, chemokine and cytokine production, and antimicrobial peptide production.
- Elucidate the effects of factors produced by epithelial cells that affect lamina propria DC function and/or T cell differentiation, including TSLP, IL-10, and TGF-β.
- Generate mouse models expressing epithelial cell-specific deletion of key genes involved in epithelial mucosal immune function and barrier function to define the function of these genes in epithelial cell regulation of immune function.

- Characterize embryonic and adult stem cell differentiation into epithelial cells focusing on the attainment of properties that relate to epithelial immune function.

Research Goal 1.23: Understand the role of antigen-presenting cells in the mucosal immune system.

The DC is a key cellular player in the mucosal immune response and, as such, plays a major role in mucosal host defense and mucosal autoimmunity.

Objectives:

- Define the factors that influence DC maturation and function to mediate T cell effector and regulatory functions within the mucosal environment.
- Elucidate the function of DC TLR signaling with respect to positive and negative DC responses.
- Generate mouse models characterized by DC dysfunction to study the role of DCs in mucosal host defense and inflammation.

Research Goal 1.24: Understand trafficking of mucosal cells to various parts of the mucosal immune system.

Researchers have begun to establish the cellular and molecular mechanisms that ensure that cells generated within the inductive areas of the mucosal immune system "home" back to the effector areas of the system.

Objectives:

- Elucidate the factors that control chemokine-chemokine receptor interactions or other cell-cell interactions that contribute to mucosal traffic patterns.
- Generate mice that lack key components of the gut homing apparatus and, thus, allow in-depth examination of gut homing mechanisms.
- Develop a systems approach to the study of lymphocyte and DC homing that integrates the many factors that affect this process.

Research Goal 1.25: Understand mucosal unresponsiveness (oral tolerance) and mucosal regulatory T cell development.

The last decade has advanced the understanding of mechanisms governing immune unresponsiveness to antigens in the mucosal environment, particularly the function of regulatory cells that suppress responses to gut antigens (including commensal microflora and food antigens). The importance of these regulatory cells has become apparent in the study of murine models of inflammation, in which it was shown that lack of regulatory cell generation leads to colonic inflammation and possibly to allergic responses to food antigens as occurs in celiac disease and food allergies.

Objectives:

- Determine the nature of the immunological milieu of the mucosa in enhancing or retarding the development of regulatory T cell functions, such as the synthesis of TGF-β, retinoic acid, IL-35, and IL-27.
- Understand the immunological mechanisms that underlie the development of food allergies and develop methods to assess these allergies.
- Elucidate the biology of regulatory T cells with relation to the function of FoxP3 and other intra-cellular factors that control regulatory cell function.
- Develop gene therapy approaches to the enhancement of regulatory T cell function to treat chronic inflammatory states.

GOALS FOR RESEARCH

Research Goal 1.26: Understand the differentiation and function of mucosal lymphocytes and other immunologically active cells.

The intestines are in a state of physiologic inflammation that is presumed to be largely in response to the local milieu and, especially, to the presence of the commensal microflora. This state is important to mucosal defense against infections and cancer but, when dysregulated, it can result in uncontrolled inflammation.

Objectives:

- Define the unique factors that mucosalize local lymphocytes and other cell types (e.g., mast cells, mesenchymal cells) and, especially, the role of the commensal microflora.
- Determine the site of induction and factors that regulate the development of mucosal effector (i.e., Th1, Th2, Th17, and NKT cell) and regulatory pathways in intestines.
- Investigate the effect of aging on these pathways.

Research Goal 1.27: Develop mucosal vaccination strategies.

A practical aspect of studying the induction of IgA and other mucosal responses arises from the fact that the mucosal system is separated from the "systemic" immune system by the homing receptors that mandate the traffic of cells originating in the inductive sites of the system to effector sites. This implies that only mucosal immunization can effectively deal with pathogenic invasion of the mucosa. This point is particularly relevant to the prevention of HIV infection given evidence that the GI tract is a major site of initial HIV development and an important reservoir of established HIV infection.

Objectives:

- Elucidate epithelial or stromal cell factors and cytokines involved in elaboration of IgA-producing B cells.
- Understand IgG responses in the mucosal immune system and the neonatal Fc transport system.
- Develop new adjuvants that target particular aspects of the mucosal immune response.
- Develop effective vaccines for the prevention of major, epidemic, enteric viral infections.

MAJOR CHALLENGES AND STEPS TO ACHIEVE THE RESEARCH GOALS

Animal models: The current range of animal models for studying digestive diseases biology could be expanded by the development of tools that permit targeted genetic studies in various animal models within specific tissues and cell types. Validation of specific promoters and/or minigenes that drive tissue- or segment-specific gene expression in mouse and other relevant models (zebrafish, *Xenopus*, *Drosophila*) is required. Efforts to strengthen digestive disease research efforts across the scientific community might include: (1) the establishment of a coordinated system that would allow for the identification, characterization, and distribution of suitable mouse lines in a defined (e.g., C57Bl/6) background, including conditional gain- and loss-of-function alleles that can be induced in a temporally regulated manner; and (2) centralized animal resources to act as a repository of useful models. Moreover, efforts to generate novel animal models of specific GI, liver, and pancreatic disorders would accelerate research on the molecular pathophysiology of disease development. The success of translational research requires experimental approaches in animal models that can be more directly compared with human outcomes.

Therefore, equipment and chemical probes could be developed that permit parallel live tissue analyses in the human and mouse intestinal tract. The latter requires interdisciplinary research among digestive diseases researchers, chemists, and biomedical engineers.

Germ-free animal facilities: Many initiatives proposed in this research plan involve the study of live experimental animals under conditions in which the commensal microflora of the gut are strictly defined and controlled. Centralized germ-free facilities would provide qualified researchers with germ-free and/or microflora-defined mice for study under various conditions. If such facilities could develop the capability of sending these mice to distant locations, research of this sort could theoretically be conducted anywhere.

Methods to characterize the microbiome: It is critically important to develop basic tools to properly investigate and understand the composition of the normal intestinal microflora. As part of this effort, there is a need to collect and organize a curated database to accommodate large amounts of 16S rDNA and metagenomic sequence data and to develop advanced bioinformatic methods for analyzing these data. In addition, microarrays need to be developed that contain rDNA sequences from all of the major human colonic species for rapid characterization of the species composition of the colonic population.

More sophisticated analysis of sequence data, especially metagenomic sequence data, to deduce the known and undiscovered activities of the intestinal microbiome will require collaboration between experts in microbial physiology and bioinformatics. Improvements in microarray technologies could eliminate the need for any amplification step (i.e., direct sampling of community DNA in the case of the 16S rDNA array and of RNA in the case of metagenomic microarrays). Advanced computational approaches, such as codon usage algorithms, would enable researchers to detect and determine the origin of mobile elements and to systematically screen such mobile elements to ascertain what accessory genes (e.g., antibiotic resistance or toxin genes) are carried.

Progress in this field could be accelerated by developing microarrays that represent genes on mobile elements found in the major groups of colonic bacteria and by making these broadly available to the scientific community at a low cost. Such methodologies could also focus on defining the minor species of the microflora that have resisted cultivation to date. Finally, the development of new and better noninvasive means of quantitating the amount of energy derived from the colonic fermentation, the rate of intestinal cell turnover, and the activity of the ENS would facilitate research on the effects of the microflora in large groups of humans or animals. Noninvasive technologies, such as a swallowed capsule or MRI (e.g., to detect the fate of swallowed dyes), to monitor changes in the composition and activities of the microflora in different segments of the GI tract are priorities for development. Resources being developed under the auspices of the Human Microbiome Project, an initiative of the NIH Roadmap for Medical Research, will be critically important for meeting these challenges.

Cell lines relevant to the digestive system: Primary cells derived from humans and animal models with defined attributes would enable *in vitro* studies to characterize the signaling pathways and secretory potential of a large variety of cell types. Important cell lines to be defined and established are those related to the intestinal epithelial (and mesenchymal) stem cells, as well as native intestinal epithelial cell and DC lines. To do so, it will be important to develop better cell-specific markers. In addition, innovative techniques are required for the long-term maintenance of cells in culture under circumstances in which the cells do not undergo major changes in their characteristics. Moreover,

central facilities to acquire and maintain these cells for distribution would be helpful.

Bioinformatics: GI research would benefit from the development and implementation of novel genomic and proteomic approaches and bioinformatics databases. Investments to develop computational biologists and bioinformatics infrastructure and to foster interdisciplinary research between GI physiologists (including immunologists and bacteriologists) and computational biologists would strengthen the field. Mammalian models that provide systems biology resolution would allow integration of information from molecular regulation research directly into studies defining organismal impact without the need to create new models. For example, it might be possible to identify the role of a protein without creating a tissue-specific conditional knock-out or transgene. Centers could be developed for cell type-specific protein profiling and disease state profiling with standardized procedures and outcomes. Development of a proteome fingerprint of cell types important to various digestive disease processes, as well as to digestive development, physiology, and immunology, in mouse and humans would promote research progress. This could be augmented by the generation of large national data and tissue banks for the application of modern genomic and proteomic technologies.

Translation of genetic findings into understanding human digestive disease: Integration of genetic discovery research with studies to understand the function of newly discovered genes is required to translate basic research into opportunities to improve human health. Academia-industry dialogue could be encouraged to expand our understanding of digestive diseases in human populations. The development and coordination of regional and national databases with appropriate serum, DNA, and tissue biobanking would provide crucial resources for the entire research community.