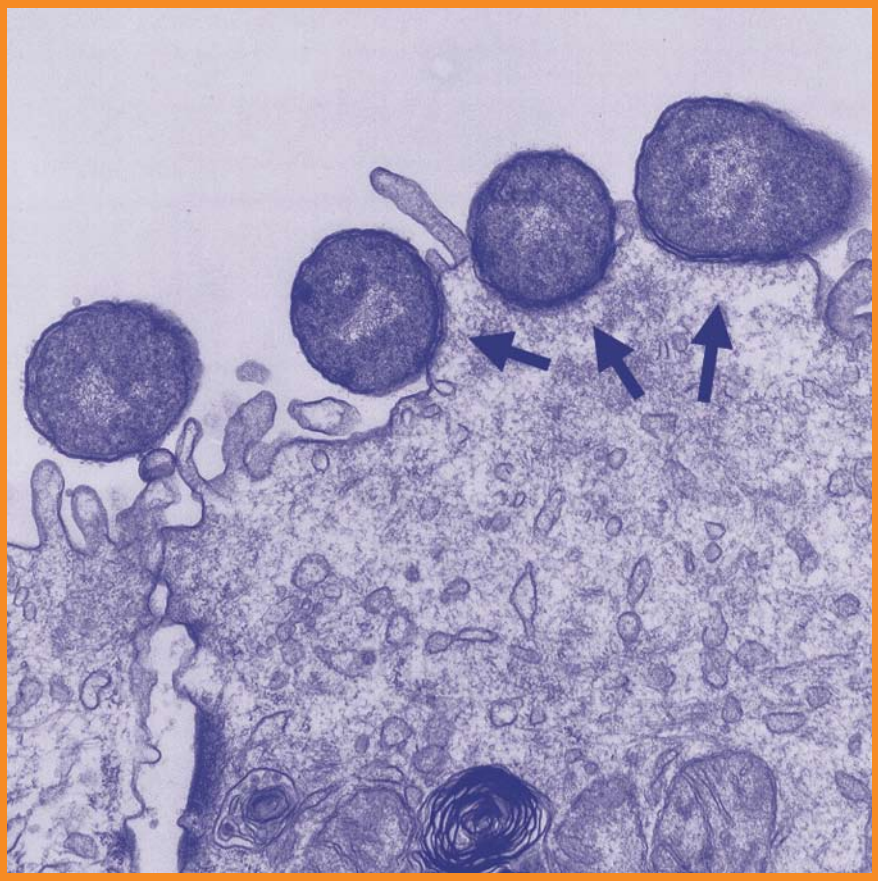

Transmission electron micrograph showing *Escherichia coli (E. coli)* bacteria attaching to intestinal epithelial cells (arrows). This strain of *E. coli* is a major cause of infantile diarrhea worldwide, which is associated with a high rate of mortality. Recent NIDDK-sponsored research advances described in this chapter shed light on how these and other bacterial strains interact with intestinal cells, contributing either to human health or disease.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. These conditions include disorders of the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. Disorders of the digestive tract exact a significant toll on many Americans each year. For example, approximately 135 million people each year suffer from non-food-borne gastroenteritis, a typically infectious inflammation of the GI tract associated with such symptoms as diarrhea, nausea, and vomiting.[1] Additionally, liver and biliary diseases affect a large portion of the population and represent a huge burden, in terms of quality of life as well as health care costs, such as the estimated 6 billion dollars spent annually in the U.S. on gallbladder disease care.[1] NIDDK-supported scientists are vigorously pursuing research to understand how widespread these diseases are across the U.S., to identify the causes of these diseases and how they progress, and to test new interventions for treatment and prevention of these costly diseases, including drugs, surgery, and behavior modification.

Diseases of the GI tract include inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis. These diseases are marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. IBD often strikes early in life, with a peak age of onset in adolescence or young adulthood. To address this condition, surgery may be required, including removal of the affected region of the intestine. Scientists are dissecting the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. Helping to catalyze the design of novel therapeutic strategies will be the continued discovery of predisposing genetic variations and their interactions, as well as other factors, such as potential autoimmune and microbial influences. Research on controlling intestinal inflammation has potential benefits not only for patients with inflammatory bowel diseases, but also for those at risk of developing colorectal cancer.

Intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroparesis is another functional bowel disorder that is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. A common cause of gastroparesis is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden, particularly in the elderly.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the small intestine is damaged when the immune system reacts to the protein gluten—a component of wheat, barley, and rye. This reaction interferes with the ability to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, growth failure. The only current treatment for celiac disease is maintenance of a gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their healthcare providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued

[1] *Sandler RS, et al: Gastroenterology 122: 1500-1511, 2002.*

advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microorganisms that inhabit the gastrointestinal tract are increasingly appreciated as powerful players in maintaining or tilting the balance between digestive health and disease. These microbes can affect intestinal health in some surprising ways, depending on their interactions with each other, with host cells, and with nutrients ingested by their host. Scientists are gaining insights into the ways these microorganisms influence the development and function of the digestive tract.

Several types of liver disease have serious adverse impacts on health, and some can lead to complete liver failure. Some liver diseases primarily affect children—such as biliary atresia, a progressive inflammatory liver disease—while others more commonly affect adults—such as non-alcoholic steatohepatitis (NASH). Some are caused by viral infection—such as hepatitis B and C—while others arise from diverse factors such as autoimmune reactions, genetic mutations, drug toxicity, and other, unknown triggers. A functioning liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. The number of livers available from deceased donors is limited, and research is of critical importance to identify and treat liver disease, preserve liver function in people with liver disease, and explore treatment options beyond cadaveric liver transplants.

The number of overweight and obese Americans has risen dramatically in the past two decades and is now at epidemic levels. Obesity is associated with numerous serious diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular factors that control appetite, metabolism, and energy storage, they are identifying potential targets for the development of new pharmacologic agents to promote safe, long-term weight loss. Investigators are also continuing behavioral research to help people achieve healthy lifestyles that include increased physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

GENETICS OF INFLAMMATORY BOWEL DISEASES

Autophagy Is Implicated in Crohn's Disease:

Scientists have recently identified a gene that increases susceptibility for Crohn's disease (CD), a major form of inflammatory bowel disease (IBD). The gene is involved in autophagy, a process cells use to eliminate unwanted cellular components by capturing and degrading them into molecules that can be recycled by the cell. This exciting discovery was made in a two-phase study supported by the NIDDK IBD Genetics Consortium.

As described in the Cross-Cutting Science chapter, genome-wide association scans are now possible which can screen individuals' complete genomes for hundreds-of-thousands of small mutations, or gene variants. In the first phase of the study, members of the IBD Genetics Consortium conducted a screen using this state-of-the-art technology to identify genetic variants that contribute to CD. The scans were performed using the DNA of 547 CD patients and 548 healthy volunteers. Surprisingly, when the genetic variants of the two groups were compared, a relatively rare variant of the IL-23 receptor gene was identified that protects against CD. (See highlights from Dr. Judy Cho's Scientific Presentation, which appears later in this chapter.)

CD is a complex genetic disease caused by inappropriate immune responses to bacteria that naturally reside in the intestine. Because CD is a complex disease involving several genetic and environmental factors, the scientists conducting this study believed that some of the genes contributing to CD may have only a modest effect on disease risk and that larger study cohorts would be required if these subtle gene variants were to be detected. Therefore, a second phase of the study was conducted, which increased the sizes of the study cohorts to a total of 946 CD patients and 977 healthy volunteers. Analyses of these larger scans identified the new CD autophagy gene. Several other significant CD-associated genetic variants were identified in these scans as well. The discovery of an autophagy gene associated with CD is an important step in understanding this complex disease. Additionally, autophagy and the signaling pathways used to initiate this process can now be explored as targets for novel drugs designed to prevent and treat CD.

Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, Green T, Kuballa P, Barmada MM, Datta LW, Shugart YY, Griffiths AM, Targan SR, Ippoliti AF, Bernard EJ, Mei L, Nicolae DL, Regueiro M, Schumm LP, Steinhart AH, Rotter JI, Duerr RH, Cho JH, Daly MJ, and Brant SR: Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet 39: 596-604, 2007.

UNDERSTANDING INTERACTIONS BETWEEN BACTERIA AND THE GASTROINTESTINAL TRACT

Of Mice, Fish, and Men—Multi-Species Studies Explore Impact of Intestinal Microbes:

Three recent studies using animal models and human samples yield new insights and renewed curiosity about the impact of the trillions of microbes that inhabit the human intestine. These studies combine new genetic knowledge and techniques with the most useful animal and bacterial models available to shine a light on the interior world of intestinal microbes.

One study characterized the functions and evolutionary adaptations of a type of bacterium that is abundant in the human intestine and known to influence host nutrient digestion. The bacterium is called *Methanobrevibacter smithii,* or *M. smithii*. This bacterium was shown in previous experiments to increase the efficiency of host digestion. The result was more calories absorbed and a heightened risk for obesity. (Additional information on how gut microbes influence obesity development is presented in the chapter on "Obesity.") Building upon these findings, researchers sequenced the bacterium's genome and compared it to other bacterial strains to identify genes that are specific to *M. smithii* and point to its unique functions inside the human intestine. They then used a mouse model that had been raised in a sterile environment to keep its intestine completely free of bacteria. In this model, they identified the unique RNAs expressed and metabolic functions performed when *M. smithii* was introduced into the mouse gut. The researchers concluded this set of investigations by exploring some potential targets in the *M. smithii* genome for future drug discovery, specifically inhibitors of the bacterial genes that enhance their host's ability to harvest energy from the diet.

In another study, resourceful researchers used advanced genomic tools and data, some swimming bacteria, and a see-through fish to track the movements and host impacts of intestinal bacteria. Their findings contrasted with previous research, which presented a static view of bacterial behavior in the gut. When watching "live" through a microscope, researchers could see the bacteria traveling through the intestine of the naturally transparent zebrafish, *Danio rerio*. Similarly to the mouse model in the *M. smithii* study, the zebrafish were raised in a sterile environment until the introduction of the intestinal bacteria *Pseudomonas aeruginosa*. This type of bacteria is usually thought of as harmful, in part because of its presence in patients with inflammatory bowel disease and cystic fibrosis. However, recent research has challenged this belief by showing beneficial effects in fish. For these experiments, the bacteria were made to glow by introducing a fluorescent gene for easier visualization. Using this simplified, highly visible system, scientists were able to observe how the bacteria navigated the intestine, swimming with their flagellum—a whip-like tail. To test the impact of this bacterial colonization on the fish, some of the bacteria were hobbled by disabling their flagellum. Immobilization of the bacteria correlated with a drop in immune responses, demonstrating the bacteria's beneficial effects on fish immunity.

A third study combined both the mouse and zebrafish models in a reciprocal transplant experiment to answer fundamental questions about the evolutionary origins and inter-species differences of intestinal microbial communities. Mammals and fish typically host very different types of microbes in their intestines. By swapping intestinal contents between these species, scientists hoped to determine how the host habitat shapes the microbial community. In these experiments, both animals were raised either under sterile conditions to keep their intestines free of microbes, or in a normal environment where microbes naturally colonize the intestine. Then, intestinal contents from a normally raised zebrafish were transplanted into a sterile mouse, and vice versa (sterile zebrafish were colonized with mouse intestinal contents). Days after this initial colonization, the intestinal contents were sampled from the transplanted animals, and DNA was sequenced to identify the bacterial species present. Remarkably, the researchers found that each animal shaped the foreign microbial community to more closely resemble its

native mix of intestinal microbes. For example, in the transplanted mouse intestine, bacteria that are abundant in the native mouse intestinal community, such as the Firmicutes, were amplified, while the zebrafish's dominant bacteria, known as Proteobacteria, were diminished. A similar phenomenon was observed in the transplanted zebrafish intestine, where the Proteobacteria, a minor type of species in the mouse's intestine, was substantially amplified in the zebrafish, while other species were reduced or absent.

Each of these studies reveals a fascinating facet of intestinal microbes, from their origins to their impact on the host's everyday functions, such as absorbing nutrients and maintaining a healthy immune system. Future studies will continue these explorations of bacterial-host interactions in the intestine and their relationship to human health.

Rawls JF, Mahowald MA, Ley RE, and Gordon JI: Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell 127: 423-433, 2006.

Rawls JF, Mahowald MA, Goodman AL, Trent CM, and Gordon JI: In vivo imaging and genetic analysis link bacterial motility and symbiosis in the zebrafish gut. Proc Natl Acad Sci USA 104: 7622-7627, 2007.

Samuel BS, Hansen EE, Manchester JK, Coutinho PM, Henrissat B, Fulton R, Latreille P, Kim K, Wilson RK, and Gordon JI: Genomic and metabolic adaptations of Methanobrevibacter smithii to the human gut. Proc Natl Acad Sci USA 104: 10643-10648, 2007.

Mechanism of Enteropathogenic *E. coli* Infection:

NIDDK-supported scientists recently uncovered, in greater detail than ever before, the strategies used by a particular food-borne bacteria to cause intestinal disease. The bacteria, known as enteropathogenic *E. coli* (EPEC), cause severe watery diarrhea, particularly in infants living in developing countries. In fact, EPEC causes far more illness and death worldwide than the *E. coli* strain O157:H7, which was responsible for the high-profile, food-related illnesses in the United States in recent years. Unlike other types of *E. coli*, EPEC does not cause illness by releasing a toxin or entering host cells, but rather by attaching to the host's intestinal cells and directly affecting their function. However, until recently, it was not fully understood how infection with EPEC caused this occasionally deadly disease.

Diarrhea often occurs when sodium chloride (NaCl) cannot be absorbed from nutrients passing through the intestinal tract. NaCl absorption is accomplished by its transport across intestinal cell membranes in a process known as coupled ion exchange. This process maintains a neutral charge in intestinal cells while absorbing the charged sodium (Na+) and chloride (Cl-) ions. In the current study, scientists gained new insight into EPEC-induced diarrhea by studying the chloride exchange process in mouse models and in cultured human intestinal cells infected with EPEC using radioactively labeled chloride. From these studies, they determined that chloride uptake by intestinal cells is diminished in the presence of EPEC. Further investigation demonstrated that EPEC secrete bacterial molecules into host cells that disrupt the transport of a chloride ion exchanger protein, DAR, to its proper location in the cell's membrane thus preventing the uptake of chloride. The resulting decrease in cellular chloride creates an imbalance of sodium and chloride ions within the cells, which in turn precipitates watery diarrhea. Based on these studies, scientists designed a molecular model detailing the cascade of events that occur during EPEC infection. This valuable model of EPEC's *modus operandi* and its consequences will enable the development of new drugs to treat and to prevent infant mortality caused by EPEC infection.

Gill RK, Borthakur A, Hodges K, Turner JR, Clayburgh DR, Saksena S, Zaheer A, Ramaswamy K, Hecht G, and Dudeja PK: Mechanism underlying inhibition of intestinal apical Cl/OH exchange following infection with enteropathogenic E. coli. J Clin Invest 117: 428-437, 2007.

LIVER DISEASE RESEARCH

Comparing Outcomes of Liver Transplantation Procedures in Patients with Hepatitis C:

Researchers who are following a large group of liver transplant recipients are gaining insights into the best use of these procedures in patients with hepatitis C. Liver transplantation is often the only treatment option available for patients with advanced liver disease due to hepatitis C, but donor livers are in limited supply. Use of portions of the liver from living donors has

expanded the pool of organs available, but may present additional complications not experienced when using organs from deceased donors. These complications could make the procedure riskier for patients with advanced liver disease, such as those individuals with chronic hepatitis C. Ongoing follow-up of patients at nine U.S. liver transplant centers in the NIDDK-sponsored Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) recently provided important information to guide the use of this procedure in patients with hepatitis C. By tracking outcomes for about 3 years after the transplant, the researchers uncovered key factors that contributed to success of the transplants in patients receiving organs from living or deceased donors. Chief among these factors was the level of experience of the transplant center in performing the more novel living donor procedure. Once a transplant center was sufficiently experienced in performing living donor liver transplants, the procedure outcome was as successful as one using a deceased donor organ in patients with hepatitis C. Based on this research, patients with hepatitis C needing a liver transplant should have equally successful outcomes with organs from either a living donor or a deceased donor, provided the transplant center staff are proficient at both procedures. This flexibility greatly increases the chances that these patients will receive a potentially life-saving transplant.

Terrault NA, Shiffman ML, Lok ASF, Saab S, Tong L, Brown RS Jr, Everson GT, Reddy KR, Fair JH, Kulik LM, Pruett TL, Seeff LB, and the A2ALL Study Group: Outcomes in Hepatitis C virus-infected recipients of living donor vs. deceased donor liver transplantation. Liver Transpl 13: 122-129, 2007.

INHERITED DISEASES

Copper Availability and Menkes Disease:

Researchers have delineated a role for copper in nerve cell activation and early central nervous system development. Copper is an essential nutrient in the diet that is rapidly absorbed by the stomach and intestine into the circulation and transported to the liver. As a critical component of many enzymes, it plays a key role in cellular respiration, iron oxidation, antioxidant defense, connective tissue formation, and the formation of nerve-conduction factors. The liver is the primary site of copper storage, and normal body levels can be reduced if copper transport is impaired, or if clinical conditions are present that cause malabsorption. The maintenance of appropriate levels of copper is critical for brain function, as well as for the development and activity of the nervous system. Clinical and experimental studies show that copper deficiency during pregnancy leads to neurologic problems and abnormal organ development in offspring.

Mutations in the gene encoding a copper-transporting enzyme known as atp7a result in Menkes disease, an X-linked, neurodegenerative disorder that can cause seizures, failure-to-thrive, and, ultimately, death. Normally, atp7a helps to maintain sufficient copper levels inside cells, such that the presence of a mutated *atp7a* gene limits copper availability. Previous clinical studies of patients with Menkes disease have shown points of contained degeneration of the gray matter of the cerebral cortex, and neuronal loss most pronounced in two specific areas of the brain—the hippocampus and cerebellum.

To uncover the specific mechanism by which copper availability affects neuronal activation, researchers over-stimulated the N-methyl-D-aspartate (NMDA) receptor on neurons in the presence or absence of a compound that binds available copper. The overstimulation of neurons—also known as excitotoxicity—is a pathological process by which the cells are damaged and killed. The experiments showed that a lack of available copper causes increased NMDA receptor-mediated excitotoxic cell death, suggesting that copper specifically protects against this type of neuronal death. Furthermore, neurons obtained from a mouse model of Menkes disease, in which the copper-transporting enzyme is synthesized but not functional, revealed an increased sensitivity to excitotoxicity. The increased sensitivity to excitotoxicity was prevented by addition of copper. Thus, these results suggest a functional interaction between NMDA receptor activity and copper availability that could form the basis of a potential treatment for Menkes disease.

To further elucidate copper-related mechanisms for defects in Menkes disease, researchers performed a genetic screen in a zebrafish model in order to investigate the role of copper in notochord formation, which is similar to early spinal development in humans. Using a genetic screen for embryos exhibiting traits

of copper deficiency, the scientists also discovered a mutation in the zebrafish gene *atp7a*. This zebrafish model of Menkes disease should allow characterization of the development of abnormalities associated with this disorder, and allow high-throughput screens for clinically relevant reagents that restore copper-dependent enzyme activity in spite of *atp7a* mutations. Furthermore, excitotoxic neuronal death from low oxygen levels or blood flow causes significant neonatal morbidity and mortality, and these studies offer possible therapeutic approaches to this problem through manipulation of copper availability. Taken together, these studies underscore the importance of copper availability in the body for ensuring healthy organ development and preventing life-threatening disease.

Schlief ML, West T, Craig AM, Holtzman DM, and Gitlin JD: Role of the Menkes copper-transporting ATPase in NMDA receptor-mediated neuronal toxicity. Proc Natl Acad Sci USA 103: 14919-14924, 2006.

Mendelsohn BA, Yin C, Johnson SL, Wilm TP, Solnica-Krezel L, and Gitlin JD: Atp7a determines a hierarchy of copper metabolism essential for notochord development. Cell Metab 4: 155-162, 2006.

Molecular Link to Autoimmunity in Wiskott-Aldrich Syndrome: Recent findings enhance understanding of the roles of a key protein and a specific population of immune cells in causing the autoimmune disease associated with Wiskott-Aldrich Syndrome (WAS). This syndrome is an inherited disorder of the immune system that is characterized by recurrent infections, bleeding due to low platelet numbers, and eczema. Many patients with WAS also have one or more immune system-related conditions, such as intestinal inflammation, arthritis, or vasculitis. However, a paradox exists in which WAS patients display both immune deficiency (inability to fight off infection) and autoimmunity (inappropriate up-regulated reactivity of immune system to "self"). WAS is caused by a mutation in the gene coding for the WAS protein. This mutated protein impairs the immune cells' ability to transmit signals from receptors on the cell surface to the cytoskeleton. More than 200 unique mutations have been characterized in the *WAS* gene. Patients with the more severe manifestations have mutations in the *WAS* gene that result in loss of protein production. Until recently, most studies have focused on understanding the defects in activation of T cells (a specific type of immune system cell) caused by impairment of WAS protein. Now NIDDK-supported scientists have identified a specific population of T cells that are negatively affected in the absence of WAS protein. This population of T cells—called regulatory T cells—functions to prevent other immune cells from attacking the body's own tissues. The scientists undertook investigations in both humans and mice. Their studies of a WAS patient whose *WAS* mutation spontaneously reverted to a functional form offer new insight into this protein's effect on the stability of regulatory T cells. Previously, this patient had a mutation in the *WAS* gene that resulted in the absence of WAS protein production with a clinical picture of lifelong, recurrent episodes of autoimmune hemolytic anemia. The revertant is predicted to restore the normal protein amino acid sequence and normal WAS protein expression. Indeed, after the mutation reverted to a functional form, the patient exhibited an improved clinical picture with a striking increase in regulatory T cells expressing WAS protein. WAS protein-deficient mice were found to develop high levels of self-reactive molecules (autoantibodies) and autoimmune disease. To gain further insight into WAS, the researchers studied mice in which the *WAS* gene had been deleted. While WAS protein-deficient mice produced normal levels of regulatory T cells in the thymus, there was a preferential loss of regulatory T cells within the overall peripheral T cell pool. These findings indicate that regulatory T cells deficient in WAS protein do not proliferate or survive as well as normal cells with a functional WAS protein. These studies provide important information regarding how the WAS protein sustains regulatory T cells, and how alterations in these key factors contribute to the autoimmune disease experienced by patients with WAS. This research further contributes to our overall understanding of the role of the regulatory T cell in preventing autoimmune disease.

Humblet-Baron S, Sather B, Anover S, Becker-Herman S, Kasprowicz DJ, Khim S, Nguyen T, Hudkins-Loya K, Alpers CE, Ziegler SF, Ochs H, Torgerson T, Campbell DJ, and Rawlings DJ: Wiskott-Aldrich syndrome protein is required for regulatory T cell homeostasis. J Clin Invest 117: 407-418, 2007.

BARRETT'S ESOPHAGUS RISK FACTORS

Elucidating Risk Factors for Barrett's Esophagus:

Scientists report that extra abdominal fat may be a risk factor for Barrett's esophagus. Barrett's esophagus is a precancerous condition where the cells lining the esophagus change in shape and organization. Short of surgical removal of the esophagus, there is no effective cure for the condition. Known risk factors for Barrett's esophagus include being male, Caucasian, or over the age of 40. Individuals with gastroesophageal reflux disease (GERD), in which stomach acid flows backward into the esophagus, also are at increased risk of developing Barrett's esophagus. In turn, the condition puts patients at greater risk of developing esophageal adenocarcinoma, which is rapidly increasing in the United States. Therefore, research to further understanding of the risk factors for Barrett's esophagus may yield information that also would provide insight into the development of esophageal adenocarcinoma.

Scientists recently examined the association between obesity and abdominal girth and the occurrence of Barrett's esophagus and GERD. Obesity is thought to be a risk factor for GERD, and extra abdominal fat could directly promote acid reflux by placing added pressure on the stomach. Obesity was determined using body mass index (BMI), a measure that takes both height and weight into account, and abdominal girth was measured simply as waist size. In this study, while there was no association of BMI with Barrett's esophagus, larger abdominal circumference was moderately associated with the condition. The association was strongest in individuals with no GERD symptoms. When GERD symptoms were included in the analysis, the association between abdominal girth and Barrett's esophagus was decreased, which was expected as GERD symptoms may partly mediate the effect of abdominal girth in the development of Barrett's esophagus. These data suggest that larger abdominal girth may be a risk factor for both GERD and Barrett's esophagus. Although the mechanism by which extra abdominal fat raises the risk of these conditions is unknown, it could act by increasing pressure on the stomach and contributing to GERD symptoms through effects on gastrointestinal mobility. The results of this research suggest that larger abdominal circumference, but not overall obesity, is a risk factor for Barrett's esophagus. Based on these findings, reduction of waist size may be advisable for patients at high risk of developing this condition or subsequent esophageal carcinoma.

Corley DA, Kubo A, Levin TR, Block G, Habel L, Zhao W, Leighton P, Quesenberry C, Rumore GJ, and Buffler PA: Abdominal obesity and body mass index as risk factors for Barrett's esophagus. Gastroenterology *133: 34-41, 2007.*

National Commission on Digestive Diseases

Diseases of the digestive system span a wide range of conditions—from functional gastrointestinal and motility disorders, inflammatory bowel disease, and celiac disease, to liver and gallbladder diseases, to pancreatic diseases and GI cancers. Collectively, these diseases represent an enormous public health burden. A strong commitment to advancing research is required to combat digestive diseases.

Since its establishment by the NIH Director, Dr. Elias Zerhouni, in 2005, the National Commission on Digestive Diseases has made substantial progress toward its goal to improve the health of the nation through advancing digestive diseases research. The Commission is responsive to the mutual interest in this research area shared by the Congress, the NIH, and the research community. Within the NIH, the NIDDK is providing leadership and support for the Commission.

As part of its charge, the Commission is assessing the state-of-the-science in digestive diseases and the related NIH research portfolio, in order to identify research challenges and opportunities for inclusion in its long-range research plan for digestive diseases. The Commission's efforts benefit from the diverse expertise of its members, who represent the academic and medical research and practice communities, the patient advocacy community, and the NIH and other Federal health agencies. In 2006, the Commission initiated a research planning process through two public meetings held near the NIH and by engaging in activities such as: (1) defining the topic areas within digestive diseases research that comprise the research plan, (2) assigning Commission members to chair topical Working Groups, (3) conducting an open call for additional experts to serve as Working Group members, and (4) laying the foundation for the Working Groups' deliberations by teleconference to identify research goals and other recommendations. Dr. Zerhouni addressed the Commission members at their November 2006 meeting to express his support and appreciation for their efforts.

During the Commission's public meeting in June 2007, Working Group chairs presented their Groups' research recommendations for specific digestive diseases, along with steps to achieve the proposed goals. The chairs then provided the Commission members with content for the topic-specific chapters of the research plan. At the following meeting in November 2007, in Chicago, Illinois, the Commission considered the entire draft research plan and invited public comments. A formal public comment period held after the November meeting invited additional stakeholder input on the draft research plan, which was posted on the internet. Following incorporation of public input and preparation of the final publication, release of the completed research plan is anticipated in 2008. The Commission's 10-year research plan will guide the NIH—along with the investigative and lay communities—in pursuing important research avenues for combating digestive diseases.

Additional information about the Commission can be found on its website: *http://NCDD.niddk.nih.gov*

Functional Dyspepsia Treatment Trial Launched

The NIDDK is sponsoring a new clinical trial to explore the causes and most effective treatment of a common digestive condition known as functional dyspepsia. Functional dyspepsia is a type of indigestion for which symptoms can include severe stomach pain or great discomfort after eating. These symptoms are thought to result from abnormal muscular activity in the stomach, which may in turn result from abnormal neural activity within the stomach or between the brain and the gut. Current treatments are limited to food restriction or drugs that affect muscular activity in the stomach.

Now a new clinical trial has been launched to test potential therapeutic agents for functional dyspepsia that act on the brain and gut. The Functional Dyspepsia Treatment Trial (FDTT) will test two FDA-approved drugs—amitriptyline and escitalopram. These drugs are traditionally prescribed as anti-depressants, but the trial will determine if they can also relieve stomach pain or discomfort after meals in patients with functional dyspepsia. Additional goals of the trial include the discovery of particular genes that predict response to these treatments and a determination of whether the response is "durable" over the long term, even after patients stop taking the drugs for 6 months. Recruitment of patients for the trial began in January 2007 and will continue for 5 years. Patients participating in the trial are between the ages of 18 and 75 and have not experienced relief from their functional dyspepsia symptoms with previous treatments.

More information about functional dyspepsia and other common forms of indigestion is available through the National Digestive Diseases Information Clearinghouse at: *http://digestive.niddk.nih.gov*

STORY OF DISCOVERY

Advances in Inflammatory Bowel Diseases Research

NIDDK support for research on genetics and immunology of the inflammatory bowel diseases (IBD) is paving the way to the development of unique and effective therapies for patients who suffer from these diseases.

IBD was described in the medical literature as early as the mid-18th Century, but it was not until the mid-20th Century that the two major subtypes of IBD—Crohn's disease (CD) and ulcerative colitis (UC)—were identified and distinguished by the area of the intestine they affect. The incidence of these diseases in western, industrialized societies increased dramatically during the 20th century. These are painful and debilitating diseases, characterized by chronic, intermittent intestinal inflammation. In CD, inflammation can occur anywhere in the alimentary tract and sometimes in other sites, but most often occurs in the end of the small bowel and beginning of the large bowel (colon). In UC, the site of inflammation is restricted to the colon, or large intestine. The leading theory for the cause of IBD is that inflammation is triggered by inappropriate immune responses to bacteria that naturally reside in the intestine and that the underlying predisposition to these inappropriate immune responses is caused by multiple interacting genes. Under normal circumstances, most bacteria residing in the gut have a beneficial or benign effect on their host, but an overly active immune system may be provoked by these bacteria in IBD.

Genetic Factors in IBD Uncovered

Studies of human twins and of animals have confirmed that genetic factors contribute to IBD. Some gene variants are specifically associated with either CD or UC, while others are involved in both diseases. The importance of genetic factors is also reflected in family studies showing the incidence of IBD to be higher among family members.

A major research breakthrough on the genetics of IBD came in 2001, with the discovery of the first IBD-associated gene, called *NOD2*. The *NOD2* gene was found to be associated solely with CD, not with UC. This landmark research, which was supported by the NIDDK, represents one of the earliest, most well-established associations in complex genetic disorders. The product of *NOD2* is a cellular protein found in immune cells, called monocytes, and in cells lining the intestinal wall. Although the mechanisms underlying the relationship of the *NOD2* gene variant to CD are not yet fully understood, the NOD2 protein is known to activate communication (signaling) pathways in response to components of bacterial cell walls, leading to a variety of immune responses.

Building on this important finding, the NIDDK in 2002 established the Inflammatory Bowel Disease Genetics Consortium (IBDGC). (For more information on the Consortium, see highlights from a Scientific Presentation by Consortium investigator Dr. Judy Cho, which appears later in this chapter).

The Consortium's efforts were greatly enabled by resources provided by the NIH-sponsored Human Genome Project and the International HapMap Project, which were major drivers in propelling research on human genetics. The Human Genome Project sequenced the 3 billion nucleotide base pairs of the human genome, a monumental effort that concluded in April 2003. Data from this project were made available to scientists around the globe to facilitate the pursuit of medical research. The International HapMap Project, published in 2005, is a catalogue of common small genetic variations called SNPs (single nucleotide polymorphisms) that occur in the nucleotide (or letter) sequences of individuals' DNA. The Genome and HapMap projects have been accompanied by great strides in the development of new rapid biomedical technologies so that hundreds

of thousands of SNPs can now be determined in single DNA samples. The genome-wide association scan based on these advances has become the cutting-edge technology for identifying genes that contribute to human disease, and was used by the Consortium to identify genetic factors in IBD.

Recently, members of the Consortium used this genome-wide association technology in a two-phase study designed to identify additional genes that contribute to CD. In this study, blood samples from CD patients and healthy volunteers were scanned for known genetic variants using over 300,000 SNPs. The first phase of the study was very successful in detecting several significant SNP associations, including a variant of a gene encoding a receptor for the cytokine (an immune system chemical) interleukin-23 (IL-23). Surprisingly, one variant of the gene was shown to protect against CD. Additional studies have shown that the IL-23 receptor is required for CD to develop in animal models.

Because the inflammatory bowel diseases are complex diseases involving the contributions of many genes, it was anticipated that genes also existed that had more subtle associations with IBD, the detection of which would require screening much larger cohorts. Therefore, a second, expanded phase of the study was conducted on a larger population of CD patients and healthy volunteers. In this second phase of the study, scientists discovered another CD associated gene, *ATG16L1*, which is involved in autophagy. Autophagy is a process by which cells capture, degrade, and recycle unwanted cellular material into useful molecules. This process has also been associated with the body's early immune response that is activated by the recognition of bacterial components. The involvement of the autophagy process has been verified by two other scientific research groups. One group identified the autophagy gene, *ATG16L1*, using a different protocol in which 72 SNPs, selected through a screening process, were used in a genome-wide association scan of CD patients and healthy controls. The other group identified a second autophagy gene linked to CD, called *IRGM*, in a major genome-wide association study that scanned 14,000 patients with seven different diseases (2,000 patients for each of the seven diseases) and a shared control set of 3,000 healthy volunteers. The study identified 27 additional disease-related genetic variations, including nine for CD, seven for type 1 diabetes, and three for type 2 diabetes.

Mapping the Molecular Pathways of IBD Development

Discovery of the IBD gene, *NOD2*, provided the first evidence linking this disease to the immune response to bacteria. The NOD2 protein is an intracellular sensor of bacterial wall components. Upon sensing the bacteria, NOD2 activates multiple molecular pathways associated with initial responses by the immune system. Extensive research continues to clarify the roles that pathways stimulated by NOD2 play in the errant activation of immune response associated with IBD.

Research on chemicals utilized by the immune system, including cytokines such as interleukins, has demonstrated the important role of pathways activated by these molecules in IBD development. Identification of the interleukin-23 receptor gene as being associated with the risk of developing IBD coincided with other research investigating the roles of IL-23 and its receptor in autoimmunity, the immune system's inappropriate reaction to the body's own tissues. Studies exploring the causes of inflammation in autoimmune disease have focused on two cytokines, IL-12 and IL-23, which have related structures, but different functions. These two molecules are dimers that each have one identical subunit, as well as one unique subunit. Antibodies against the common, shared subunit of the two cytokines inhibit inflammation in both animals and in human CD. More recent studies in mice have shown that IL-23, not IL-12, is responsible for inflammation.

STORY OF DISCOVERY

In one study examining the roles of IL-12 and IL-23 in IBD, scientists used a mouse model infected with bacteria known to induce inflammation and then analyzed IL-12 and IL-23 subunit expression in the intestine. The mice responded to bacterial infection with increased production of the common subunit of both interleukins and the unique subunit of IL-23, but not the IL-12 unique subunit, demonstrating that inflammation is dependent on IL-23, not IL-12. Furthermore, when antibody was introduced to block the unique IL-23 subunit, inflammation was markedly reduced, confirming that IL-23 is essential for inflammation in the intestine. Confirmation that IL-23, not IL-12, is required for intestinal inflammation was made in another study using two mice strains with double mutations. Both strains contained a mutation that causes them to spontaneously develop inflammation resembling CD. Additionally, the IL-12 unique subunit was inactivated in one strain and the IL-23 unique subunit was inactivated in the second strain. Mice with mutations in the IL-12 unique subunit developed colitis; however the IL-23 unique subunit mutants remained disease free, confirming that active IL-23 is essential for intestinal inflammation. These results point to selective targeting of IL-23 as a potential new therapeutic approach for human IBD.

Recent research has also refined our understanding of the types of immune cells involved in IBD, and how they interact with key molecular pathways. IL-23 has been shown to induce the production of other inflammatory cytokines by immune cells called monocytes and macrophages. IL-23 also activates a recently identified subtype of helper T cells (T_H cells), called T_H17 cells. Until recently, only two major subsets of T_H cells had been identified: T_H1 cells, which secrete molecules that destroy intracellular microbes and are associated with CD; and T_H2 cells, which secrete molecules that destroy extracellular microbes and are associated with UC. The newly-discovered T_H17 cells secrete the inflammatory cytokines TNF-alpha, IL-6, and IL-17, and are thought to be particularly important in causing tissue inflammation in immune diseases, including IBD. Thus, not only has IL-23 been implicated as an important cytokine in IBD, it appears that the cytokine acts through a very specific type of T cell that has only recently been identified. These discoveries suggest important new pathways to be explored to develop treatments for CD.

New Treatments for IBD

The elucidation of new disease genes and the molecular responses they initiate is key to developing drugs that prevent and treat IBD. Two examples from recent years involve molecules known as TNF-alpha and PPAR-gamma. The cytokine TNF-alpha is now recognized as a major factor in the inflammatory immune responses associated with IBD. The drug infliximab was the first recombinant antibody designed to bind to TNF-alpha, thereby preventing it from engaging with receptors that activate inflammatory responses. Infliximab was initially thought to be effective only in treating and maintaining remission of CD, but has now been show to be an effective treatment of UC.

The peroxisome proliferator activated receptor-gamma (PPAR-gamma) regulates gene expression in the nuclei of immune cells and epithelial cells that line the colon and is known for its effects on tumor suppression in the colon and on attenuation of colitis. PPAR-gamma expression was found to be impaired in cells lining the colons of UC patients, indicating a potential role in the treatment UC. Mutant mice with minimal expression of PPAR-gamma in their colon epithelial cells were given a substance that induces colitis, in order to determine if PPAR-gamma plays a protective role against developing UC. Mutant mice exhibited higher levels of molecules that promote inflammation and increased susceptibility to experimental colitis when compared with control mice. Rosiglitazone, a drug used for the treatment of type 2 diabetes, activates the PPAR-gamma receptor. When rosiglitazone was administered to the mice, the severity of the induced colitis was decreased and cytokine production was suppressed

in both mutant and control mice, demonstrating that PPAR-gamma plays a role in protecting against colitis. Because administration of rosiglitazone was effective in reducing colitis symptoms in mutant mice expressing minimal levels of PPAR-gamma, as well as control mice, it is possible that rosiglitazone may also act independently of PPAR-gamma in suppressing inflammation.

The efficacy of rosiglitazone in treating UC in humans was recently tested in a multicenter clinical trial supported by the NIDDK. Patients participating in the trial had mild-to-moderate UC and had been previously treated with the drug 5-aminosalicylate, the most common treatment for UC, but had not responded well or were intolerant to the drug. After receiving either rosiglitazone or a placebo, patients were assessed for improvement in their condition. After 12 weeks, 44 percent of patients given rosiglitazone had clinical remission compared to 23 percent of patients given placebo. These data demonstrate that rosiglitazone is effective in the short-term treatment of patients with mild-to-moderate UC who did not benefit from other treatments. Further long-term studies must still be conducted to assess use of this class of drug as a maintenance therapy for UC, and to determine whether they provide an additional new treatment option for patients suffering from UC.

Genetics of Inflammatory Bowel Diseases (IBD): IL23R as an IBD Susceptibility Gene

Dr. Judy Cho

Dr. Judy Cho is an Associate Professor in the Department of Medicine and Genetics at the Yale School of Medicine and the Director of Yale's Inflammatory Bowel Disease Center. A leader in the field of inflammatory bowel disease (IBD) research, Dr. Cho and her colleagues are widely recognized for their 2001 discovery of the first known gene to increase susceptibility to Crohn's disease—the NOD2/CARD15 gene. Dr. Cho is the Chair of NIDDK's Inflammatory Bowel Disease Genetics Consortium Steering Committee and Principal Investigator of the Consortium's Data Coordinating Center. Recently, the NIDDK IBD Genetics Consortium identified IL-23R as another IBD susceptibility gene. Dr. Cho presented this clinical research study to the NIDDK Advisory Council at their February 2007 meeting. The following are highlights from her presentation. (Additional information on IBD-related research conducted by Dr. Cho and other NIDDK-sponsored researchers is presented in this chapter's "Story of Discovery.")

The inflammatory bowel diseases are chronic, intermittent intestinal inflammations which are thought to result from inappropriate responses by the immune system to bacteria normally found in the intestine. Symptoms include diarrhea, abdominal pain, intestinal bleeding, and, in cases of childhood onset, growth retardation. IBD occurs frequently in young people—with a peak age of onset between 15 and 30 years of age. Because IBD is largely seen in industrialized societies, some researchers have suggested that it may be associated with changes in intestinal microbial populations during the industrialization process.

The two major subtypes of IBD are ulcerative colitis (UC) and Crohn's disease (CD), which are distinguished by the area of the intestines affected. The site of inflammation with UC is restricted to the colon, or large intestine. In CD, inflammation is found in the small intestine and often affects both the small and large intestines. Ileal CD, which targets a part of the small intestine known as the ileum, is the most common form of Crohn's disease.

Although little is understood regarding its etiology, IBD is known to involve complex interactions between multiple genes, as well as the microbial environment of the intestine. Two genetic associations with IBD had been well-established, the *NOD2* gene, identified by Dr. Cho and her colleagues, and a variant in another area of the genome called *IBD5*. The discoveries of these genetic variations provided two pieces of the complex IBD puzzle, but did not fully explain the incidence of IBD. Thus, the search continued for other genes associated with this disease.

Searching for Additional IBD Genes

The prevalence of Crohn's disease is several times higher in the Ashkenazi Jewish population of European ancestry than in the non-Jewish population of European ancestry. In this genome-wide association study, over 300,000 naturally occurring genetic variations were screened in Ashkenazi Jewish and non-Jewish patients and healthy controls. These variations, known as SNPs (single nucleotide polymorphisms) are small differences in an individual's DNA sequence that can have varying disease consequences ranging from causing major genetic diseases such as cystic fibrosis or sickle cell anemia to more subtle effects that alter disease risk. SNPs are valuable disease biomarkers used in both research and clinical diagnoses.

SCIENTIFIC PRESENTATION

The study began with a genome-wide screen of non-Jewish ileal CD patient and control cohorts. Ileal CD patients were selected exclusively to minimize genetic differences within the patient cohort. The screen identified three SNPs having highly significant associations with CD. Two of the three SNPs were located in the previously identified CD susceptibility gene, *NOD2*. However, the third was located in the IL23 receptor gene (*IL23R*). Surprisingly, this uncommon gene variant was found to confer protection against CD.

Ileal CD patients of Ashkenazi Jewish ancestry and their controls were then screened for *IL23R* markers. Highly significant differences were observed in the frequency of the protective marker in the two study groups. For example, this marker was identified in only two percent of CD patients in contrast to seven percent of the controls, supporting its protective role in preventing CD.

Following the two screens, the research team conducted a study to determine the frequency of the transmission of *IL23R* markers in nuclear IBD families consisting of children who were affected by IBD (CD, UC, and indeterminate IBD) and both of their parents. The study revealed that the protective variant of *IL23R* was much less likely to be passed down from parents to their IBD-affected children. Both Jewish and non-Jewish families with this marker were protected against developing CD; however, only the non-Jewish population showed a similar protective effect against UC.

The unexpected identification of a gene variant that protects against the risk of IBD has given new insights into the molecular underpinnings of this disease. These findings substantiate a hypothesis, supported by recent immunological studies, that the *IL23R* gene is required for the manifestation of clinical IBD. Importantly, these research results also provide potential therapeutic targets for its prevention and treatment.

The Inflammatory Bowel Disease Genetics Consortium (IBDGC)

In 2002, the NIDDK established the Consortium to provide the research resources necessary to take advantage of the wealth of genetic information provided by the NIH-sponsored Human Genome Project in elucidating the disease mechanisms of IBD. The infrastructure established to accomplish the Consortium's mission includes a Data Coordinating Center, which oversees genetic analysis, database analysis, and coordination between six Genetic Research Centers. The Centers recruit patients and healthy volunteers for IBD study cohorts, submit patient blood samples and phenotype data to a repository, and conduct genetic research studies. Governance of the Consortium is provided by a Steering Committee consisting of Consortium scientists and a NIDDK health science administrator.

As Chair of the Steering Committee and head of the Data Coordinating Center and of one of the research centers, Dr. Cho has played a significant role in the development of the Consortium. In describing the Consortium's major advantages, Dr. Cho identified:

- Synergy of expertise provided by gastroenterologists who are primarily interested in IBD and geneticists whose interests include IBD and complex disorders;
- Stringent quality control attained through sample and data uniformity;
- Ability to recruit the large numbers of patients required to identify the genes responsible for IBD;
- Availability of resources necessary for high risk, high priority projects—including genetic studies of disease differences in minority populations; and
- Knowledge of priorities and opportunities provided by NIH oversight.

The Consortium has developed collaborations with outside investigators, providing valuable data, genotyping services, and research resources. For

example, immortalized cell lines are being derived from patients' blood samples. These cell lines and the DNA extracted from them will be linked to the patients' phenotypic data and stored for use in Consortium research projects. They will also be made available to the broader scientific community. Additionally, control datasets representing different populations are being analyzed and made available on the web. This approach provides major research effectiveness and cost-saving advantages for future IBD studies.

A Vision for Future IBD Research

Dr. Cho described her vision for the future of IBD research and her major priorities. These include developing models of disease risk, developing biomarkers, predicting disease course, and finding ways to prevent disease. These endeavors all have as their underpinnings the identification of the multiple genes that contribute to IBD and the elucidation of their interactions with each other and their environment. Even genes with limited direct associations with IBD may have significant biological consequences that must be considered in designing important risk models. Biomarkers that reflect genetic variation and the molecular consequences of gene expression are important research indicators of disease risk, disease prognosis, and patient response to therapies. Thus, biomarkers will serve as major drivers in the development of new approaches to the prevention and cure of IBD.

The IBD genome-wide association study presented by Dr. Cho has continued to yield important genetic discoveries. An expansion of this study identified three new IBD susceptibility genes which are described in the IBD Story of Discovery, also in this chapter.

Howard Klein

Taking Life—and Hepatitis B—By the Horns

Two degrees from the Juilliard School of Music…. Classical pianist….Former music critic for *The New York Times*….Director of the Rockefeller Foundation's arts program….Board president for the Carter Family Memorial Music Center Foundation in Virginia….Linguist….Wine connoisseur and collector….Happily married and father of two sons and one stepson….

It's fair to say that 76-year-old Howard Klein is an accomplished Renaissance man. He's also a person with hepatitis B who credits the quality of life he currently enjoys to the treatment and personal attention he has received from clinical trials sponsored by the NIDDK.

Howard Klein

About Hepatitis B

The virus that causes hepatitis B is one of at least five different hepatitis viruses (hepatitis A to E virus) that can result in liver disease in humans. Hepatitis B makes the liver inflamed and swollen. Over time, the virus compromises the liver's ability to perform its bodily functions, which include the organ's ability to fight infections, stop bleeding, process drugs and toxins in the blood, as well as store energy for when the body needs it. In its most extreme forms, hepatitis B can lead to severe liver damage, liver cancer, and the need for a liver transplant.

Thanks to research supported by the NIH and other organizations, hepatitis B has been effectively prevented and controlled in the United States and many other countries through vaccines that protect against the virus and public health education programs. Screening of blood donations also has contributed to a reduction of hepatitis B cases. However, the disease is far from entirely eradicated, especially in individuals who live in or emigrate from countries where the disease is more common, such as parts of Asia.

It's fair to say that 76-year-old Howard Klein is an accomplished Renaissance man. He's also a person with hepatitis B who credits the quality of life he currently enjoys to the treatment and personal attention he has received from clinical trials sponsored by the NIDDK.

Because it is a blood-borne disease, hepatitis B can be transmitted in several different ways (see sidebar). Many people who are infected don't know it because they feel perfectly healthy. Howard, for example, never manifested any symptoms of hepatitis B prior to diagnosis. It was diagnosed through follow-up blood work for another serious illness he was experiencing. That's where Howard's story begins.

Howard's Story

In 1984, Howard was diagnosed with a severe case of lymphoma, a cancer of the lymphatic system. At the time, he was told that he had a 20 percent chance of surviving the disease. His weight

PATIENT PROFILE

dropped to 125 pounds and he required a massive blood transfusion to save his life. Howard believes he contracted hepatitis B through the transfusion, and his doctors agree. However, because he manifested no symptoms, he wasn't diagnosed with hepatitis B until 1995, when his lymphoma was in remission.

Thanks to research supported by the NIH and other organizations, hepatitis B has been effectively prevented and controlled in the United States and many other countries through vaccines that protect against the virus and public health education programs. However, the disease is far from entirely eradicated…

"I was in California on a wine-tasting trip with one of my sons when I got a call from my doctor," Howard says in a somewhat amused tone of voice. "He'd just received the results of my blood work and said I had serious liver problems and not to drink any wine." His doctor also recommended that Howard go to the NIDDK, one of 27 research institutes of the NIH. Howard was finally diagnosed with chronic hepatitis B. Since 1996, Howard has taken part in several NIDDK clinical trials that have tested various pharmaceutical agents for treatment of hepatitis B.

At NIDDK, Howard started out on a drug called lamivudine, which is taken orally once a day. The then experimental treatment looked promising, but, over time, Howard became resistant to the drug. Next, he was put on an interferon drug, a synthetic version of the naturally produced protein used by the body to fight viruses by boosting the immune system. Interferon attempts to prevent the hepatitis B virus from further damaging the liver by inactivating the virus and removing it from the body. This drug is administered much like insulin is for diabetes, with self-injections either into the stomach or leg, which Howard found discomforting.

"I really had problems with self-injecting interferon into my body," he says. As it turned out, his discomfort turned out to be a moot point. His interferon treatment followed the same path as the lamivudine. At the beginning of treatment, Howard responded well, but ultimately, his liver did not respond to the interferon and the hepatitis B virus remained active.

In the meantime, prior to and during the early years of participating in the NIDDK clinical research, Howard's liver, as a result of his chronic hepatitis B, was undergoing an unusually rapid onset of cirrhosis. Cirrhosis occurs when scar tissue replaces normal, healthy liver tissue, blocking the flow of blood through the organ and preventing it from working as it should. Cirrhosis is a leading cause of death in the United States.

"In 2000, my doctor at NIH showed me how deadly my cirrhosis had become. I was really bad off," says Howard. Howard's hepatitis was having an adverse impact on his life and career as a professional musician. "The years 2000 to 2003 were particularly difficult," adds Howard. "Music is very demanding because your brain has to think ahead. You're not just in the moment; you know what's coming and you have to be there. When I'd try to learn a new piece of music or perform, I always felt fatigued and sluggish. No pain, just a general sense of malaise." In 2003, however, Howard was put on a combination drug therapy of lamivudine and adefovir dipivoxil, which, like lamivudine, is taken orally—and it worked.

Howard has benefited from the rapid expansion in recent years of pharmaceutical agents available to treat hepatitis B. The variety of hepatitis B drugs means that, if patients eventually develop resistance to long-term treatment with one drug, they can be switched to another one or put on a combination of drugs that is effective.

"God, I'm lucky!"

"As a result of the combination drug therapy, Howard's prognosis is excellent," says Theo Heller, M.D., the

physician-scientist who has been following Howard all these years at the NIDDK while Howard participated in research studies. "If we performed a biopsy today, I doubt we'd find any cirrhosis," he adds.

But that doesn't mean that Howard can let down his guard. Although his blood tests are all normal and hepatitis B virus levels are below detection, he still has the hepatitis B antigen in his blood. "If he stops his combination drug treatment, his hepatitis will come straight back," says Dr. Heller.

Howard says he sees 76 as the new 56 and adds that there are still plenty of things he'd like to accomplish in his life. "If you're interested in life, you challenge yourself," he says emphatically.

In the face of all this, Howard remains as productive as ever. In addition to his lead role with the Carter Family Memorial Music Center Foundation, Howard continues to perform as a musician. Last year, for example, he played two concerts at the Goethe Institut in New York with his son, who is an opera singer, performing Schubert's famous song cycle *Winterreise*.

Howard says he sees 76 as the new 56 and adds that there are still plenty of things he'd like to accomplish in his life. "If you're interested in life, you challenge yourself," he says emphatically.

So far, Howard has managed to avoid the most serious repercussions of hepatitis B, including liver cancer or the need for an organ transplant. He is currently in an NIDDK-sponsored study of the natural history of liver disease, which is tracking the long-term effects of treatment, and he credits the NIDDK, through its research programs, for giving him this "extended" life opportunity.

"God, I'm lucky!" Howard says in a voice full of energy, hope, and promise.

Hepatitis B spreads by contact with an infected person's blood, semen, or other body fluids.

You could get hepatitis by:

- Having sex with an infected person without using a condom;
- Sharing drug needles;
- Having a tattoo or body piercing done with dirty tools that were used on someone else;
- Getting pricked with a needle that has infected blood on it;
- Living with someone who has hepatitis B;
- Sharing a toothbrush or razor with an infected person;
- Traveling to countries where hepatitis B is common; or
- Receiving a blood transfusion in the 1980s or earlier, before effective screening of donor blood for the hepatitis B virus.

An infected woman can give hepatitis B to her baby at birth.

You CANNOT get hepatitis B by:

- Shaking hands with an infected person;
- Hugging an infected person; or
- Sitting next to an infected person.