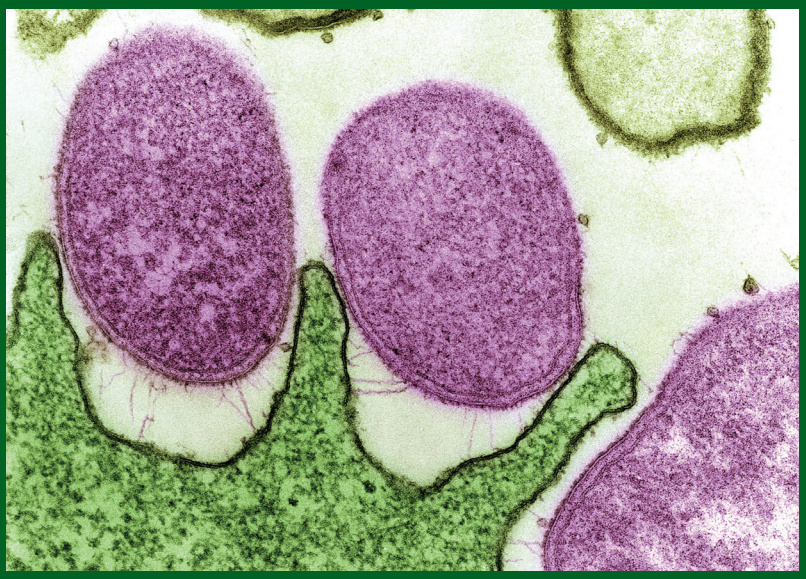

**Colorized electron micrographs of *Helicobacter pylori* bacteria (on top in blue; on bottom in purple) in the human stomach. NIDDK-funded researchers are elucidating important interactions between bacteria and the gastrointestinal tract that contribute to human health and disease. As described in a research advance in this chapter, analysis of the *Helicobacter pylori* genome has shed light on genetic alterations that allow some strains of this common stomach bacterium to become more effective pathogens, causing severe inflammation (gastritis) that can lead to cancer.**

*Top image: Credit: David McCarthy / Photo Researchers, Inc.*

*Bottom image: Credit: SPL / Photo Researchers, Inc.*

# 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 tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. Disorders of the digestive tract—such as irritable bowel syndrome and inflammatory bowel disease—exact a significant toll on many Americans each year. 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.*

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 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 and is now at epidemic levels. Obesity is associated with numerous serious diseases, including type 2 diabetes, heart disease, and cancer. While multiple factors contribute to obesity, caloric intake clearly plays a key role in weight gain. 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 next chapter.)

Intestinal disorders 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 experience 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.

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. 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 identification 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.

The microorganisms that inhabit the gastrointestinal tract are 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 cells of their host. Scientists are gaining insights into the ways these microorganisms influence the development and function of the digestive tract.

Some digestive diseases can be triggered by the body's reaction to certain foods. 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 health care providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued 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 IL-23 Receptor Is a Major Susceptibility Gene for Inflammatory Bowel Disease:

The two major forms of inflammatory bowel disease (IBD) — Crohn's disease and ulcerative colitis—are thought to arise from a combination of genetic susceptibility and environmental factors. Previous studies have identified mutations in the *NOD2* gene as playing a role in the development of Crohn's disease in humans. Building on this finding, the NIDDK established the IBD Genetics Consortium to speed the search for other susceptibility genes in this complex disease. To identify additional genes, the researchers performed a genome-wide association study by testing more than 300,000 genetic variants in people with and without Crohn's disease. For long stretches, a given DNA sequence may be identical in two different people. However, every so often, one (or more) of the nucleotides varies at a site called a single nucleotide polymorphism or "SNP." The researchers found that, out of the hundreds of thousands of SNPs examined, three were strongly associated with Crohn's disease. Of those, two were in the previously known *NOD2* gene, and the third SNP was in a gene encoding the interleukin-23 (IL-23) receptor. While characterizing the various polymorphisms in the IL-23 receptor gene to determine which ones were the most harmful, the scientists found a polymorphism that appears to protect against development of Crohn's disease. Taken together with previous findings, this important discovery offers new hope for better therapies for patients with this chronic disease.

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## "OMICS" TECHNOLOGIES ENABLE UNDERSTANDING OF THE HUMAN DIGESTIVE TRACT AND ITS MICROBIAL COMMUNITY

Research on human health and disease has been greatly enhanced by "omics" technologies. These technologies unlock the vast amounts of information stored in the collection of human genes (genomics);

their products—RNAs expressed (transcriptomics), and proteins translated (proteomics); as well as the many small molecules participating in metabolic processes (metabolomics). These approaches are now being applied with exciting results to understanding the complex functions of the human digestive tract, including studies into the unique regenerative properties of intestinal stem cells and of the microbial world teeming within. Examples follow.

**Unique Gene Expression of Adult Gut Stem Cells:** Researchers recently used genomic analyses to catalog genes expressed by adult stem cells responsible for renewing the lining of the stomach and small intestine. Because very few stem cells are present in the normal gut, investigators used cells from a mouse model engineered to overproduce them. They found that gastric and intestinal stem cells express a unique set of genes not found in stem cells from other organs. This gene catalogue represents an important tool for investigators to use for elucidating the specialized functions of adult gut stem cells, thereby hastening the time when these cells could be used to treat gastrointestinal diseases.

**Importance of the Gut Microbial Community:** The human gastrointestinal tract, particularly the large intestine, is home to at least 10 trillion microorganisms. Recent "omics"-based studies have proven extremely helpful for shedding light on the importance of the gut microbial community in human health and disease. Recently, a group of researchers initiated an in-depth study of the collection of microbial genomes (microbiome) in the healthy human intestine. Using stool samples from two healthy adults, they catalogued the genetic diversity of microorganisms in the human large intestine and further defined their distinctive functions within the human body. This effort to catalog gut microbial genes, and the functional attributes associated with the proteins they produce, provides a foundation for future studies into how the gut-microbiome partnership is important for normal human metabolism and intestinal health.

In fact, investigators are already exploring the role of the gut microbiome in human disease using genomic analyses of microbial species from "humanized" animal models and patient samples. For example, researchers have recently used transcriptomic analysis to understand how interactions among specific gut microbial species can affect host energy balance. For these analyses, they used a "humanized" mouse model, raised so that the gut was free of microbes and then colonized with gut microbes commonly found in humans. They found that two microbial species in particular—*Methanobacter smithii* and *Bacteroides thetaiotaomicron*—have a cooperative relationship in digesting fiber that leads to more efficient intestinal nutrient absorption and energy storage as fat. Further research building on this work could lead to new ways to address both obesity and undernourishment.

Researchers have also used genomic analysis to understand digestive disease caused by *Helicobacter pylori* infection, which is extremely common in the U.S. and other countries. While most people develop a mild case of stomach inflammation from the infection, a select few develop more severe, chronic inflammation that can progress to gastric cancer. Upon detailed genomic analysis of bacterial strains isolated from several patients who developed severe gastric disease, results revealed a common gene "signature" among these strains. Also uncovered were unique genetic changes in some strains that allowed them to survive in the stomach as inflammation progressed to gastric cancer. These findings could aid in identifying patients in whom *H. pylori* infection will likely cause severe disease, so that it can be prevented or treated before progressing to gastric cancer.

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## LIVER DISEASE RESEARCH

### Search for Reasons Underlying Different Hepatitis C Treatment Responses:

Chronic hepatitis C is a major cause of chronic liver disease and the need for liver transplantation in the U.S. Unfortunately, there is a variable response rate to the available standard therapy for chronic hepatitis C—a combination of the drugs peginterferon and ribavirin. African Americans with hepatitis C are less responsive to treatment than Caucasian Americans. To investigate possible reasons for this difference and identify ways to improve treatment regimens, the NIDDK-funded Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C) is comparing treatment responses in groups of African Americans and Caucasian Americans. Recent results from this study have more accurately characterized the racial difference in response to treatment. Results showed a significantly lower viral response in African Americans as early as week four of treatment, and a higher rate of "breakthrough," with a return of elevated viral levels later in the course of treatment. The investigators were also able to rule out several potential explanations for the difference in treatment response, including higher viral levels before treatment, sex, age, weight, extent of fibrosis in the liver, and amount of medication taken. Research in this study population is continuing in search of possible virologic, immunologic, and genetic factors underlying the cause of reduced treatment response in African Americans with chronic hepatitis C.

*Conjeevaram HS, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucase TE, Afdhal N, Brown RS, Belle SH, Hoofnagle JH, Kleiner DE, and Howell CD, for the Virahep-C Study Group. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. Gastroenterology 131: 470-477, 2006.*

### Measures of Success in Hepatitis C Treatment:

The standard therapy used to treat chronic hepatitis C consists of a combination of the drugs peginterferon and ribavirin given for approximately 6 months or a year. However, this therapy proves, ultimately, to be ineffective in a large proportion of people with chronic hepatitis C. In an effort to monitor the success or failure of the treatment, current guidelines call for the use of assays to measure hepatitis C virus (HCV) RNA levels during and after treatment as an indicator of lasting response and clearance of the viral infection. Recently, a more sensitive assay of HCV RNA was developed, called the transcription-mediated amplification or "TMA" assay, though its usefulness for monitoring treatment response was unknown. Researchers involved in the NIDDK-supported Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) Trial put the new assay to the test using samples from their study population—patients with chronic hepatitis C who did not respond to prior treatment with interferon. Compared to an older HCV RNA assay, the TMA assay proved more sensitive in detecting HCV RNA and predicting earlier in the course of treatment whether a sustained response was likely. Based on the promising results in this subset of patients, further studies are warranted to determine the usefulness of the TMA assay in monitoring and predicting treatment response in other patients with chronic

hepatitis C. If the results are applicable to other patient groups, the assay could represent a better way to predict non-responsiveness to standard therapy, and also allow for early discontinuation of therapy in patients who would not benefit from further treatment.

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# *National Commission on Digestive Diseases*

Since its establishment by the NIH Director 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 the Long-Range Research Plan that it has been tasked with developing. 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. The Commission convened for two public meetings during 2006 to initiate the research planning process through such activities as: (1) defining the 13 topic areas within digestive diseases research that make up the research plan, (2) assigning Commission members to chair 13 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 and other means. The Working Groups are convening to identify research goals and other recommendations for inclusion in this effort. The resulting 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*

# *Launching the New Celiac Disease Awareness Campaign*

The NIDDK launched the Celiac Disease Awareness Campaign on July 18, 2006. "We now know that celiac disease is more prevalent than previously thought and that it often remains under-diagnosed," said Dr. Griffin Rodgers, Acting Director of NIDDK. "Through the campaign, we hope to increase physician awareness of the disease, resulting in earlier diagnosis and better outcomes for celiac patients."

Celiac disease is an autoimmune disorder that interferes with the absorption of nutrients from food. Individuals with this condition experience an inappropriate response by their immune systems to gluten, a protein that is found in wheat, barley, and rye, causing a wide range of symptoms such as gas, diarrhea, abdominal pain, delayed growth, skin rashes, infertility, and osteoporosis.

Once thought to be rare, celiac disease is now known to affect nearly one percent of the U.S. population. Although there is no cure, most patients can avoid symptoms of the disease by maintaining a gluten-free diet. However, because of the vast array of symptoms, celiac disease frequently goes undiagnosed.

In June 2004, the NIH held a Consensus Development Conference on Celiac Disease, sponsored by the NIDDK and the Office of Medical Applications of Research. A Consensus Statement prepared by experts from the Conference recommended that the NIDDK lead an awareness campaign for physicians, dietitians, nurses, and the public about celiac disease. Based on this recommendation, the Celiac Disease Awareness Campaign was developed, with coordination among the professional and voluntary organizations working on celiac disease. The campaign offers fact sheets, booklets, practice tools for health professionals, NIH research information, and resources from professional and voluntary organizations that focus on celiac disease.

The Celiac Disease Awareness Campaign web page, located at *www.celiac.nih.gov*, features such informational items as awareness campaign news, educational material and resources, examples of celiac disease research, celiac disease organizations, and a link to the National Digestive Diseases Information Clearinghouse.

# PATIENT PROFILE

## Ed McGrenaghan

*Gastroparesis Compounds Diabetes Treatment*

Despite having been diagnosed with type 1 diabetes at age four-and-a half and experiencing complications from the disease starting in pre-adolescence, Ed McGrenaghan, now 50, says he has been very active most of his life.

"I was involved in numerous organizations, including four Boy Scout troops and three volunteer firehouses, and I played on a number of sports teams," says Ed, who admits that getting married at age 35 slowed him down a bit. About 6 years ago, however, his health began to decline.

Part of Ed's left foot was removed as a result of an infection that would not heal due to his diabetes. About a year and a half later, on a New Year's Eve, Ed suffered a heart attack and had a stent inserted to open one of his coronary arteries. He has neuropathy damage to nerves in his feet, legs, and fingertips. Ed also has osteoarthritis and was recently diagnosed with something called adhesive capsulitis, more commonly referred to as "frozen shoulder." Although not directly related to his diabetes, the frozen shoulder is painful and difficult to treat, and the prescribed steroids can increase blood glucose levels—something that people with diabetes struggle to control.

To complicate matters even more, Ed has a condition called gastroparesis. Gastroparesis is a disabling stomach disorder that often occurs in people with type 1 or type 2 diabetes. Gastroparesis can worsen one's diabetes by making it difficult to control blood glucose levels. But thanks to research advances, Ed is finding it easier to manage both his gastroparesis and his type 1 diabetes.

Ed McGrenaghan

**WHAT IS GASTROPARESIS?**

Gastroparesis is a disorder characterized by symptoms of and evidence for gastric (stomach) retention in the absence of mechanical obstruction. One of the most common causes of gastroparesis is diabetes. Although not completely understood, it is thought that gastroparesis happens when the vagus nerve to the stomach is damaged and no longer controls the movement of food through the digestive system. Diabetes can damage the vagus nerve if blood glucose levels remain high over a long period of time. It is caused when high blood glucose levels injure the blood vessels that carry oxygen and nutrients to the vagus nerve. When the vagus nerve is damaged due to lack of nutrients and oxygen, the muscles of the stomach and intestines do not work properly, and the movement of food is slowed or stopped. Food that lingers too long can harden into solid masses, called bezoars, which may cause nausea, vomiting, and obstruction in the stomach. In addition, damage to the vagus nerve may account for some of the severe symptoms of gastroparesis

such as nausea, vomiting, and bloating, independent of the slowed movement of food.

When food that has been delayed in the stomach finally enters the small intestines and is absorbed, blood glucose levels rise. Since gastroparesis makes stomach emptying unpredictable, a person's blood glucose levels can be erratic and difficult to control. This problem is especially dangerous for people, like Ed, who have either type 1 or type 2 diabetes.

**Signs and Symptoms of Gastroparesis**

- Heartburn
- Nausea
- Vomiting of undigested food
- An early feeling of fullness when eating
- Unintentional weight loss
- Abdominal bloating
- Erratic blood glucose levels
- Lack of appetite
- Gastroesophageal reflux

**LIVING WITH GASTROPARESIS AND DIABETES**

Although Ed was officially diagnosed only 5 years ago, on reflection he thinks that the origins of his gastroparesis may date back almost 40 years, when he began experiencing chronic stomach ulcers at age 11. These have persisted most of his life. "At around 26 years old, I experienced frequent light vomiting for 3 or 4 months, but back then, they didn't have the diagnostic tests they have today," says Ed.

When he was in his mid-30s, Ed recalls episodes in which he would be engaged in conversation at the dinner table and had to excuse himself to rush to the bathroom, where he would have "dry heaves" and diarrhea. According to Ed, this went on for 3 years. His doctors told him that what he was experiencing was related to diabetes-induced neuropathy.

It was shortly after having had part of his foot removed 5 years ago that Ed's gastroparesis was diagnosed. "I would start vomiting at 2 a.m. and continue through to 2 p.m. the following day—and I had diarrhea at the same time," says Ed. These episodes also entailed numerous visits to the emergency room because of the dehydration that ensued. All of this was going on at the same time Ed was trying to control his blood glucose levels to keep his diabetes in check.

Ed talks about a lot of this in the past tense now, because 2 years ago, a battery-operated gastric neurostimulator was surgically implanted to help control his nausea and vomiting associated with gastroparesis. The gastric neurostimulator is an option for people whose nausea and vomiting do not improve with medications. "It's been a godsend," Ed says of the device. "It has given me back a lot of my freedom and doesn't restrict me in any way."

Since having the gastric neurostimulator implanted, Ed says that he has had only two vomiting episodes, one of which he truly believes was more viral than gastroparesis-related. However, the device doesn't do anything to relieve his episodes of diarrhea, which Ed says he still experiences about three times a month, "but they are much less severe than they used to be."

**TREATING GASTROPARESIS**

Unfortunately, there is no cure for gastroparesis. The primary goal for individuals like Ed, who also have diabetes, is to regain control of their blood glucose

## PATIENT PROFILE

levels. Treatments include insulin, oral medications, changes in what and when to eat, and, in severe cases, feeding tubes and intravenous feeding.

Ed is acutely aware of the need to control his blood glucose levels. He has three brothers with diabetes, one of whom died of a massive heart attack associated with the disease.

Although research has produced several drugs to treat gastroparesis, Ed relies primarily on his gastric neurostimulator and an insulin pump, which he started using about 18 months before having the stimulator implanted. Prior to using these two devices, Ed's hemoglobin A1c (HbA1c) value (a measure of glucose levels over time) was in the range of 11 or 12 percent. HbA1c values provide an excellent indirect measure of how well a person's blood glucose has been controlled over the preceding two to three months, with lower values indicating better control. Since using both the gastric neurostimulator and insulin pump, the HbA1c value has dramatically dropped to 8 or 8.2 percent, he says. It is currently recommended that all persons with diabetes try to maintain HbA1c values as close to normal as safely possible (7 percent or less).

In addition, Ed watches his diet and tries to eat five or six smaller meals a day in lieu of three full ones. "I just stopped eating as much," says Ed. "As time goes on, I'm able to read myself better. As soon as I put something in my stomach I can feel it start to swell. It tells me when to stop." Ed's wife also has been a big help to him. "She works in the medical field and has access to lots of medical information. She knows what she's doing when she helps me. I couldn't ask for a better mate."

That's not to say Ed's life is back to what it was 20 years ago when he was working and volunteering his time to numerous organizations. Five years ago, Ed had to give up his job as horticulturist for a private garden center because of his deteriorating health. "Now, on a good day, I could probably work a couple of hours," says Ed, who spends most of his days at home doing household chores or taking care of others in the family, who may be ill at the time. "With all my body has been through, it just doesn't have the stamina it used to have."

On the positive side, both the gastric neurostimulator and insulin pump "bring a lot of relief," says Ed.

There is much more that needs to be learned about gastroparesis and improving its management. To address these issues, the NIDDK recently funded the "Gastroparesis Clinical Research Consortium," which consists of a network of clinical centers and one data-coordinating center working cooperatively to conduct rigorous clinical research to elucidate the functional changes associated with this disorder and to develop better treatments.

## STORY OF DISCOVERY

# *Drug-Induced Liver Injury*

Many Americans rely on approved drug therapies to manage their health problems. But in some rare cases, the cure itself can cause disease, as with adverse reactions to drugs. The liver, due to its central location and key role in processing and detoxifying foreign chemicals—including drugs—is one of the most common sites for adverse drug reactions, which can range from mild injury to acute liver failure, leading to the need for a liver transplant, or even resulting in death. Research supported by the NIH has played an integral role in advancing knowledge about drug-induced liver injury. These advances include deciphering the process by which liver cells detoxify drugs; identification of disease processes and risk factors; and revealing the magnitude of drug-induced liver injury in the U.S. due to the commonly used analgesic, acetaminophen.

**Drug-Induced Liver Injury in the U.S.:** While some instances of drug-induced liver injury can be traced to the inherent toxicity of a drug, many cases are unpredictable—resulting from the combination of a drug's properties and an individual's unique susceptibility. The problem is compounded by several factors, including the large number of drugs available, frequent concomitant use of multiple drugs, limitations of the current post-marketing surveillance system, and lack of specific diagnostic tests for drug-induced liver injury, which can mimic every other known form of liver disease. Beyond its significant burden on individuals and threat to the public health, drug-induced liver injury represents a major drain on the U.S. economy as the most common reason for either halting development of a promising new drug or bringing regulatory action (warnings or withdrawals) against drugs already on the market.

**Understanding How Drugs Harm the Liver:** Since 1923, when a therapeutic drug for gout was recognized as causing liver injury in some individuals, cases of drug-induced liver injury have risen as more drugs came on the market. From the time this phenomenon was first described, researchers have focused on determining the underlying mechanisms and disease processes at work. Over the past several decades, studies sponsored by many NIH Institutes, including the NIDDK, have made significant contributions to understanding how drugs are metabolized and how they cause liver disease.

Beginning in the 1950s, NIH-supported researchers helped to decipher the three-step process by which liver cells detoxify drugs and other foreign compounds. In "Phase 1," a complex of enzymes metabolizes the drug to a reactive intermediate. NIH-sponsored researchers working from the 1950s to 1970s contributed to the identification of a key component of this enzyme complex called cytochrome P450, and found that its activity could be influenced by genetic variations and other factors. NIH-supported research also contributed to the discovery of "Phase 2," in which the drug metabolite is inactivated by addition of a chemical group before being transported by "Phase 3" proteins out of the liver and into bodily fluids for excretion.

In the 1970s and 1980s, research performed by NIH intramural and extramural investigators using cell

## STORY OF DISCOVERY

culture and animal models uncovered the mechanisms of liver injury due to specific drugs. In the case of acetaminophen—an ingredient in many over-the-counter pain medications sold in the U.S.—high levels of the reactive drug metabolite were found to be directly toxic to liver cells, resulting in cell death. Since then, further progress has been made in understanding the variety of mechanisms by which drugs can cause liver injury. Clinical research supported by the NIH since the 1980s also contributed to identifying several risk factors for liver injury caused by drugs, including age, gender, genetic make-up, nutritional status, pre-existing disease, simultaneous use of other drugs, and alcohol intake. Efforts to characterize the spectrum of disease due to drug-induced liver injury and to predict its clinical course were advanced by one of the pioneers of the field—Dr. Hyman Zimmerman at the Department of Veterans Affairs—who collaborated with NIH-supported researchers and developed a rule that predicts mortality for drug-induced liver injury associated with jaundice. A diagnostic tool consisting of a series of questions called the "RUCAM" was developed in 1989, based on recommendations from an international conference of experts. The RUCAM is still used to diagnose drug-induced liver injury, but its use is problematic, in that its interpretation varies widely, even among experts. Diagnosis continues to be based largely on exclusion of other types of liver disease, although it has benefited indirectly from NIH research on better ways to diagnose liver diseases from other causes.

**Current Research Advances on Drug-Induced Liver Injury:** In some cases, liver injury caused by drugs leads to acute liver failure, for which the only practical available therapy is liver transplantation. Historically, limited data were available on the causes and outcomes of drug-induced acute liver failure. The NIDDK-sponsored Adult Acute Liver Failure Study Group was founded in 1997, based on investigator-initiated efforts to address this problem by expanding knowledge about natural history, causes, and outcomes. The Group has collected samples and data needed to conduct retrospective as well as forward-looking studies that more closely examine the problem of acute liver failure in the U.S., focusing largely on cases caused by drugs.

In 2002, the Group published their ground-breaking finding that liver injury due to acetaminophen use had risen dramatically in recent years to become the most frequent known cause of acute liver failure in the U.S. Building upon this important observation, the Group recently developed an assay to directly identify cases of acetaminophen-induced acute liver failure by measuring unique compounds in the serum—an advance that could facilitate diagnosis and allow more accurate estimates of prevalence. In 2005, the Group expanded its focus to study the problem in children. The Pediatric Acute Liver Failure Study Group and the adult-focused Group now include 25 U.S. sites, where clinical trials of a therapy are being conducted to improve patient survival.

The NIDDK-led Drug-Induced Liver Injury Network, established in 2003, is another major research effort aimed at studying this health problem. Based on recommendations from an October 2000 scientific conference, this Network of five clinical centers and one data coordinating center is enabling research on liver toxicity due to prescription drugs, as well as complementary and alternative medicines. Studies aim to develop better tools for directly diagnosing,

and ultimately preventing, drug-induced liver injury, as well as enhancing knowledge of disease processes. Network investigators are currently testing a new, consensus-based diagnostic tool for drug-induced liver injury and comparing it to the RUCAM. The Network is also sharing information on cases with the FDA, and is evolving into a resource for the national clinical community and the public. Additionally, the Network is planning to identify additional cases and apply genome-wide screening techniques, which can aid in assessing the role of genetic variability. Ultimately, researchers hope to identify genetic or biological markers that indicate an individual's risk of developing drug-induced liver injury and then combine this with information on potentially toxic drug signatures. Such a body of knowledge could enable more predictive and personalized medical care in which health care providers are better equipped to determine whether a drug is safe for a particular patient or subset of patients to use.

**Future Research Plans:** The evolution of this field of research illustrates the continuing benefits of NIH-supported research, as well as collaboration with other Federal agencies and industry. Major NIH research programs, such as the Drug-Induced Liver Injury Network and the Acute Liver Failure Study Group, as well as investigator-initiated research efforts directed toward goals in the trans-NIH *Action Plan for Liver Disease Research,* are helping to address the problem of drug-induced liver injury.

# SCIENTIFIC PRESENTATION

## Translational Research on Inflammation and Colorectal Cancer

*Dr. Raymond N. DuBois*

*Dr. Raymond DuBois is the B. F. Byrd Professor of Molecular Oncology, Professor of Medicine, Cancer Biology, and Cell/Developmental Biology, and Director of the Vanderbilt-Ingram Cancer Center. He received his M.D. and Ph.D. degrees from the University of Texas Health Science System before training in internal medicine and gastroenterology at The Johns Hopkins University Hospital, including research training with Nobel Laureate Dr. Daniel Nathans at The Johns Hopkins University Medical School, as a Howard Hughes Medical Institute Research Associate. In 1991, he accepted a position at Vanderbilt University School of Medicine as an Assistant Professor of Medicine and Cell Biology. From 1998 to 2003, he served as the Director of Gastroenterology, Hepatology and Nutrition. He has received numerous awards in recognition of his work, which focuses on colorectal cancer prevention. The following are highlights based on a scientific presentation Dr. DuBois gave at a meeting of the Institute's National Advisory Council in February 2006.*

Dr. DuBois discussed colorectal cancer (CRC), and his research on its connection to inflammation. CRC is the third most common type of cancer, and also the third most common cause of cancer death in the U.S. However, the disease need not be fatal. When caught early, while tumors are still localized, survival rates are excellent. Because treatment is so much more effective when it comes early in the disease than when it comes later, surveillance is critical in older Americans, as well as in younger people who are genetically predisposed to the disease.

Of course, preventing the development of cancer, when possible, is even better than diagnosing it early. CRC can be prevented by identifying pre-cancerous growths called adenomas before they progress to overt cancer, and in some cases by altering diet or lifestyle. In addition, recent advances in our understanding of the molecular events that lead to CRC are beginning to suggest preventive approaches. Chemoprevention—the use of a drug or combination of drugs that could inhibit some cancer-related events—could be a viable option. Dr. DuBois' presentation focused primarily on this approach.

### INFLAMMATION AND THE MOLECULAR UNDERPINNINGS OF CRC

Studies have shown that people who take aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) may have a lower risk of CRC than those who do not. Furthermore, inflammation caused by certain infectious diseases and other medical conditions (such as obesity) predispose people to particular types of cancer. These observations suggest a connection of inflammation to cancer that the DuBois lab and others have sought to elucidate. Inflammation may:

- Inhibit "programmed cell death" and suppress immunity, two of the body's key defenses against cancer;
- Promote growth of arteries and veins that can potentially feed a growing tumor;
- Enhance the ability of cancer cells to invade other tissues (spread); and
- Promote genetic changes that can lead to cancer.

Although inflammation has many causes, the body can trigger inflammation through production of chemicals called "prostaglandins." Cyclooxygenase enzymes are an essential component of the pathway that produces prostaglandins, and these are the enzymes that aspirin and many other anti-inflammatory painkillers act to inhibit.

Work in the last three decades has firmly established that progression from normal tissue to cancer is a multi-step process, in which a series of genetic and other changes within pre-cancerous cells remove critical constraints on dangerous cell division and proliferation. In CRC in particular, there are usually mutations in a protein known as APC. The DuBois lab and others noted that also there are frequently increased amounts of a cyclooxygenase enzyme called COX-2 present in pre-cancerous colorectal cells than in the surrounding tissue, and that even more COX-2 is often present in the cells after they have progressed to overt cancer. A larger amount of COX-2 in the cells suggests that they are potentially generating high levels of inflammatory signals, i.e., prostaglandins. These observations suggest that changes in COX-2 may be an important part of the multi-step progression to CRC, and that inflammatory signaling via prostaglandins may contribute to carcinogenesis.

Indeed, not only does reducing inflammation reduce the risk of cancer, but also high levels of inflammatory signals have been shown to enhance risk. The amount of COX-2 produced in healthy tissues is regulated, so that excessive signaling does not occur. When scientists deliberately remove this regulation in experimental animals, they create a situation in which the protein is always produced at high levels in a particular organ. Under those conditions, that organ becomes very likely to develop tumors. Additionally, animals that are genetically predisposed to develop CRC, but which lack receptors for prostaglandins, experience a markedly lower rate of growth and progression of their cancer than do mice with the same genetic susceptibility, which also have an intact inflammatory signaling pathway.

**CLINICAL TRIALS USING COX-2 INHIBITORS**

The clear connection between COX-2 and CRC makes the protein an excellent target for chemoprevention. The DuBois group therefore participated in a clinical trial to test a specific COX-2 inhibiting drug called celecoxib for efficacy in patients with a familial predisposition for CRC. Eighty-three patients were randomly assigned to receive either placebo or one of two different doses of celecoxib. Because their genetic predisposition is so powerful, all of the patients in this trial had a large number of pre-cancerous polyps at the outset of the study. Their polyps were counted and measured before and after the 6 month trial. Patients receiving the drug had, on average, a significant drop in the number and size of polyps compared to patients receiving the placebo. Moreover, patients taking the higher dose of the drug had a markedly better reaction than those taking the lower dose.

Interestingly, some patients had little or no response to the drug, although others responded very well—in some cases having all of their polyps disappear during the trial. A second study was later performed using blood samples taken from patients in the trial previously described, to try to detect differences between the two groups, in order to target the therapy only to those who are likely to benefit from it. In the later

study—not performed by the DuBois group—the researchers were able to determine that a particular protein was present in the blood of patients who did not respond to the celecoxib treatment, but not in the responsive patients. This type of information could be used in the future to determine who would or would not be likely to benefit from anti-inflammatory treatment to prevent or delay CRC.

Of course, people with a known familial predisposition for CRC actually represent a small fraction of total CRC cases. That is the reason it is so important for all people over the age of 50 to be screened by a colonoscopy procedure, which permits physicians to visualize and biopsy the colon for signs of cancer. Doctors can immediately remove any pre-cancerous adenomas they find during colonoscopy, which will often be all the treatment a patient needs. However, 30 to 40 percent of patients will experience re-growth of the adenomas within 3 years after such removal. Can anti-inflammatory chemoprevention improve the odds of staying cancer-free? The answer is clearly "yes," because aspirin is known to reduce 3-year recurrence rates by about 20 percent. Aspirin inhibits both COX-2 and COX-1, a related enzyme not thought to be involved in cancer.

Trials have also been under way to test celecoxib and other selective COX-2 inhibitors. Preliminary indications are that specific inhibition of COX-2 may be more effective than aspirin in preventing recurrence of adenomas. However, data made public since these trials began indicate that there is a significant risk of cardiovascular side effects in patients taking COX-2 inhibitors. Because aspirin may actually have cardiovascular benefits, it may eventually prove to be the better chemopreventive agent for some or all patients at risk of CRC. Further analysis will be necessary.

**OTHER POTENTIAL THERAPEUTIC TARGETS IN THE INFLAMMATORY PATHWAY**

While studies are focusing on COX-2 because it is responsible for an early step in the body's chemical process of generating prostaglandins, other investigations are exploring additional avenues to combat CRC. For example, inhibitors also exist for enzymes that promote later steps of prostaglandin production, and some of these are currently in preliminary clinical trials to test their efficacy as possible preemptors of CRC. Some of these enzymes are tissue-specific, and may potentially offer a target that is specific to the intestinal tract, thus avoiding some of the side effects associated with COX-2 inhibition.

In principle, it is also possible to interrupt inflammation at the point where the prostaglandin signal is received—at its so-called receptor. Because the COX-2 pathway generates a variety of related prostaglandins, each with a particular receptor and signaling cascade, it may be possible to finely tune chemoprevention of CRC by targeting specific prostaglandin receptors. Thus, this approach may allow interference with cancer progression, while avoiding cardiovascular problems or other unwanted side effects of COX-2 inhibitors.

Accordingly, the DuBois lab has sought to better understand inflammatory signaling downstream of COX-2. In particular, they have focused on identifying the harmful effects of a particular prostaglandin known as $PGE_2$. They found that administering $PGE_2$ over a period of time to mice with a genetic predisposition to CRC led to a dramatic increase in the number of adenomas in the animals' intestines. Most adenomas found in untreated mice in this strain of mice are in the colon, and indeed, treatment with $PGE_2$ increased the number and the size of the adenomas found

there. $PGE_2$ treatment also led to formation of a large number of adenomas in the animals' small intestines. These studies suggest that there may be potential therapeutic benefits to finding ways to inhibit $PGE_2$.

To pursue this, the DuBois group focused on a protein called PPAR-delta because its expression is typically elevated in CRC, and it is also elevated as a result of inflammation. They found that when they administered $PGE_2$ to mice with the genetic predisposition for CRC, but which also lacked PPAR-delta, the $PGE_2$ had no cancer-promoting effect. These data suggest that PPAR-delta may be a downstream effector of $PGE_2$, and may logically represent a potential therapeutic target.

Interestingly, PPAR-delta is also known to have a role in metabolism. Data from other groups suggest that stimulation of PPAR-delta activity can cause weight loss and improve lipid profiles and insulin sensitivity in overweight rodents. Thus, PPAR-delta stimulation may be a therapeutic option for obesity, diabetes, and hypertension, even as PPAR-delta inhibition might potentially have benefits for prevention of CRC. The DuBois lab treated the CRC-susceptible mice which had PPAR-delta with a compound to stimulate its activity. They found a significant increase in the size and number of adenomas after stimulating PPAR-delta. Whether an effect such as this would be observed in humans or even in animals that are not genetically susceptible to CRC is unknown; however, these data suggest caution is warranted when considering the advisability of either inhibiting PPAR-delta to prevent CRC or stimulating it as a treatment for other conditions. Several investigators are currently attempting to develop means of stimulating PPAR-delta to achieve benefits with respect to metabolism without side effects in the colon.

Another potential therapeutic target in the inflammatory pathway is EGFR, a growth-factor receptor that can also be stimulated by $PGE_2$. The DuBois lab found that inhibiting EGFR with a drug called erlotinib reduced adenoma growth and number in CRC-susceptible mice. Interestingly, they found that erlotinib combined with celecoxib, the COX-2 inhibitor, resulted in a nearly complete disappearance of adenomas in these animals. These results suggest that anti-inflammatory therapy could be combined with erlotinib or a related compound to create a chemopreventive treatment that may be more effective than either single drug in reducing the danger of CRC in some CRC-susceptible people.

**CONCLUSIONS**

Dr. DuBois' presentation underlined the need for effective means of preventing CRC in patients who may otherwise develop the disease. Dr. DuBois discussed the role and component parts of the inflammatory pathway within the body, and particularly within intestinal tissues. He demonstrated that inhibition of inflammation at various stages in this pathway is a viable option for preventing CRC, and finished with data showing that a combination of chemopreventive agents may be more effective than a single drug. At the same time, Dr. DuBois pointed out that the cardiovascular side effects of selective COX-2 inhibition and the positive and negative impacts of targeting particular components of the COX-2 pathway, such as PPAR-delta, are important reminders that great care must be taken in bringing new treatments into practice.