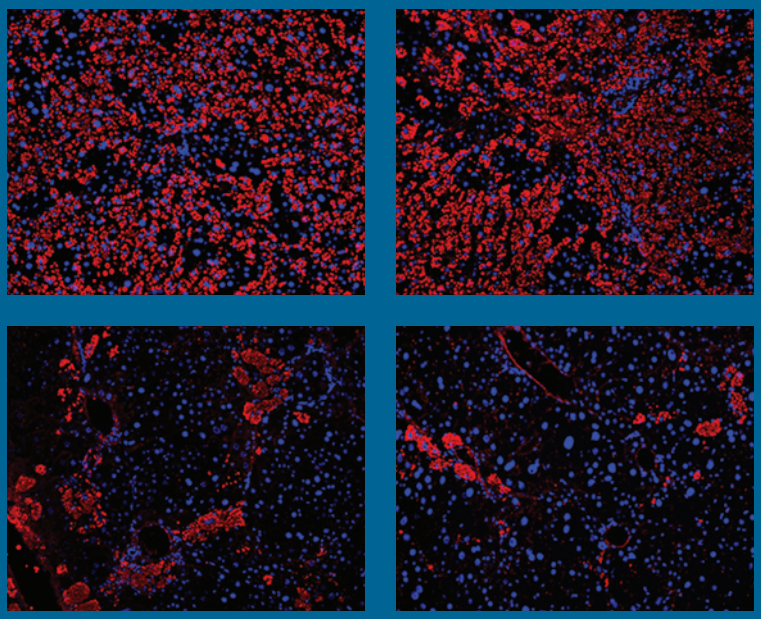


These sections of liver tissue, taken from a mouse model of liver damage associated with the disease alpha-1 antitrypsin deficiency, show reduced accumulation of the mutated alpha-1 antitrypsin protein (in red) in mice given the drug carbamazepine (lower two panels) compared to untreated mice (upper two panels). As described in this chapter, researchers have found that this drug, previously used for other conditions, shows promise in pre-clinical studies as a treatment for alpha-1 antitrypsin deficiency, a form of genetic liver disease that affects children as well as adults.

Images provided by Drs. Tunda Hidvegi and David H. Perlmutter from Hidvegi T, Ewing M, Hale P, Dippold C, Beckett C, Kemp C, Maurice N, Mukherjee A, Goldbach C, Watkins S, Michalopoulos G, and Perlmutter DH: An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. Science 329: 229-232, 2010. Reprinted with permission from AAAS.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the U.S. each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. In 2004, more than 35 percent of all emergency and outpatient hospital visits—some 100 million—were associated with a diagnosis of a digestive disease.[1] While some digestive diseases are common and others quite rare, collectively, they exact a significant toll on public health in terms of their effects on quality of life, years lost due to premature death, and costs associated with hospitalization and pharmaceutical and surgical interventions. To reduce the public health burden associated with digestive diseases, NIDDK-supported scientists are vigorously pursuing research to better understand how widespread these diseases are across the U.S. and in specific population groups, to identify the causes of these diseases and how they progress, and to test new interventions for prevention and treatment of these costly diseases, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. The continued discovery of predisposing genetic variations and potential autoimmune and microbial influences will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer. Screening programs for colorectal cancer are aimed at reducing mortality through early detection, particularly in those individuals at higher risk.

Diseases of the stomach and intestines include some of the most common digestive diseases, such as peptic ulcer disease, which is typically caused by an infection with the bacterium *Helicobacter pylori* or use of non-steroidal anti-inflammatory drugs. Stomach and 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. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus transform into an intestinal type of cell, is associated with a heightened risk of esophageal cancer, the most rapidly rising cancer in the U.S. 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

[1] *Everhart JE, editor. The burden of digestive diseases in the United States. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: U.S. Government Printing Office, 2008; NIH Publication No. 09-6443.*

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 immune system reacts to the protein gluten—a component of wheat, barley, and rye—and results in damage to the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only current treatment for celiac disease is maintenance of a strict 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 microorganisms that inhabit the GI tract are important factors in maintaining or tipping 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 GI microorganisms influence the development and function of the digestive tract, as well as other systems throughout the body, such as those with immune and metabolic functions.

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis, and their complications. Acute pancreatitis is typically caused by gallstones, while common causes of the chronic form include inherited genetic factors and heavy alcohol use. In both forms, digestive enzymes attack the pancreas from within, causing inflammation and pain. Research has elucidated genetic factors contributing to pancreatitis that may lead to ways to treat or prevent this disorder.

The liver is an organ within the digestive system that performs many critical metabolic functions, as well as distribution of nutrients such as fats. When the liver is functionally compromised by disease, this can have serious adverse effects on health and can sometimes lead to complete liver failure. Some liver diseases primarily affect children, such as biliary atresia (a progressive inflammatory liver disease), while others generally affect adults, such as a form of nonalcoholic fatty liver disease (NAFLD) known as nonalcoholic steatohepatitis. In recent years, however, NAFLD has been increasingly diagnosed in children in the U.S. as well, concurrent with rising overweight and obesity. While some forms of liver disease are caused by viral infection such as hepatitis B and C, or by genetic mutations such as alpha-1-antitrypsin deficiency, others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, research is of critical importance to identify liver disease early, preserve liver function in people with liver disease, and develop new treatment options, including transplants performed with liver tissue from living donors.

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, and environmental factors that influence appetite, metabolism, and energy storage, they are identifying potential avenues for the development of new intervention strategies to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

Other nutrition-related disorders under investigation involve specific alterations in nutrient metabolism, such as an inherited form of copper deficiency called Menkes

disease. NIDDK-supported research has enhanced knowledge of how this nutritional disorder and others develop, and how they can best be treated.

GENETICS OF INFLAMMATORY BOWEL DISEASES

Genetic Risk Factors Associated with Inflammatory Bowel Diseases in Children:

Researchers have identified five new genetic variations that predispose children to developing inflammatory bowel diseases (IBD). The two major forms of IBD—Crohn's disease and ulcerative colitis—are marked by chronic and destructive inflammation in the intestinal tract. While the precise causes of IBD are unknown, there is a strong genetic component that predisposes individuals to developing these diseases. Previously, nearly 50 genetic risk factors had been identified as associated with IBD. Many of these genetic factors involve components of the immune system that control intestinal inflammation. These factors, however, were typically identified in adults with IBD. Since some clinical aspects of IBD in children differ from those in adults, it was important to extend these studies to identify genetic risk factors associated with the development of IBD in pediatric populations.

An international team of researchers has carried out the largest genome-wide association (GWA) study to date for identifying genetic variants associated with IBD in children. In a GWA study, scientists scan thousands of genomes for genetic variants that are more common in individuals with a disease—such as a form of IBD—than in healthy individuals. In this study comparing children with IBD and healthy children, the researchers identified variants in five new regions of the genome that increase children's risk of developing IBD. One of the most prominent risk factors identified for Crohn's disease was found near the *IL27* gene. This gene codes for a protein that is involved in an immune response previously implicated in the pathogenesis of Crohn's disease. The researchers showed that colon cells from children with Crohn's disease had much lower levels of *IL27* gene activity than cells from healthy individuals, suggesting that the risk variant reduces the amount of protein made from the *IL27* gene. In addition to the five new genetic regions, the pediatric population also has many of the genetic risk factors previously identified in adults with Crohn's disease or ulcerative colitis. This implies that IBD involve similar biological pathways in adults and children, but that IBD in children may involve some distinct pathways as well. Defining the genetic variations that predispose individuals to developing IBD enables researchers to gain insight into the causes of these diseases and potentially develop strategies to help detect, treat, and prevent early-onset IBD in children.

Imielinski M, Baldassano RN, Griffiths A, et al. Common variants at five new loci associated with early-onset inflammatory bowel disease. Nat Genet 41: 1335-1340, 2009.

New Genetic Risk Factors Associated with Ulcerative Colitis: Researchers have identified new genetic risk factors for ulcerative colitis (UC), one of the major forms of IBD. UC causes inflammation and ulcers in the lining of the rectum and colon. Previous genetic analyses have suggested that UC and Crohn's disease—the other major form of IBD—share some but not all of the susceptibility genes that would predispose some individuals to develop these diseases. However, to date, far fewer genetic risk factors have been identified in people with UC as compared to Crohn's disease.

In an effort to identify additional genetic variations underlying UC, an international team of researchers, including investigators from the NIDDK IBD Genetics Consortium, carried out two new GWA studies comparing the genetic variation of individuals with UC to the genomes of healthy individuals. In addition, they analyzed these new data sets in conjunction with data from a previously reported study to increase the sample size and also the likelihood of identifying UC-associated genetic variants. From this combined analysis, the scientists identified at least 30 distinct risk factors for ulcerative colitis, about half of which were previously unknown. In addition, this analysis revealed genetic variants that Crohn's disease and UC share in common; several variants previously known only for their association with Crohn's disease were found to be associated with UC. To help determine the biological implications of the genetic associations, the researchers examined the activity of candidate genes located near five genomic regions containing UC-associated variants. The activity of these genes in different tissues and cell types highlighted the potential importance of

several cellular functions in the pathogenesis of UC, including the integrity of the cells lining the intestine, which normally form a barrier between the intestine's contents and the rest of the body; the response to cellular "stress," such as the presence of improperly shaped proteins within a cell; and the induction and resolution of inflammation. This combined analysis enhances the understanding of disease mechanisms underlying UC, and how they overlap or are distinct from Crohn's disease. However, researchers estimate that these findings explain only a fraction of the genetic risk for UC. Therefore, further research is needed to identify additional susceptibility genes that improve our knowledge of UC and inform future strategies to manage this disease.

McGovern DPB, Gardet A, Törkvist L, et al. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. Nat Genet 42: 332-337, 2010.

Variants in Gene Cluster Associated with Chronic Inflammatory Diseases: An international network of researchers has uncovered a set of genetic variants in a region of the human genome that is associated with chronic inflammatory diseases, such as IBD. This genomic region, called the major histocompatibility complex (MHC), contains a cluster of genes that play an important role in the immune system. A subset of MHC genes—referred to as *HLA* genes in humans—encodes a set of proteins that present foreign molecules to the immune system to generate a response that fights infection. While this response is designed to be protective, the *HLA* genes have been implicated in a number of autoimmune diseases, in which the body mounts an inappropriate immune response against itself. The intrinsic genetic complexity and variability in the MHC region, however, has limited the ability of researchers to identify the precise genetic variants associated with autoimmune disease.

Researchers collaborating through a network spanning the U.S. and Europe have now identified genetic variants within the MHC that are unique to and shared across multiple chronic inflammatory diseases with autoimmune features, including Crohn's disease and ulcerative colitis—the two major forms of IBD—and several other diseases. By scanning the MHC region of thousands of DNA samples from healthy individuals and patients with these diseases, the researchers were able to uncover the most common genetic variants associated with these diseases. For Crohn's disease and ulcerative colitis, the genetic scan identified a version of the *HLA-DRB1* gene as being the primary genetic factor within the MHC region associated with disease susceptibility. In addition, the large number of samples used in this study allowed the researchers to identify additional, secondary genetic variants within the MHC that may also contribute to the risk for these diseases. These results suggest that a complex pattern of genetic variants across the entire MHC region is associated with the risk of developing IBD and other chronic inflammatory diseases of autoimmune origin. In addition, the researchers found a set of genetic variants that is shared among the different chronic inflammatory diseases, which may shed light on a common pathogenic mechanism that causes these diseases.

International MHC and Autoimmunity Genetics Network (IMAGEN). Mapping of multiple susceptibility variants within the MHC region for 7 immune-mediated diseases. Proc Natl Acad Sci USA 106: 18680-18685, 2009.

BACTERIA AND VIRUSES IN THE HUMAN INTESTINE

Bacterial "Census" Reveals that Healthy People Host Distinct Communities of Microbes: Researchers have developed a catalogue of the diversity and variation in bacterial species that reside on or within the body of healthy people. The human body is host to an enormous ecosystem of microorganisms. This microbial community—or microbiota—contains nearly 100 trillion organisms, with the number of bacterial cells on or in the human body outnumbering human cells by almost 10 to 1. The resident bacterial communities provide important functions that aid in metabolism, help prevent infections, and train the immune system. Since these traits are critical for normal health and may, if altered, contribute to disease, it is important to understand the diversity and variation of the human microbiota at different body sites, among individuals, and over time. Although previous studies revealed the diversity of the bacterial communities residing at distinct body sites, an integrated view of the human microbiota across the entire body is needed to fully define the genetic diversity that contributes to normal health.

Using state-of-the-art DNA sequencing methods, researchers have taken a census of the bacterial communities across several body sites of healthy individuals. The researchers collected samples of the bacterial communities from different body sites—including the gut, mouth, ears, nose, hair, and various skin surfaces—of healthy volunteers on several occasions over a 3-month period. After isolating the bacterial DNA from these samples, the scientists analyzed the DNA sequences of a particular gene known to vary among different bacterial species to determine the diversity of bacterial species present at different sites for each individual. They found that the composition of the bacterial communities was determined mostly by their location on or in the body, with the different body sites having distinct community members. These communities were dominated by four groups of related bacteria, with no one particular group found on all of the body sites of any individual on any given day in this study. In addition, the bacterial community composition at some body sites, such as in the gut, varied considerably between different people. However, each individual's "personalized microbiota" appeared to be relatively stable over time. By defining the composition and variation of the microbiota in healthy individuals, researchers now have a baseline for detecting changes in the microbiota that may be associated with human diseases.

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, and Knight R. Bacterial community variation in human body habitats across space and time. Science 326: 1694-1697, 2009.

Exploring the Viruses that Reside in the Human Intestine: Scientists have identified and characterized viruses that live in the human intestines. The Human Microbiome Project (HMP) was initiated by NIH to generate a comprehensive characterization of the microbes that inhabit the human body, as well as their genetic material or genomes—collectively referred to as the microbiome—and to analyze their roles in health and disease. The HMP and other studies of microbes that reside within people have yielded significant information about the content of the human microbiota. These studies have focused primarily on bacteria in the microbiome. Now, scientists have identified the intestinal viral populations in order to explore the role of viruses in relation to the broader microbial community and its human hosts. Most of the viruses identified and characterized in this new study were "bacteriophages," a type of virus that infects bacteria.

The researchers began by isolating viruses from stool samples collected from identical twin sisters and their mothers at three time points over 1 year. A molecular biology technique was used to amplify the small amounts of viruses in the samples. The DNA from the viral populations of each individual's intestine—the "virome"—was then sequenced. The researchers also isolated and sequenced the DNA from bacteria present in the stool samples. By comparing sequences of the viruses and bacteria in the samples with database sequences of known microbes, they were able to identify individual microbial species. In contrast to past research showing that bacterial communities in the intestines of twin pairs and their families were more similar than those of unrelated individuals, analysis of the viral species diversity revealed that the array of viral types was unique to the individual. The diversity of intestinal viruses from each individual was found to be very low and stable throughout 1 year. The most abundant types of bacteria present were those that likely would be infected by the specific forms of viruses identified. The researchers also noted the dominance of types of viruses that infect bacteria, but do not destroy them. Interestingly, this relatively peaceful co-existence differs from the "predator-prey" dynamic of viral-bacterial interactions observed in sludge and other microbial ecosystems that have been studied.

This study attests to the potential benefits of studying intestinal viruses as an important part of research on the human microbiome. Understanding the interactions between intestinal viruses and their bacterial hosts may shed light on the relationships of these microbes with their human hosts. Also, with increased knowledge, the viral component of the intestinal microbial population may serve as a biomarker of microbial response to treatment interventions and progression of disease.

Reyes A, Haynes M, Hanson N, et al. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 466: 334-338, 2010.

Immune Cell Surface Protein Links Gut Bacteria to Diet and Protection Against Inflammation: Microbes residing throughout the human body are now being appreciated for their contributions both

to human health and disease. Scientists have shown that the community of microbes living in the human gut has "co-evolved" with its host to the point that a well-balanced gut microbial community is essential for healthy functioning of the digestive system, as well as the immune system, to prevent conditions such as IBD and other inflammatory or autoimmune conditions. For example, gut microbes perform many functions that humans are incapable of, such as harvesting certain nutrients from the foods we consume. Some bacterial species break down dietary fiber into short-chain fatty acids in the large bowel. These fatty acids have been shown to have a beneficial, anti-inflammatory effect on conditions such as IBD, and are known to act directly on cells in the gut or elsewhere by latching onto components of the cell's surface known as "receptors," including one called GPR43 in humans, or Gpr43 in mice.

In this study, researchers aimed to identify the mechanism by which this type of fatty acids, produced as a result of bacterial fermentation of dietary fiber, have a protective effect against IBD and other inflammatory conditions, such as arthritis and asthma. They showed that the presence of intestinal bacteria reduces disease severity using a mouse model that mimics a form of IBD known as ulcerative colitis. For this experiment, they compared mice raised conventionally with those raised in a bacteria-free environment, before and after their guts were repopulated with bacteria. Based on prior knowledge of microbial effects on IBD via production of short-chain fatty acids, which act through receptors like GPR43, the scientists utilized microarray screening technology to identify immune cells that produce high amounts of GPR43/Gpr43 in humans and mice, respectively. In mice genetically engineered to lack Gpr43 and treated to model ulcerative colitis, immune cells did not respond normally to short-chain fatty acids, and these fatty acids did not reduce intestinal inflammation as in wild-type mice. The results of this experiment indicate that short-chain fatty acids act through Gpr43 on the surface of immune cells to exert their protective effect against intestinal inflammation. Similar results were seen in mouse models of arthritis and allergic airway inflammation.

This study identifies how interactions between by-products of bacterial metabolism and a receptor on the surface of immune cells act to protect against IBD and other inflammatory conditions, such as rheumatoid arthritis and allergic inflammation of the airways. These interactions could provide a target for manipulating immune responses in these conditions through such means as diet and prebiotic/probiotic supplementation.

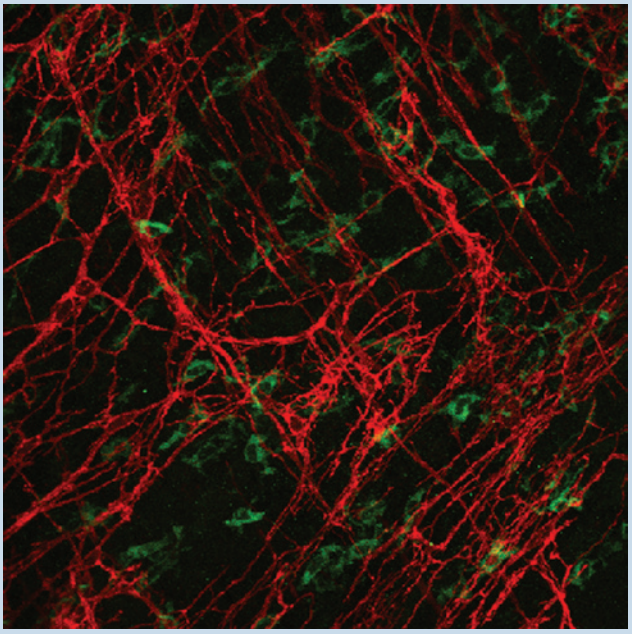
Maslowski KM, Vieira AT, Ng A, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 461:1282-1286, 2009.

Bench to Bedside Journey for Gastroparesis Treatment

Research supported by NIDDK is translating successes made in the laboratory into a potential new therapy for people with gastroparesis. Gastroparesis is a disorder characterized by delayed emptying of stomach ("gastric") contents into the intestines, due to abnormal function of the muscles of the stomach and intestines. It is referred to as a "motility" disorder, because the muscles do not properly move contents along in the gastrointestinal tract. The muscle dysfunction results from damage to nerves and tissues, which is most commonly caused by diabetes. Retention of food in the stomach leads to such unwelcome symptoms as recurrent nausea, vomiting, bloating, weight loss, and pain in the abdomen.

Through basic research supported by NIDDK since 2004, a group of scientists at the Mayo Clinic in Minnesota have studied the mechanisms underlying gastroparesis. A major focus of their research was on interstitial cells of Cajal (ICCs) that are found throughout the gastrointestinal tract and play an important role in controlling motility. These cells function as intestinal "pacemakers," regulating contraction of muscles in the gastrointestinal tract, similar to a pacemaker for the heart. Gastroparesis is associated with a loss of ICC function. The researchers discovered that they could reverse this disorder in a mouse model by administering an agent that protected the ICCs from oxidative stress associated with diabetic gastroparesis.[1] The agent they used, hemin, is a biological product of red blood cells; it boosts the production of an enzyme called heme oxygenase-1 (HO-1), which helps reduce oxidative stress. Hemin has been used in the past to treat an inherited condition called acute intermittent porphyria.

The investigators subsequently conducted a study in humans demonstrating activation of the HO-1 enzyme in response to a pharmacologically relevant and well-tolerated dose of hemin.[2] Now, they are conducting the first randomized controlled clinical trial to test whether hemin therapy can improve gastric emptying and other symptoms in patients with gastroparesis. The researchers hope that this clinical trial, based on knowledge gained from years of careful labwork, may soon yield an effective medication for gastroparesis. A news story featuring the trial and one of its participants was broadcast in August 2010 on an ABC News affiliate in Minnesota.



This image shows the network of cells, called ICCs (interstitial cells of Cajal), from the stomach of a mouse. The ICCs (shown in red) reside in muscle layers of the stomach and exist in close relationship with immune cells called macrophages that produce the enzyme HO-1 (in green). HO-1 protects the nearby ICCs from damage. Reduced levels of HO-1 result in loss of ICCs and of key neuronal factors, leading to the development of gastroparesis. Scientists are testing whether therapy with the biological agent hemin can help people with gastroparesis through increased production of HO-1, thereby allowing repair of the ICC network, re-expression of key neuronal factors, and normalization of gastric emptying. Image provided by Dr. Gianrico Farrugia.

In addition to supporting the ongoing work of this group of investigators, NIDDK sponsors other research on gastroparesis through its Gastroparesis Clinical Research Consortium. The Consortium performs clinical research to develop effective treatments for this disorder. The Consortium's studies include multi-center clinical trials testing existing pharmaceuticals, such as the antidepressant nortriptyline, as potential treatments to improve gastroparesis symptoms. Additional information on the Consortium is available at: *www.jhucct.com/gpcrc/default.asp*

[1] *Choi KM, et al. Gastroenterology 135: 2055-2064, 2008.*

[2] *Bharucha AE, et al. Clin Pharm Ther 87: 187-190, 2010.*

BIOENGINEERING APPROACHES TO TREATING FECAL INCONTINENCE

A Potential Step Toward Treating Fecal Incontinence—Functioning Bioengineered Anal Sphincter Implants in Mice: In research that may have implications for future development of treatment for fecal incontinence, scientists have successfully sustained physiologically functional, implanted bioengineered internal anal sphincters (IAS) in mice. The IAS is a ring-like muscle located at the end of the rectum. An incompetent IAS muscle is a primary cause for the uncontrolled release of stool that occurs in people with fecal incontinence, a condition that places devastating emotional, social, physical, and economic burdens on people who are affected by it.

In a recent study, scientists used smooth muscle cells obtained from mouse IAS to bioengineer three-dimensional IAS rings. The cells were grown on special plates containing a mold around which the cells could create the three-dimensional ring structure. Once an IAS was formed, it was implanted into a small pocket made surgically under the skin of a recipient mouse. The key challenge for the scientists in this study was to promote the development of a vascular network that would supply blood to the implant, ensuring its survival over an extended period of time. In an effort to stimulate the growth of new blood vessels, the researchers placed a tiny pump with a catheter alongside the nascent IAS in the surgical pocket. The pump was able to provide a constant flow of a growth factor called FGF-2 to the new implant and surrounding tissue. Examination of the implants and surrounding tissue 25 days after surgery revealed that the scientists' approach was successful. The IAS implants that continually received growth factor thrived. New blood vessels emerged in the surrounding tissue, the bundles of muscle cells forming the IAS structure appeared healthy, and there was no evidence of rejection of the implants, which had been constructed using donor mice cells.

In a subsequent study, the scientists compared the physiological functions of bioengineered IAS before and after they were implanted in mice for 4 weeks. IAS were first tested for spontaneous basal tension (the state which prevents uncontrolled release of stool) by attaching one end of the IAS to a stainless steel pin fixed to the center of a culture plate containing a physiological solution and attaching the other end, without stretch, to a movable tension measuring arm. To test functionality, the IAS was bathed in solutions to stimulate or relax the IAS muscle, and the force exerted on the tension arm was measured. By comparing the tensions for pre- and post-implantation IAS, the researchers demonstrated that the implanted IAS had retained physiological integrity. The bioengineered muscles were able to contract and relax in a manner that mimicked the natural *in vivo* function of the IAS.

This study's successful implantation in mice of bioengineered IAS that retained physiological functionality presents an opportunity for new studies that one day may be translated into bioengineered IAS for people suffering from incontinence. This would be an enormous benefit for these people, greatly improving their daily lives and alleviating the social and financial burdens associated with this disorder.

Hashish M, Raghavan S, Somara S, et al. Surgical implantation of a bioengineered internal anal sphincter. J Pediatr Surg 45: 52-58, 2010.

Raghavan S, Miyasaka EA, Hashish M, et al. Successful implantation of physiologically functional bioengineered mouse internal anal sphincter. Am J Physiol Gastrointest Liver Physiol 299: G430-G439, 2010.

CELIAC DISEASE RESEARCH

New Genetic Variants Associated with Celiac Disease Are Identified: Scientists have uncovered new genetic variants that are associated with the risk of celiac disease and have linked these variants to four pathways of the immune system. Celiac disease is a complex genetic disease that can cause damage to the intestine, resulting in poor absorption of nutrients, painful digestive and other symptoms, and other serious complications. These symptoms occur when people with the disease eat grains containing gluten—such as from wheat, rye, and barley—which provokes an abnormal immune response that attacks their intestine. For children, celiac disease can have devastating consequences, such as impaired growth and development, while adults may experience anemia, bone loss, and other complications.

In an earlier study, scientists conducted a genome-wide association (GWA) study to identify two gene variants that are required for celiac disease, and 12 chromosome regions that are associated with a risk for the disease. Although these findings were impressive, it was determined that all of the known variants did not account entirely for the genetic risk of celiac disease. In the new study, scientists set out to identify variants that may have smaller, yet critical, effects on disease risk. This was accomplished with a larger GWA study that included DNA samples from a larger number of patients with celiac disease and healthy volunteers. The samples were analyzed using a denser concentration of probes to identify differences in the DNA sequences of the patients compared with those of the volunteers. This approach was successful in uncovering 13 new chromosome regions that are associated with celiac disease, and 13 additional chromosome regions with suggestive associations with celiac disease. Many of these regions were found to contain genes with functions related to the immune system. In addition, uncovering the genetic variants led the scientists to identify four specific immunological pathways that are relevant to the pathogenesis of celiac disease. The scientists also found that more than half of the variants associated with celiac disease correlate with the extent to which nearby genes are turned on or turned off (expressed), indicating that the variants may increase the risk of celiac disease by influencing the expression of other genes. These new findings have advanced knowledge of celiac disease and may also have important implications for other autoimmune diseases, such as type 1 diabetes.

Dubois PC, Trynka G, Franke L, et al. Multiple common variants for celiac disease influencing immune gene expression. Nat Genet 42: 295-302, 2010.

Mixed Effects of Undiagnosed Celiac Disease in Older Adults: Scientists studying the consequences of undiagnosed celiac disease in a population of American men and women 50 years of age and older found that undiagnosed celiac disease did not increase the risk of death over the 10-year period of the study, although other health consequences were observed. Celiac disease is an autoimmune disease caused by intolerance of the gluten proteins found in many grains. Although there is no cure for celiac disease, it can be treated effectively with a gluten-free diet. Previous research findings from different studies have differed with respect to whether undiagnosed celiac disease increases rates of premature death. Now scientists have surveyed a group of older men and women in the general population over a 10-year time period to determine how people who are 50 years of age or older may be affected by undiagnosed celiac disease.

For this study, scientists screened frozen blood samples from almost 17,000 people living in Olmsted County, Minnesota, using assays for particular antibodies that are characteristic of celiac disease. People who had not been diagnosed with clinical celiac disease but whose blood samples tested positive for the disease with two different antibody assays were classified as having undiagnosed celiac disease. Blood samples that tested negative were used for the study's control group. Analysis of the data from this screening determined that approximately 0.8 percent of the people whose blood samples were screened had undiagnosed celiac disease. The medical records of the undiagnosed celiac group and the control group were then reviewed for more than 100 potential medical conditions, or cases of death, over the 10-year period after the blood samples had been collected. The records showed that, among the people whose blood samples had tested positively, approximately 15 percent subsequently received a clinical diagnosis of celiac disease. In contrast, no individuals in the control group were diagnosed with celiac disease. This study did not find an increase in mortality among people with undiagnosed celiac disease, although other health risks and potential benefits were observed in this group. The undiagnosed celiac group had increased risk of osteoporosis and hypothyroidism, but they also had lower BMIs (body mass index) and cholesterol levels.

The aim of this study was to determine the effects of undiagnosed celiac disease on older men and women. However, the mixed study results do not clarify whether awareness of undiagnosed celiac disease in cases where there are no clinical symptoms provides a net benefit to the individual. It thus remains unclear whether screening of the general public for celiac disease is warranted.

Godfrey JD, Brantner TL, Brinjikji W, et al. Morbidity and mortality among older individuals with undiagnosed celiac disease. Gastroenterology 139:763-769, 2010.

PREDICTORS, GENETICS, AND TREATMENT OF NONALCOHOLIC FATTY LIVER DISEASE

Identifying Noninvasive Predictors of Nonalcoholic Fatty Liver Disease: Using a database of demographic, clinical, and laboratory information on patients with nonalcoholic fatty liver disease (NAFLD), scientists have evaluated the utility of these noninvasive variables to diagnose and predict severity of nonalcoholic steatohepatitis (NASH)—a more serious form of NAFLD that is accompanied by liver injury, inflammation, and fibrosis. Currently, a liver biopsy, where a physician removes a sample of liver tissue to examine under a microscope, is the only definitive way to diagnose NASH and to identify disease severity. However, a liver biopsy is an invasive procedure that has challenges with patient acceptance, costs, and sampling variability, as well as risks associated with it being an invasive test. It would, therefore, be beneficial to develop noninvasive methods for diagnosing NASH using routinely obtained clinical and laboratory data.

The NIDDK-supported NASH Clinical Research Network has collected clinical and laboratory information on 1,266 adults with diagnosed or suspected NAFLD who have participated in the Network's clinical studies. As part of these studies, most of the participants also received liver biopsies. Of the study participants who received a liver biopsy, 57 percent were diagnosed with having "definite" NASH. By analyzing the demographic, clinical, and laboratory data for these individuals, the scientists found that patients with NASH were more likely to be women, have diabetes, and show signs of metabolic syndrome. There was not a significant difference in age, measures of obesity, and ethnicity for those with biopsy-diagnosed NASH compared to those without. In addition, measures of liver enzyme levels, which are common laboratory tests for assessing liver function and potential liver damage, did not appear to be useful screening tools for diagnosing NASH in patients with NAFLD. However, liver enzyme levels were reliable measures for predicting the most advanced stages of liver injury resulting from NASH. To develop a tool for diagnosing the presence and severity of NASH, the researchers developed a predictive model based on the demographic, clinical, and laboratory information available. As more information was included in the model, the researchers were better able to predict advanced stages of liver injury in adults with NAFLD.

Researchers in the NASH Clinical Research Network will continue to follow these patients to better understand the causes and natural history of NAFLD. By identifying additional clinical or demographic "markers" associated with disease, researchers may be able to develop more robust, noninvasive measures for predicting the presence and severity of NASH.

Neuschwander-Tetri BA, Clark JM, Bass NM, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology 52: 913-924, 2010.

Metabolic Syndrome Is a Potential Predictor of Liver Damage in Children: A new study indicates that criteria for metabolic syndrome may identify children who are at greatest risk for severe liver damage caused by NAFLD. NAFLD is caused by the accumulation of fat in the liver of people who drink very little or no alcohol. It is the most common cause of pediatric chronic liver disease in North America. In adults, NAFLD has been associated with metabolic syndrome, a constellation of symptoms that places people at risk for cardiovascular disease and type 2 diabetes. Recent studies suggest that this association may also place children at risk. In response to these findings, scientists conducted a study to analyze the metabolic syndrome-NAFLD relationship in children and adolescents.

Overweight and obesity are the primary risk factors for NAFLD and are part of metabolic syndrome. Other components of metabolic syndrome include high blood pressure, low HDL (good) cholesterol, high triglycerides, and insulin resistance (an indicator of type 2 diabetes or diabetes risk). The children and adolescents who participated in the current study were all enrolled in the NASH Clinical Research Network, a multi-site clinical network established by NIDDK to assess the causes, natural history, and therapy of NAFLD. The scientists conducting this study used liver biopsies to assess liver damage and clinical tests to measure the components of metabolic syndrome. Children in the study were diagnosed with metabolic syndrome if they had three of the five criteria mentioned above. Everyone who participated in the study had NAFLD, and 25 percent were found to have metabolic syndrome as well. Deeper analyses of the comparisons of the frequency and severity

of symptoms of NAFLD and metabolic syndrome revealed important correlations between them. Scientists used several criteria for diagnosing the severity of liver disease, including the amount and pattern of liver fat that was present, ballooning or enlargement of liver cells, and the degree of liver tissue scarring. Analyses of these conditions demonstrated that all of these criteria were significantly associated with metabolic syndrome. Central obesity (large waist circumference) and insulin resistance, however, were the features that were most consistently associated with the severity of liver damage caused by NAFLD.

This study uncovered important relationships between metabolic syndrome and liver damage that results from pediatric NAFLD. If future clinical studies confirm this correlation, these relatively noninvasive methods—compared to liver biopsy—may be used to evaluate the risk and progression of liver disease in children and adolescents with NAFLD.

Patton HM, Yates K, Unalp-Arida A, et.al. Association between metabolic syndrome and liver histology among children with nonalcoholic fatty liver disease. Am J Gastroenterol 105:2093-2102, 2010.

Genetic Variants Associated with Nonalcoholic Fatty Liver Disease: Two large studies using data from the NASH Clinical Research Network and other sources have found associations between genetic variants and NAFLD diagnosed by liver biopsy. In recent years, NIDDK-supported investigators have conducted genome-wide association studies to identify genetic factors that could predispose some individuals to develop NAFLD. For example, researchers uncovered a variant in a gene called patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), which codes for a type of enzyme involved in lipid metabolism. A variant of *PNPLA3* is known to be associated with elevated liver enzymes and with excess fat in the liver, which serve as noninvasive markers of NAFLD. However, the gold standard test for diagnosing NAFLD remains the liver biopsy, in which key structural changes characteristic of the disease can be observed under the microscope. Two research groups recently conducted studies that tested whether these genetic variants were associated with NAFLD, when it was diagnosed using the more definitive test of a liver biopsy.

In one study, scientists used genomic data from adults and children participating in the NASH Clinical Research Network, as well as patients with NASH who participated in clinical studies at the NIH Clinical Center. The majority of the study participants were Caucasian. The researchers determined whether these individuals carried any of the six genetic variants previously associated with elevated liver enzymes and excess liver fat. Of these variants, the *PNPLA3* variant was most strongly associated with several features of NAFLD observed in the liver biopsy. In children with NAFLD, the *PNPLA3* variant was associated with earlier disease development. The study also associated the *PNPLA3* variant and other genetic variants with more severe forms of NAFLD in adults.

In a separate study, another group of researchers used genomic data and samples collected by the Network and other consortia to test whether genetic variants were associated with biopsy-confirmed NAFLD, as well as features of metabolic syndrome. They chose to focus on seven genetic variants that had been associated with NAFLD in previous studies using liver imaging of excess liver fat and liver function tests. For data on individuals with NASH, they used samples from the NASH Clinical Research Network, which they compared to samples from ancestry-matched controls studied through the Myocardial Infarction Genetics Consortium, which is supported by the National Heart, Lung, and Blood Institute. The study also focused on individuals of European ancestry who participated in these consortia, in order to reduce genetic variability in the study population. By analyzing genomic data from these sources and associating it with cases of NAFLD confirmed by microscopic evaluation of liver biopsies, the researchers found that out of the seven genetic variants analyzed, only the *PNPLA3* variant was strongly associated with the disease and its severity. Additionally, using data from consortia conducting genome-wide association studies related to elevated blood lipids, type 2 diabetes, and obesity, the group showed that this gene variant was specifically associated with NAFLD, but not with aspects of metabolic syndrome.

These studies demonstrate a strong association between gene variants such as *PNPLA3* and biopsy-confirmed NAFLD. Genetic analyses such as these shed light on the multiple metabolic pathways involved in this form of liver disease, which may lead to the development of

therapies targeting these pathways. Additional studies of NAFLD genetics are needed that involve other racial/ethnic groups to identify genetic factors that could contribute to differences observed in NAFLD prevalence.

*Rotman Y, Koh C, Zmuda JM, Kleiner DE, Liang TJ, and the NASH CRN. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) with histological severity of nonalcoholic fatty liver disease. Hepatology 52: 894-903, 2010.*

Speliotes EK, Butler JL, Palmer CD, et al. PNPLA3 *variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. Hepatology 52: 904-912, 2010.*

Identification of a Major Genetic Risk Factor for Nonalcoholic Fatty Liver Disease: Researchers have identified genetic variants that impart a high risk of NAFLD, a common condition that is associated with risk for more serious liver disease, as well as type 2 diabetes, cardiovascular disease, and liver cancer. NAFLD—essentially the abnormal accumulation of fatty deposits in the liver in the absence of chronically high alcohol intake—is most prevalent in obese individuals, and is therefore linked to excessive consumption of calories. However, many people with obesity do not have NAFLD, while some people of normal weight do. Most people with NAFLD experience no symptoms early in the course of the disease. The fat deposits observed in NAFLD are comprised primarily of a group of molecules called triglycerides. In the blood, triglycerides are typically associated with a class of proteins called apolipoprotein C. Previous research had shown that two variants in the *APOC3* gene, which encodes one of these proteins, were associated with elevated blood levels of triglycerides.

To test whether these *APOC3* variants affect the likelihood of NAFLD, researchers studied a group of 95 Asian Indian men who were sedentary, but neither obese nor alcoholic, and who had not been previously diagnosed with disease. The researchers found that 38 percent of the men who had at least one of the two high triglyceride *APOC3* variants also had NAFLD, while none of the men who lacked both variants had the disease. This was a strong indication that *APOC3* has a significant impact on risk of NAFLD in Asian Indian men. To further assess the impact of *APOC3* on risk for NAFLD, the researchers then examined a group of 163 apparently healthy men from other ethnic groups. They found that 9 percent of those with the high risk variants actually had the disease, but again, found no NAFLD in the men without them. The researchers also observed insulin resistance—which is known frequently to lead to type 2 diabetes—in the men who had NAFLD, but not in the men without the disease, whether or not they had the high risk *APOC3* variants. Encouragingly, when the researchers provided a 3- to 6-month dietary intervention to seven of the Asian Indian men who had both NAFLD and insulin resistance, they found that liver fat content fell and insulin resistance abated—suggesting that a healthful lifestyle can help prevent NAFLD and insulin resistance even in people with high risk forms of *APOC3*. These results shed light on the genetic basis for NAFLD, while providing hope that this common liver disease may be preventable even in those at high genetic risk.

Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med 362: 1082-1089, 2010.

Largest Trial in Adults with Nonalcoholic Steatohepatitis Shows Benefits of Treatments: NASH is a serious and increasingly common form of NAFLD in the U.S. Its features include excess fat and inflammation in the liver similar to alcoholic liver disease, but they occur in people who drink little to no alcohol. Though its precise causes are unknown, it is typically associated with obesity, type 2 diabetes, hypertension, elevated lipids in the blood, or other features of metabolic syndrome, which affects a growing number of adults and children in the U.S. However, NASH can also affect people who are of normal weight and do not have diabetes or other signs of metabolic syndrome. The disease often goes undetected for years, until an abnormality in liver function unrelated to other common causes of liver disease is noticed incidentally through a measure such as elevated liver enzymes in the blood. Long-term NASH may develop into severe cirrhosis, liver cancer, and/or liver failure requiring a transplant. Currently, there are no specific, Food and Drug Administration-approved treatments available for NASH.

To test potential treatments for NASH in adults, NIDDK's NASH Clinical Research Network conducted a clinical trial called the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Nonalcoholic Steatohepatitis trial, or "PIVENS." This multi-center clinical trial tested whether treatment with pioglitazone, an insulin-sensitizing drug used for diabetes, or the antioxidant vitamin E could improve NASH in non-diabetic adults, in comparison to placebo. Non-diabetic adults were studied in order to test the insulin-sensitizing drug in a population that had not already been treated with this type of drug. Participants were treated with a once-daily dose of vitamin E, pioglitazone, or placebo for 2 years, followed by 6 months of evaluation after treatment. Liver biopsies and other clinical samples were taken to assess whether features of NASH, such as fibrosis, inflammation, and fat in the liver, improved during the course of treatment. Although only vitamin E significantly improved an overall measure of NASH, both vitamin E and pioglitazone improved some of the features of this disease, including a reduction in liver enzyme levels, as well as fat and inflammation in the liver. However, many participants taking pioglitazone also experienced the unhelpful side effect of weight gain.

The PIVENS trial is the largest randomized controlled clinical trial to date in patients with NASH. The results from this trial represent an important milestone in the search for effective treatments for this common form of liver disease. While these results are promising, especially for vitamin E, patients should consult a physician before using such high-dose vitamin E or pioglitazone long term for the treatment of NASH. Future research will be needed to determine whether the benefits of vitamin E and pioglitazone extend to adults with both diabetes and NASH, or will continue with minimal risks during long-term treatment. Some trial participants have chosen to continue participating in the Network's prospective, longitudinal follow-up study, so that they can continue to contribute to our knowledge of this disease and its long-term management.

Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. New Engl J Med 362: 1675-1685, 2010.

For additional information on the PIVENS trial and other NASH research efforts, please see the Patient Profile and Story of Discovery in this chapter.

ADVANCES ON RARE FORMS OF GENETIC LIVER DISEASES

A Promising Treatment for Alpha-1 Antitrypsin Deficiency: Researchers have used modified cell lines and mouse models mimicking the conditions of alpha-1 antitrypsin deficiency to show dramatic benefits of a drug already approved for other indications, which works by activating a cellular process called "autophagy." Alpha-1 antitrypsin deficiency is the most common form of genetic liver disease in children, and a cause of cirrhosis and liver cancer in adults as well. This disease is the result of a mutation in the alpha-1 antitrypsin (alpha-1 AT) protein, which is produced in the liver and released into the blood to exert a protective effect on the lungs. This mutation causes the alpha-1 AT protein to have an aberrant, misfolded shape and to be retained inside the liver cells, where it accumulates and damages the liver. Basic research has shown that liver cells have some limited defenses against the mutated protein in the form of pathways that degrade unnecessary or abnormal proteins; one of these is the autophagy pathway. Based on this knowledge of mechanisms by which the mutated alpha-1 AT protein is degraded by processes such as autophagy, researchers set out to test whether a drug that boosts autophagy could effectively treat the liver disease associated with alpha-1 antitrypsin deficiency.

Starting from a list of drugs that were recently shown to enhance autophagy of proteins that, like mutated alpha-1 AT, accumulate in cells, researchers selected the drug carbamazepine based on its extensive safety profile in humans. Carbamazepine is currently prescribed as an anticonvulsant and mood stabilizer. The researchers first tested carbamazepine in a human cell line that was genetically altered in the laboratory to produce a mutated form of the alpha-1 AT protein. The drug markedly reduced the amount of accumulated protein in the cells by enhancing its degradation. Further testing showed that the drug acted by boosting autophagy beyond the cell's usual response to accumulated protein. Additional testing in mouse cell lines that were modified to inactivate autophagy or proteasomal degradation pathways indicated that both pathways are used to some extent by carbamazepine to enhance disposal of the mutant alpha-1 AT protein. The researchers then turned to a mouse model that mimics liver disease associated with alpha-1 antitrypsin

deficiency. Mice had been genetically altered to produce the human mutated alpha-1 AT protein, as well as produce a green fluorescent protein in cells where autophagy was occurring to allow easy detection of signs of this cellular process under the microscope. Two weeks of treatment with carbamazepine in this mouse model decreased levels of the mutated protein in the liver, where signs of ramped-up autophagy were found. In these mice, the treatment also reduced liver fibrosis, a primary feature of liver disease associated with alpha-1 antitrypsin deficiency.

This study demonstrates the power of combining basic research on cellular processes underlying disease with knowledge of existing therapeutics that target these processes in order to identify promising treatments that may work for multiple diseases. These pre-clinical studies in mice show the potential of the autophagy-enhancing drug carbamazepine as a treatment for liver disease associated with the genetic liver disease alpha-1 antitrypsin deficiency. However, future clinical studies will be needed to test the benefits of this treatment in pediatric and adult patients with the disease.

Hidvegi T, Ewing M, Hale P, et al. An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. Science 329: 229-232, 2010.

Viral-Mediated Gene Repair Corrects Hereditary Tyrosinemia Mutation in Mouse Liver: Scientists have successfully repaired a mutation in the gene linked to the metabolic disease hereditary tyrosinemia type 1 (HT1) in mice using a specially engineered virus to transport the normal gene sequences, thereby preventing and ameliorating the associated liver damage. HT1 is a rare and fatal metabolic disease caused by genetic mutation leading to deficient activity of the enzyme fumarylacetoacetase. This enzyme catalyzes the last step in the cellular pathway that breaks down the amino acid tyrosine, which is a component of proteins and can be obtained from food. In humans with insufficient activity of this enzyme, metabolic compounds in the pathway build up in liver and kidney cells to toxic levels, causing cell death. Liver damage in patients with HT1 can range from acute liver failure during the first months of life to a progressive chronic liver disease after the first years of life. Therapeutic options for this condition are limited to dietary restriction of tyrosine, liver transplantation, or treatment with an agent called NTBC, which interrupts a different part of the tyrosine metabolic pathway in a way that prevents the toxic compounds from accumulating. However, none of these approaches are optimal or are capable of addressing the underlying enzyme deficiency before severe damage to the liver and other organs occurs. Developing a strategy to repair the underlying genetic defect in patients with HT1 has the potential to directly treat or even prevent this disease.

To test their treatment strategy, the research team used a mouse model with a mutation in the gene coding for the fumarylacetoacetase enzyme, which develops a disease similar to human HT1, but in an accelerated manner. They also designed a tool for gene repair consisting of an adeno-associated virus fused to a segment of the normal gene that encodes the enzyme. Adeno-associated viruses had previously been studied in animal models and humans as potential vehicles for delivering gene therapy. The normal gene sequences carried by the virus can be used by a cell to replace the mutation in its native, mutant gene, thus enabling production of the normal enzyme and treatment of HT1. Neonatal and adult mice with the mutated enzyme were given NTBC to protect against fatal liver injury and injected either with their virus-based gene repair kit or with saline. The scientists then stopped administering NTBC and observed signs that the gene for the defective enzyme had been successfully repaired in mutant mice given the virus carrying the normal gene. For example, the liver cells of the mice began to produce the corrected enzyme and even show a growth advantage as they repopulated the liver with healthy cells. Liver function tests demonstrated that the underlying liver disease was almost completely corrected by gene repair in the mutant mice.

This study provides a proof of principle in an animal model for the use of gene repair to correct an inherited metabolic liver disease. With additional research, targeted gene repair using the adeno-associated virus may prove to be an effective treatment for HT1 and other similar diseases resulting from mutation of a single gene.

Paulk NK, Wursthorn K, Wang Z, Finegold MJ, Kay MA, and Grompe M. Adeno-associated virus gene repair corrects a mouse model of hereditary tyrosinemia in vivo. *Hepatology 51: 1200-1208, 2010.*

TREATMENT FOR ACUTE LIVER FAILURE

Successful Treatment for Early-Stage Acute Liver Failure Not Caused by Acetaminophen: Results of a recent clinical trial testing a new treatment for patients in the early stages of acute liver failure (ALF) due to causes other than acetaminophen overdose showed improved outcomes with the treatment. ALF is a rare but devastating condition for which the only therapy currently available is liver transplantation. The majority of ALF cases in the U.S. are caused by toxicity from an overdose of the over-the-counter pain reliever acetaminophen. Fortunately, cases of acetaminophen-related ALF can be successfully treated if caught in the early stages, with an agent called N-acetylcysteine (NAC), which neutralizes a toxic product of acetaminophen metabolism. Researchers speculated that this antidote might have beneficial properties that could prove useful in treating cases of ALF resulting from other causes, including other forms of drug-related injury, autoimmune hepatitis, and hepatitis B.

The NIDDK's Acute Liver Failure Study Group conducted a clinical trial across 24 U.S. sites to test whether NAC treatment could improve survival and reduce the need for liver transplantation in patients with ALF from causes other than acetaminophen toxicity. After patients were given an intravenous infusion of NAC or placebo for 72 hours, survival and transplantation rates were assessed 3 weeks and 1 year later. Results were compared across groups of patients based on their stage of disease prior to treatment. Although no significant differences in overall survival emerged, patients with less advanced disease who were given NAC showed improved survival without the need for a transplant, compared to those given placebo. NAC was also well-tolerated, with uncommon and minor side effects.

Based on the results of this trial, NAC shows promise as a safe and effective treatment for early-stage, non-acetaminophen-related ALF, a condition for which no other therapeutic option currently exists beyond liver transplantation. Future studies may explore optimal dosing and duration of this treatment, as well as predictors of patient response and the physiologic basis of this response, in order to achieve the greatest benefit from this treatment for ALF resulting from causes other than acetaminophen toxicity.

Lee WM, Hynan LS, Rossaro L, et al. Intravenous N-acetylcysteine improves transplant-free survival in early stage non-acetaminophen acute liver failure. Gastroenterology 137: 856-864, 2009.

COMPLICATIONS OF ANTIVIRAL THERAPY IN CHILDREN WITH HEPATITIS C

Infrequent Eye-Related Complications from Treatment of Chronic Hepatitis C in Children: Researchers have determined that eye-related (ophthalmologic) complications resulting from standard antiviral therapy for chronic hepatitis C are uncommon in children, contrasting with the higher adult rate of these complications.

The standard therapy for chronic hepatitis C—a combination of antiviral drugs known as peginterferon and ribavirin—has been shown in adults to be effective at suppressing viral infection. However, peginterferon has also been associated with adverse effects, including eye-related complications, such as retinopathy. Much less had been known about how children with chronic hepatitis C respond to antiviral therapy in terms of outcomes and complications. The Peginterferon and Ribavirin for Pediatric Patients with Chronic Hepatitis C (Peds-C) Study was a prospective, randomized controlled clinical trial to assess outcomes of peginterferon therapy, given with either ribavirin or placebo, in children with chronic hepatitis C. While the main trial was completed in 2008, a long-term follow-up study of children participating in the trial is currently underway.

Recently, scientists in the Peds-C Study Group collected information on eye-related complications in children with chronic hepatitis C who were treated with peginterferon, with or without ribavirin. The children underwent periodic eye exams before and after treatment. The researchers found that the prevalence of eye-related complications, such as retinopathy, is low in these children, particularly in comparison with the higher rates observed in adults treated for chronic hepatitis C. These results provide information that is useful to health care providers caring for children with chronic viral hepatitis C who are treated with antiviral therapy. While eye-related complications appear to be relatively uncommon for

these children, the severity of these complications when they do occur requires that they continue to be monitored for their occurrence.

Narkewicz MR, Rosenthal P, Schwarz KB, et al. Ophthalmologic complications in children with chronic hepatitis C treated with pegylated interferon. J Pediatr Gasteroenterol Nutr 51: 183-186, 2010.

GENETICS OF PRIMARY BILIARY CIRRHOSIS

New Genetic Risk Factors Discovered for Primary Biliary Cirrhosis: Researchers have identified new genetic variants and confirmed previously identified genetic risk factors that are associated with primary biliary cirrhosis (PBC). PBC is a chronic autoimmune disease characterized by inflammation and damage to the bile ducts, which may ultimately lead to liver damage, cirrhosis, and end-stage liver disease. This disease is believed to be triggered by an autoimmune response in which the body's immune system inadvertently attacks and destroys specific cells lining the bile ducts. As is the case with many other autoimmune diseases, PBC has a strong genetic component. Researchers previously identified genetic variants in three specific regions of the genome that are more frequently found in people with PBC than in healthy individuals. These regions contain genes that are involved in mediating inflammatory responses.

In a new study, researchers have confirmed these genetic associations in a new population of individuals with PBC. In addition, by combining their results with those of the original genome-wide association study, the researchers were able to identify genetic risk factors in three additional regions of the genome that are associated with PBC. One region—the *IRF5-TNPO3* locus—is of particular interest because of its integral role in regulating immune responses. After determining the DNA sequence of this region in patients and healthy individuals, the researchers identified two variants that account for nearly all of the association with PBC in this genomic region. These variants have been associated with other autoimmune diseases and are known to affect the activity of a gene involved in mediating immune responses. This study also identified two other gene regions with variants that increase risk for PBC and have been associated with other autoimmune diseases, including type 1 diabetes, Crohn's disease, celiac disease, and rheumatoid arthritis.

By defining the genetic variants associated with disease, researchers may be able to gain insight into the molecular factors that trigger the onset and progression of PBC. These insights could possibly be extended to other diseases as well, since there appears to be considerable genetic overlap between the risk for PBC and other chronic autoimmune diseases.

Hirschfield GM, Liu X, Han Y, et al. Variants at IRF5-TNPO3, *17q12-21, and* MMEL1 *are associated with primary biliary cirrhosis. Nat Genet 42: 655-657, 2010.*

STORY OF DISCOVERY

Rise of a "Hitherto Unnamed" Liver Disease—Nonalcoholic Steatohepatitis

In recent decades marked by an obesity epidemic in the U.S., researchers have witnessed the rise of a form of liver disease that now appears to affect more American adults, as well as a growing number of the nation's children, than any other—nonalcoholic fatty liver disease. This form of liver disease includes a more severe condition known as nonalcoholic steatohepatitis. Researchers are greatly advancing progress in understanding this disease and in developing potential treatment strategies.

In the 1950s, studies of obese veterans first documented a new form of liver disease characterized by excessive fat in the liver that occurred in individuals who did not report heavy alcohol consumption or have any other known causes of liver injury. However, cases such as these were largely dismissed as related to presumed hidden alcohol abuse. A report in 1980 described several obese patients with diabetes who, though they also did not abuse alcohol, had fat accumulation in the liver accompanied by liver inflammation—indicators that, at the time, were associated only with alcoholic hepatitis. By this point, clinical investigators had begun to realize that people with this condition really were not abusing alcohol. The investigators thus coined the term "nonalcoholic steatohepatitis" (or NASH) to describe what they referred to as a "hitherto unnamed" form of liver disease.

Understanding Disease Processes and Development

The NIDDK sponsored early research on this newly recognized form of liver disease to identify the biologic processes involved and chart the course of disease development and progression. In the 1990s, studies of liver biopsies from obese patients with or without diabetes identified some of the key morphologic changes that take place in NASH. They also charted disease progression in these patients, some of whom developed scarring, or fibrosis, which can progress to cirrhosis. Furthermore, these studies pointed to a link between NASH and insulin resistance, a condition that is also associated with type 2 diabetes. Additional studies in the early 2000s confirmed the link between NASH and insulin resistance, as well as other metabolic abnormalities, such as increased fatty acid oxidation and oxidative stress in the liver. NASH is now thought to be part of a spectrum of nonalcoholic fatty liver disease (NAFLD), which includes simple steatosis (excess fat in the liver) that, with time in some cases, can develop inflammation and other cellular changes characteristic of NASH.

Determining Prevalence and Risk Factors

In 2003, NIDDK supported one of the first population studies to estimate the prevalence of NAFLD, including NASH, in the U.S. using data from the Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey. In this study, major risk factors associated with these diseases in the U.S. included overweight with central distribution of fat (in the abdomen), as well as elevated insulin levels indicative of insulin resistance. However, researchers were also learning that, while NAFLD is often associated with obesity, there are cases of normal weight individuals with the disease. This research and subsequent studies supported by the Institute have shown that the prevalence of NAFLD/NASH varies widely among different ethnic and gender groups, with Hispanics and some Asian sub-groups, such as Asian Indian men, having a higher prevalence, African Americans having a lower prevalence, and Caucasians in between. In the mid-2000s, research by NIDDK grantees found that NAFLD was also found in a large number of American children and adolescents, with similar racial/ethnic differences observed.

STORY OF DISCOVERY

Finding Treatments

Building on the earlier research identifying risk factors that contribute to NASH/NAFLD, such as insulin resistance and oxidative stress, NIDDK-sponsored investigators conducted some of the first clinical research to identify potential therapies for this disease. In the 2000s, NIDDK intramural and extramural scientists conducted pilot studies of therapies for NASH, including the insulin-sensitizing drugs metformin and pioglitazone, as well as the antioxidant vitamin E, which improved NASH after short-term treatment. Progress in developing animal models to define disease mechanisms and test new treatments for NASH/NAFLD included studies of a mouse model deficient in serotonin, which provided evidence that this chemical may serve as a future target for NASH therapy.

In 2002, NIDDK created the NASH Clinical Research Network to assemble a large, well-characterized study population for clinical research on the causes, natural history, complications, diagnosis, and therapy of NAFLD, particularly NASH, in both children and adults. Collaborators supporting the Network have included the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, the National Institute on Minority Health and Health Disparities, and the pharmaceutical industry, which provided some of the funding and medications used in clinical trials. Building on the earlier results from pilot studies, in the late 2000s, the Network conducted the largest randomized controlled clinical trial to date of adult NASH therapy, called the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Nonalcoholic Steatohepatitis trial, or "PIVENS." PIVENS showed promising improvements in aspects of adult NASH in response to 2-year therapy with the natural form of the antioxidant vitamin E and the insulin-sensitizing drug pioglitazone. (For additional information on the PIVENS trial, please see a related research advance and Patient Profile in this chapter.) A similar clinical trial in children conducted by the Network, called the Treatment of Nonalcoholic Fatty Liver Disease in Children trial, or "TONIC," tested whether 2-year treatment with the natural form of vitamin E, the insulin-sensitizing drug metformin, or placebo improved pediatric NAFLD. In an early announcement of trial results presented at the 2010 meeting of the American Association for the Study of Liver Diseases, although neither vitamin E nor metformin treatment normalized liver enzymes in children with NAFLD compared to placebo, vitamin E treatment did improve some important features of NAFLD and may reverse NASH in children.

The NASH Clinical Research Network is planning additional studies in adults and children, including a trial to evaluate whether treatment with a drug called obeticholic acid, which is derived from a human bile acid, is a safe and effective treatment for adult NASH.

The Network is also conducting an ongoing, prospective follow-up study of participants in the PIVENS and TONIC trials to collect additional information on NASH/NAFLD in both adults and children. Data and samples collected by the Network are available for ancillary studies by the wider research community.

Identifying Genetic Factors

In recent years, NIDDK-sponsored investigators have identified genetic factors that could predispose some individuals to developing NAFLD. In 2008, a study scanned the genomes of participants in a large population-based study to uncover a variant in a gene called *PNPLA3* that was strongly associated with NAFLD and was more common among Hispanic individuals with higher liver fat and inflammation. In 2010, studies of genomic data from participants in the NASH Clinical Research Network, as well as patients with NASH studied at the NIH Clinical Center, found that the *PNPLA3* variant was also associated with earlier disease development in children. These and other recent studies have identified additional regions of the genome associated with NAFLD, including a gene known as *APOC3*.

STORY OF DISCOVERY

More to Discover

In the future, NIDDK anticipates new discoveries from the NASH Clinical Research Network and efforts by individual investigators. In these ways, NIDDK will continue its efforts toward preventing and treating the once "unnamed" disease of NASH, as its name becomes increasingly familiar.

Bacteria and the Immune System Work Together To Peacefully Coexist in the Intestine

Dr. Charles O. Elson III

Dr. Charles O. Elson III is Professor of Medicine and Microbiology and the Basil I. Hirschowitz Chair in Gastroenterology at the University of Alabama at Birmingham. Dr. Elson received his M.D. from Washington University in St. Louis, and he completed a residency in internal medicine at Cornell, an NIH fellowship in gastroenterology at the University of Chicago, and a postdoctoral fellowship in the Metabolism Branch at the National Cancer Institute. Dr. Elson is an elected member of the Association of American Physicians, an elected Fellow of the American College of Microbiology, former President of the Society for Mucosal Immunology, and has served on the National Diabetes and Digestive and Kidney Diseases Advisory Council. His research, which has been supported by the NIDDK, has made seminal contributions to understanding the regulation of immune responses in the digestive tract. At the February 2010 Advisory Council meeting, Dr. Elson presented advances from his research on the cellular and molecular mechanisms controlling the immune response to bacteria in the intestine; the following are highlights from his presentation.

The Intestinal Microbiota

The human intestine is host to an enormous ecosystem of microorganisms. This microbial community—or microbiota—is very complex. And, with a population of nearly 100 trillion organisms, the number of bacterial cells in the intestine outnumbers human cells by almost 10 to 1. While the presence of intestinal microbes has been appreciated for over a century, there is growing interest in understanding the composition of the microbiota and its role in shaping human health and disease. Recent advances—through initiatives such as the Human Microbiome Project—have started to define the diversity of bacterial species and bacterial genes present in the intestines. This has revealed aspects of the microbial community's metabolic functions and its influence on the development of the intestinal immune system.

The microbiota is established in the intestine shortly after birth. Humans and other animals tend to live in harmony with their gut microbes throughout their life. Given how intimate this relationship is, researchers have puzzled over what allows us—humans and our microbes—to peacefully coexist. Why doesn't the immune system normally attack this mass of resident microbes encountered in the intestines? How does the intestine maintain the balance, or "homeostasis," between friend and foe?

Intestinal Homeostasis in Mice and Men

Dr. Elson and his research group have studied human intestinal biology and disease using mice as an informative model. In particular, Dr. Elson's team has studied a strain of mice, called C3H/HeJBir, that spontaneously develops inflammation of the colon (colitis). They discovered that this mouse has a strong immune response to some component of the microbiota. To learn more about this immune response, the researchers focused on the role of T cells, a type of immune cell that recognizes and responds to specific molecules, such as bacterial components. Remarkably, when they isolated T cells from C3H/HeJBir mice with colitis and transferred these T cells to other mice lacking their own immune cells, the recipient mice developed colitis.

Dr. Elson next wondered what parts of the bacteria in the intestine might be activating these T cells

to generate the aberrant immune response that causes colitis. Using a molecular screening technique, Dr. Elson's team identified a number of T cell-activating bacterial components, and many turned out to be proteins known as flagellins. Flagellins make up the tail-like structures, called flagella, that some bacteria use to move.

Based on these findings, the scientists decided to test more directly whether T cells reactive to a bacterial flagellin can cause intestinal inflammation. To do this, they generated T cells that respond to one specific type of flagellin called CBir1. When they transferred these CBir1-specific T cells into other mice (which were deficient in their own immune system), the mice developed colitis. Interestingly, T cells that were artificially activated (*i.e.,* not in response to the bacterial flagellin) did not cause intestinal damage. These results supported the idea that an abnormal immune response to specific components of the microbiota causes intestinal inflammation.

In a collaboration facilitated by his NIDDK Program Project grant, Dr. Elson and his colleagues then partnered with other scientists to translate this exciting result very rapidly into understanding of human disease. They found that patients with Crohn's disease—a form of inflammatory bowel disease (IBD)—had elevated levels of antibodies specific for this bacterial flagellin. Antibodies are produced by another type of immune system cell. In addition, the presence of these antibodies was predictive of a more complicated disease course for these people. This was an exciting example of how basic research discoveries made in animal models can be translated to advance knowledge of human disease.

Interestingly, results from a very different line of research—human gene mapping—are also starting to converge with the T cell experiments in mice. Dr. Elson pointed out that in genomic studies of IBD, scientists have identified human genetic variants that are associated with immune system function in a way that's related to the inflammatory reaction caused by T cells in the mouse model. This is an exciting example of how results from animal models complement studies of humans to enhance our understanding of disease.

Suppressing an Adverse Immune Response to Gut Bacteria—the Role of Immunoglobulin A

Having found that this very potent bacterial trigger of inflammation is present in the intestine and associated with disease course in humans, Dr. Elson was interested in understanding how the intestinal immune system normally deals with its presence—what keeps T cells from causing an inflammatory response to this ever-present bacterial structure in a healthy intestine?

To address this question, Dr. Elson created a mouse model whose T cells are specific for only the CBir1 flagellin. Much like the C3H/HeJBir mouse strain, it was expected that a mouse with T cells specific for CBir1 would also develop colitis. Surprisingly, these mice turned out to be healthy. Their intestinal tissues were normal, and they did not have the expected elevated levels of flagellin-specific antibodies in their blood. Something was preventing the CBir1-specific T cells from becoming activated.

Further investigation uncovered new pieces to this puzzle. Although the mice did not have antibodies to CBir1 flagellin in their blood, Dr. Elson found that the mice had very high levels of a different type of CBir1-specific antibody in their intestines. There are several different types of antibodies. The most common type in blood is called immunoglobulin G (IgG). The most abundant type of antibody in the intestine, on the other hand, is immunoglobulin A (IgA). Dr. Elson's team found that the mouse model with CBir1-specific T cells had a large amount of intestinal IgA specific for the CBir1 flagellin. Interestingly, they found that normal mice also had a very high level of CBir1-specific IgA in their intestines. (In their previous experiment, the T cells from C3H/HeJBir mice caused colitis in mice lacking an immune system, as those mice also lack IgA.)

These findings suggested that IgA may play an important role in the normal response to gut microbes. Dr. Elson's team designed an elegant series of experiments to determine what precisely the CBir1-specific IgA was doing in the intestine. First, they isolated the CBir1-specific T cells from their mice and labeled the T cells with a dye. This dye allowed them to monitor when the T cells become activated. They then transferred the labeled CBir1-specific T cells back into different mice, and fed the mice CBir1, thinking that a large amount of this bacterial flagellin introduced into their gut might activate the T cells. When they transferred the T cells into normal mice and fed them CBir1 flagellin, the T cells remained inactive. It turned out that if they transferred the T cells into mutant mice that do not produce any IgA, however, the T cells were robustly activated by orally administered CBir1 flagellin. These results suggested that, under normal conditions, IgA in the intestine prevents this bacterial flagellin from activating its cognate T cells.

Preventing Destructive Immune Responses—A Molecular Team Effort

Dr. Elson's experiments established that IgA is important in restricting the T cell response to flagellin from the intestinal microbiota. However, there are different types of T cells with very different functions in the body. Previous studies have shown that a type of T cell known as regulatory T cells (Tregs) are abundant in the tissue lining the intestine and that they are involved in inhibiting immune responses. Unlike the T cells that are activated by bacteria to promote inflammation, Tregs produce a factor called TGF-beta, which in addition to inhibiting immune responses, also turns on production of IgA by antibody-producing cells. This raised the question as to whether IgA and Tregs operate independently or if somehow they cooperate to suppress the immune response to resident gut bacteria.

In another elegant series of experiments, Dr. Elson and his research team demonstrated that Tregs are, in fact, important in inducing the IgA response to CBir1 flagellin. In their study, the researchers used an experimental technique to reduce the number of Tregs in otherwise normal mice, and then assessed whether there was a resulting effect on IgA levels. When they depleted the levels of Tregs, the scientists found that the total amount and CBir1-specific amount of IgA dropped to negligible levels compared to mice that have normal levels of Tregs. From the results of these and related experiments, Dr. Elson and his team concluded that Tregs are indeed important for inducing the IgA response to this specific microbial product.

Gut Microbes and the Immune System Work Together To Prevent Infection

In addition to shedding light on how adverse immune reactions are prevented against normal gut microbes, Dr. Elson's research also suggests how IgA, Tregs, and the microbiota work together to help prevent infection by invading bacteria.

It is well known that in addition to its metabolic properties, the normal resident gut microbiota helps prevent infection in the intestine. That is to say, the presence of the microbiota itself makes it difficult for potentially pathogenic bacteria to take up residence and cause infection. However, it might not be expected that the combination of Tregs and IgA would be beneficial to fighting pathogenic bacteria, because Tregs also shut off the immune response that could help stave off infection.

Dr. Elson explained that this apparent paradox is reconciled when considering IgA and Tregs as working in concert with the microbiota. The IgA molecules provide a protective layer on the surface of cells lining the intestine. In this position, IgA can bind to flagellins—or other bacterial structures—from the normal microbiota and provide a foothold for the resident bacteria to live in the intestine and carry out their metabolic functions. Since Tregs are also responsive to these normal gut bacteria, they provide

a second line of defense by stimulating production of IgA and suppressing a potentially harmful inflammatory response. This further stabilizes the microbiota and helps to maintain resistance against invasion by pathogenic bacteria.

These findings have important implications for the development of oral vaccines targeting immune responses in the gastrointestinal tract. In addition to existing vaccines that are typically given by injection and generate antibodies in the bloodstream, scientists have also tried to develop vaccines that could be administered orally to protect against infectious bacteria. Some of the challenges that have been encountered in developing oral vaccines could be explained by Dr. Elson's research. That is, the mechanisms that normally prevent inflammatory responses against resident gut bacteria may also be restraining the intestinal immune system from mounting a lasting protective reaction to oral vaccines.

Conclusions

In summary, Dr. Elson's research is illuminating how animals and their resident intestinal microbes peacefully live together. The results he presented demonstrate a regulatory role of IgA in maintaining intestinal homeostasis. In addition, he showed that Tregs, which are abundant in the tissue lining the intestine, work in a coordinated fashion with IgA and the microbiota to maintain homeostasis and restrict an adverse, inflammatory immune response. Various aspects of Dr. Elson's discoveries in mouse models of intestinal inflammation have already been translated to inform our understanding of IBD in humans. These studies point to the biological pathways regulating the immune response to intestinal bacteria as being important in human health and disease.

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David Warren

Nonalcoholic Steatohepatitis (NASH) Study Finds Promising Treatments for Hidden Liver Disease

David Warren

A few years ago, 62-year-old David Warren was loving life. Retired for 3 years after 32 years of working for the U.S. Postal Service and happily married, David went about life doing what he likes best—gardening and playing the stock market from his home computer.

However, after a routine annual checkup, David's blood work showed that his liver enzymes were elevated, a sign of injury or disease in the liver. "I'd been taking a statin [a drug that can sometimes elevate liver enzymes] to help control my cholesterol, and so my primary care physician sent me to Duke University Hospital for a liver biopsy to find out whether taking the statin was the cause of my elevated enzyme count," says David.

When his biopsy report came back, David learned that something else was causing his liver damage. It turned out that he had a form of liver disease called nonalcoholic steatohepatitis (NASH), which is characterized by excess fat and inflammation in the liver. If left undiagnosed and untreated, over time NASH can lead to liver failure—requiring a liver transplant—or to the development of hepatocellular carcinoma, a form of liver cancer.

When they received the diagnosis, David and his wife were both shocked and scared. "We couldn't believe it," says David. "We always associated liver disease with drinking—and I don't drink."

Immediately after receiving his NASH diagnosis, David was asked if he'd be willing to participate in a clinical trial called "PIVENS." Supported by NIDDK, with additional support from private industry, PIVENS is a trial within NIDDK's NASH Clinical Research Network, which was established to study the natural history, disease processes, and therapy of NASH in both adults and children. The PIVENS trial tests treatments for NASH in adults, while another clinical trial, called "TONIC," focuses on treating NASH in children.

David readily agreed to participate in the PIVENS trial.

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About NASH

Recognized as a specific medical condition in 1980, NASH is believed to be on the rise in the U.S., most likely, researchers say, as a result of the epidemic

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increase in obesity. Although most people with NASH, like David, do not manifest symptoms for several years, a great danger of this disease is that it can lead to a cirrhotic liver, a condition in which the liver develops extensive scar tissue that stiffens blood vessels and distorts the internal structure of the liver. Over time, NASH can lead to significant scarring of the liver and liver cirrhosis. Liver cirrhosis may progress to liver failure, ultimately requiring liver transplantation. Currently, there are no specific Food and Drug Administration-approved treatments available for NASH.

Although the specific cause or causes of NASH are unknown, in addition to its link with obesity, the disease also is typically associated with insulin resistance or even type 2 diabetes, hypertension, elevated lipids, or other features of metabolic syndrome. Oxidative stress—a state which produces chemicals called free radicals that can cause damage to the body's proteins, membranes, and genes—also is thought to play a role.

At the time of his NASH diagnosis, David had many of the common risk factors for developing the disease, including high blood pressure, high cholesterol, high triglycerides, and insulin resistance.

"I've been insulin resistant, or pre-diabetic, for probably 4 or 5 years," says David. "My mother had diabetes, and I'm pretty sure my father did, too," he adds. Never morbidly obese, David was aware, however, that he was carrying too much weight and that he could afford to lose a few pounds.

Therefore, even prior to his NASH diagnosis, and concerned about his insulin-resistant condition turning into full-blown type 2 diabetes, David began exercising and watching his diet. "I use an elliptical rider and a recumbent bike, as well as work in my flower garden 2 or 3 hours a day," he says. It may have been enough to tip the scales in his favor.

The PIVENS Trial

PIVENS is short for Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Nonalcoholic Steatohepatitis. With enrollment starting in 2005, PIVENS was conducted at eight clinical centers around the country participating in the NASH Clinical Research Network, including the Duke University center where David participated in the trial, and a data coordinating center. It is the largest randomized, placebo-controlled clinical trial of NASH therapy to date.

As its name implies, PIVENS was designed to study whether the antioxidant vitamin E or the drug pioglitazone, which improves insulin sensitivity in cells, could be used to treat adults with NASH, like David, who were not currently or previously taking an insulin-sensitizing drug and who don't currently have diabetes.

Over a 2-year period, David and other PIVENS volunteers were given once-daily doses of either pioglitazone, vitamin E, or a placebo. During the trial, participants did not know which agent they were taking. Liver biopsies were taken before and after the trial to reveal whether these agents improved signs of NASH in the liver, the main focus of the trial. Other information collected on participants included measures of liver health and metabolic fitness, as well as any side effects from these agents.

In May 2010, results of the PIVENS trial were announced in the *New England Journal of Medicine*, showing that vitamin E significantly improved NASH in adults based on a constellation of disease features, including liver inflammation, fibrosis, and fat accumulation. Although pioglitazone did not significantly reduce this overall measure of NASH in the liver, it showed some positive effects, including more normalized liver enzymes and reduced fat in the liver.

To David's and his wife's great relief, as the months of his participation in the study went by, his NASH

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did not progress. By the end of the study, a biopsy of his liver no longer showed the changes characteristic of NASH. "The disease is well under control, and I'm no longer taking any medication for it," says David.

"I can't see why people wouldn't volunteer for these studies," says David. "It's a win-win for everybody. It changed my life."

Since the completion of the treatment phase of the study, David has continued to participate in the 6-month follow-up phase. He learned that he had been taking pioglitazone every day during the treatment phase of the study, and the only side effect he experienced was weight gain, which was also observed in other study participants taking the drug.

After the treatment phase (discontinuing the drug), through exercise and a healthy diet, David has lost 18 pounds, "but my family physician said I should lose at least another five," he says.

An added benefit of David's participation in the clinical trial was that doctors were able to discover that he had severe sleep apnea—and thus start him on a treatment for this condition from which he had been suffering for years. Untreated, sleep apnea can lead to heart attack, heart failure, irregular heartbeats, and diabetes. "For years I would wake up gasping for breath, but wasn't quite sure what was wrong with me," says David. Now he sleeps with an air mask, and reports sleeping very well at night.

"I can't see why people *wouldn't* volunteer for these studies," says David. "It's a win-win for everybody. It changed my life."

David is now loving life again. He enjoys working in his garden more than ever, and continues to adapt to whatever comes his way. "I used to plant vegetables, but the deer seem to like them more than I do," he laughs.

For additional info on the PIVENS trial and other NASH research efforts, please see related content in the research advance section and Story of Discovery in this chapter.