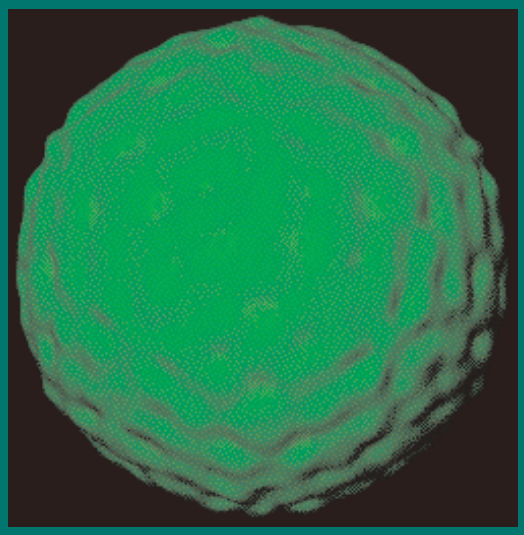
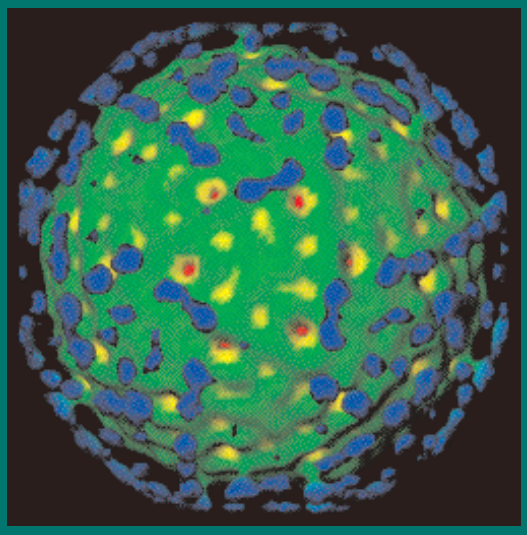
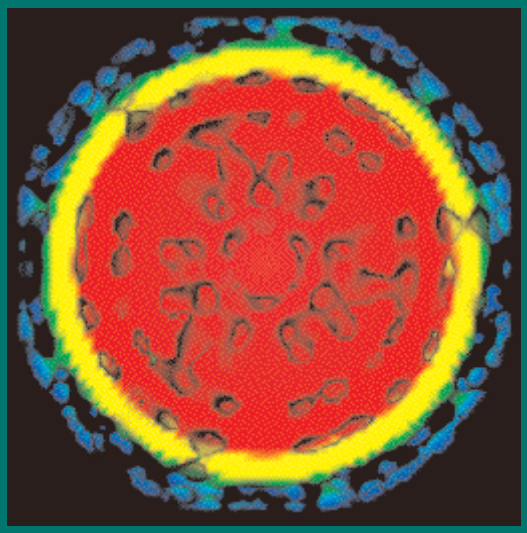






At left are reconstructed images of a recombinant hepatitis C virus (HCV)-like particle that was produced in a cell culture system. These images are three-dimensional representations of the HCV particle at high resolution. Visualizing the virus in this way enables researchers to understand how it interacts at the molecular level with compounds important in fighting against HCV infection, such as antibodies. Top: The exterior surface of the HCV particle. Middle: The HCV particle labeled with antibodies (shown in blue), which bind to proteins on the surface of the viral coat. Bottom: A cross-sectional view of the antibody-labeled HCV particle shown in the middle panel. The internal structure of the virus is shown in red. For more information about recent exciting advances in HCV research, see the Feature entitled, "Small Cells Yield Big Breakthrough in Hepatitis C Research," in this chapter.

Images courtesy of Dr. T. Jake Liang, Chief, Liver Diseases Branch, NIDDK Division of Intramural Research.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. These conditions include disorders of the gastrointestinal tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. Disorders of the digestive tract—such as irritable bowel syndrome and inflammatory bowel disease—exact a significant toll on many Americans each year. NIDDK-supported scientists are vigorously pursuing research to understand how widespread these diseases are across the U.S., to identify the causes of these diseases and how they progress, and to test new interventions for treatment and prevention of these costly diseases, including drugs, surgery, and behavior modification.

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

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

Intestinal disorders include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may 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.

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. To address this condition, surgery may be required, including removal of the affected region of the intestine. Scientists are dissecting the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. Helping to catalyze the design of novel therapeutic strategies will be the continued identification of predisposing genetic variations and their interactions, as well as other factors, such as potential autoimmune and microbial influences.

The microorganisms that inhabit the gastrointestinal tract are powerful players in maintaining or tilting the balance between digestive health and disease. These microbes can affect intestinal health in some surprising ways, depending on their interactions with cells of their host. Scientists are gaining insights into the ways these microorganisms influence the development and function of the digestive tract. Some digestive diseases can be triggered by the body's

reaction to certain foods. In individuals with celiac disease, the small intestine is damaged when the immune system reacts to the protein gluten—a component of wheat, barley, and rye. This reaction interferes with the ability to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, growth failure. The only current treatment for celiac disease is maintenance of a gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their healthcare 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.

LIVER DISEASE RESEARCH

A New Model System To Study Hepatitis C: Hepatitis C virus (HCV) is a major cause of liver disease worldwide. Current therapies for HCV are not optimally effective and a vaccine is not yet available to prevent the disease. Clinical progress has been hampered because the virus grows poorly in culture and does not produce infectious viral particles. To solve this research problem, scientists recently designed a molecular HCV replication system that is capable of producing viral particles *in vitro*. HCV was detectable inside of the cells and mature viral particles were found in the culture medium. These particles were able to infect cultured cells and an animal model of HCV. With this advance, researchers will be better able to study the life cycle and biology of this virus, and to test antiviral compounds as potential therapies for the liver disease it causes.

For more information about exciting research advances in the study of hepatitis C, see the Feature in this chapter.

Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Krausslich HG, Mizokami M, Bartenschlager R, and Liang TJ: Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 11: 791-796, 2005.

NAFLD, a Disease Without a Treatment: Recently, a clinical trial was launched to study fatty liver disease in children. The trial, termed TONIC for "Treatment of Nonalcoholic Fatty Liver Disease (NAFLD) in Children," is part of a larger NIDDK initiative, the Nonalcoholic Steatohepatitis Clinical Research Network, which is also supporting adult studies of this disease. The major characteristic of NAFLD, as its name implies, is the accumulation of fat in the liver. If inflammation and liver injury are also present, this condition is known as nonalcoholic steatohepatitis or "NASH." NAFLD is very similar to the better known condition of alcoholic liver disease; however, it is seen in children and in adults who drink little or no alcohol. NAFLD is associated with overweight and obesity and occurs in a high proportion of persons with diabetes. The rate of NAFLD in children has increased sharply and appears to correlate with the rapid rise recently in the rate of childhood obesity.

Although most children with this disease feel well and do not experience symptoms, researchers are concerned that, as they grow into adulthood, the disease may progress, increasing their risk for cirrhosis, particularly if they drink alcohol or contract viral hepatitis. Currently, there are no safe or effective drugs recommended to treat children or adults with NAFLD. If cirrhosis occurs, liver damage can be so severe that a liver transplant is the only treatment option.

The NIDDK and investigators involved in the TONIC trial hope that it will uncover the underlying conditions that contribute to the development and progression of NAFLD, as well as test the safety and effectiveness of new treatments. Results from previous small pilot studies using antioxidants, such as vitamin E, or the insulin-sensitizing drug metformin showed that these agents may improve the condition of patients with NAFLD and may delay or possibly prevent the progression to more serious liver disease. Extensive safety and efficacy data already exist on the use of metformin for the treatment of type 2 diabetes in children.

The TONIC trial will enroll 180 girls and boys, ages 8-15, with NAFLD. Because of the association of NAFLD with obesity, 90 percent of the children enrolled in the trial are expected to be obese. However, children with other liver diseases or diabetes will be excluded from the trial. This trial has three arms in that participants will receive either vitamin E, metformin, or a placebo, for a period of two years.

As the first randomized, controlled clinical trial for children with NAFLD, TONIC is expected to provide a platform to conduct rigorous studies on how safe and

effective vitamin E and metformin are as treatments. Data from this trial, along with the adult trials supported by the NIDDK through the NASH Clinical Research Network, will contribute to greater understanding of NAFLD, advance treatment options, and improve outcomes for children and adults with this disease.

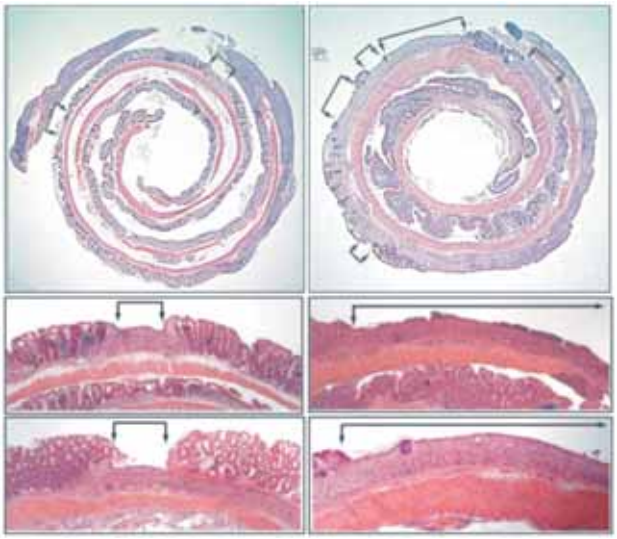
Molecular Factors Underlying Liver Development: Understanding how a single fertilized egg develops into a complex, multicellular organism is one of the most fascinating questions in all biology. Scientists studying liver development have found that the *Foxa1* and *Foxa2* genes play an important role in the developing liver. It is thought that embryonic liver development proceeds in a two-stage process whereby factors first make the tissue "competent" to respond to subsequent organ-specific signals that direct tissue differentiation into specific organs. To explore this area, investigators derived a strain of mice lacking the *Foxa1* gene entirely and lacking the *Foxa2* gene in endoderm—the embryonic tissue that gives rise to the liver. Embryos in which these genes were made non-functional (knocked out) were smaller than normal counterparts and failed to develop an embryonic liver. When normal mouse endoderm is grown in culture in the presence of certain growth factors, the tissue begins to express proteins characteristic of mature liver. In contrast, endoderm from *Foxa1/Foxa2* knockout embryos grown under these conditions did not express liver-specific proteins, suggesting that this tissue is not able to respond to the growth factors. This observation suggests that the proteins encoded by *Foxa1* and *Foxa2* genes act early in liver differentiation as competence factors that render the tissue able to respond to subsequent signals. This experimental system may be very useful in the study of organ development, because the endoderm gives rise to many tissues, including the gut, pancreas, thyroid, and lungs.

Lee CS, Friedman JR, Fulmer JT, and Kaestner KH: The initiation of liver development is dependent on Foxa transcription factors. Nature 435: 944-947, 2005.

New Imaging Methods for Hepatic and Renal Fibrosis: A common problem faced by those studying diseases of the liver and kidney is how to monitor disease progression, particularly how to assess the fibrosis that occurs in these organs as part of the disease process. In fiscal year 2007, the NIDDK plans to solicit research applications to develop non-invasive imaging methods that will allow early detection of renal and hepatic fibrosis and will facilitate monitoring of disease progression. An essential component will be cooperation between investigators who develop cutting-edge imaging methods and investigators who are familiar with the disease issues relevant to fibrosis. This effort will have significant impact on the ability of investigators to pursue novel therapies for many kidney and liver diseases, because it promises to provide a cheaper and more reliable measure of outcomes than current methods. The NIDDK also has plans to pursue a Program Announcement, to be addressed through the Small Business Innovation Research program, that will reinforce and complement the studies funded through the research solicitation on fibrosis imaging.

NEW MODELS SHED LIGHT ON INFLAMMATORY BOWEL DISEASE

Insights into Crohn's Disease from Two New Mouse Models: Crohn's disease (CD) is a chronic inflammatory bowel disease thought to arise from a combination of genetic susceptibility and environmental factors. Previous studies have identified mutations in the *Nod2* gene as playing an important role in the development of CD in humans. To better clarify the role of *Nod2*, researchers have developed two new mouse models of the disease. One group of researchers generated mice lacking the *Nod2* gene entirely. The animals were resistant to infection from bacteria introduced intravenously, but were more susceptible than normal animals to infection resulting from orally-administered bacteria. This finding highlights the protective role *Nod2* plays in fighting bacterial infection in the gut. Another group of researchers generated mice in which the normal *Nod2* gene was replaced with the most common mutant form seen in human CD. A chemical agent that damages the cells lining the intestine caused greater weight loss, increased mortality, and more severe colonic ulcerations in animals with the mutant *Nod2* gene than in normal animals. This ulceration could be minimized by the co-administration of antibiotics, suggesting that the increased damage resulted from interactions among the chemical, the bacteria present in the intestine, and the *Nod2* gene. Immune cells cultured from mice with the mutant *Nod2* gene showed increased production of a number of pro-inflammatory proteins including one of the interleukins (IL-1beta). *Nod2* mutant mice treated concomitantly with an agent that blocks IL-1beta activity and the chemical agent



Scientists use animal models to study diseases and evaluate potential new therapeutic approaches. Research has indicated that mutations in the *Nod2* gene likely play an important role in the development of Crohn's disease, a chronic inflammatory bowel disease, but the precise role of these mutations is unknown. Shown above are histological sections (at varying magnifications) of large intestine taken from normal mice (left) and from mice with mutant *Nod2* genes (right) after they had been treated with a chemical that causes ulcers. The arrows in the panels denote the borders of ulcers. Mice with the mutant gene exhibit substantially more inflammation and ulceration in their intestines, which provides further experimental evidence that this gene contributes to inflammatory bowel disease.
Image courtesy of Dr. Martin F. Kagnoff and reprinted with permission from Maeda et al. Science 307: 734-738. Copyright 2005 AAAS.

showed improvements with respect to weight maintenance and colonic ulcerations, compared to mutant animals not receiving the blocking agent. Together, these studies identify important new roles for *Nod2* in the development and progression of CD.

Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, and Flavell RA: Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 307: 731-734, 2005.

Maeda S, Hsu LC, Liu H, Bankston LA, Iimura M, Kagnoff MF, Eckmann L, and Karin M: Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. Science 307: 734-738, 2005.

BACTERIA IN THE GUT: CONTRIBUTION TO SENSITIVITY TO RADIATION

Important Insights into the Causes of Side Effects of Radiation Therapy: Scientists have recently shed new light on a very old treatment for cancer. Radiation therapy (sometimes called radiotherapy) was first used to treat cancer more than 100 years ago, and it remains a critical part of the treatment approach for almost half of cancer patients. Radiation kills living cells, but is particularly damaging to cells that divide frequently, such as cancer cells. However, the damage to other tissues caused by radiation therapy can limit the usefulness of the approach. Scientists have recently found evidence that the degree of radiation sensitivity in the intestine may be influenced in part by the trillions of bacteria that reside there. The researchers showed that mice raised in a germ-free environment are more resistant to the side effects of radiation than mice grown in the conventional way. Bacteria play a valuable role in the digestive process for all mammals, including people, but scientists are just beginning to appreciate the complex physiological interaction between animals and the bacteria that reside in their bodies. One effect the microbes have is that they decrease the expression level of a particular protein that may help protect against radiation. This finding raises the possibility that radiation therapy might be enhanced if physicians are able to either manipulate the microbial environment of the intestines or influence the expression level of this protein. Physicians may one day be able to reduce side-effects and improve survival in patients receiving radiation therapy.

Crawford PA, and Gordon JI: Microbial regulation of intestinal radiosensitivity. Proc Natl Acad Sci U S A 102: 13254-13259, 2005.

LINK BETWEEN FASTING AND A RARE DISEASE

A Protein that Couples Nutritional Status to Heme Synthesis: Researchers studying a protein that helps regulate metabolism during fasting have found an important link to understanding porphyria. Porphyria is a rare but serious condition characterized by acute attacks of abdominal pain, severe psychiatric and neurological problems, and sensitivity to sunlight. The attacks occur in susceptible people as a result of fasting or of taking certain

drugs or hormones. Susceptibility is caused by any of several rare mutations that interfere with biosynthesis of an essential iron-containing compound called "heme." Heme is a central constituent of hemoglobin, a major component of red blood cells, required for ferrying oxygen throughout the body. Heme is also used in every cell of the body as part of the energy-utilization machinery; in the liver, where it plays a vital role in neutralizing toxins, as well as many drugs; and in a variety of other proteins throughout the body. Unfortunately, heme is actually toxic when there is too much of it around, so its production is tightly controlled. Researchers studying PGC-1alpha, a protein that helps the body respond to cold temperatures by boosting energy utilization, noted that the protein is also up-regulated in the liver in response to fasting. Because fasting is also known to trigger attacks of porphyria, the scientists tested whether PGC-1alpha is also responsible for up-regulating heme biosynthesis—and found that it is. These results not only help explain what triggers porphyria attacks, they also clarify how the attacks can be blunted by treatment with glucose. They represent another piece in the complex puzzle of how the body maintains energy balance.

Handschin C, Lin J, Rhee J, Peyer AK, Chin S, Wu PH, Meyer UA, and Spiegelman BM: Nutritional regulation of hepatic heme biosynthesis and porphyria through PGC-1alpha. Cell 122: 505-515, 2005.

NATIONAL COMMISSION ON DIGESTIVE DISEASES

On September 20, 2005, the NIH Director announced the establishment of the National Commission on Digestive Diseases, which will work to improve the health of the nation through advancing digestive diseases research. The Commission is responsive to the mutual interest in this area shared by the Congress, the NIH, and the research community. Within the NIH, the NIDDK is providing leadership and support for the Commission, which will be active for two years as it develops a long-range research plan.

As part of its charge, the Commission will assess the state-of-the-science in digestive diseases and the related NIH research portfolio, in order to identify research challenges and opportunities for inclusion in the plan. The resulting 10-year plan will guide the NIH—along with the investigative and lay communities—in pursuing important research avenues for combating digestive diseases. The Commission's efforts will benefit from the diverse expertise of its members, representing the NIH and other Federal health agencies, the academic and medical research and practice communities, and the patient advocacy community. Additional information on the Commission can be found on its website: *http://NCDD.niddk.nih.gov.*

PUTTING A SPOTLIGHT ON CELIAC DISEASE

Celiac disease was once thought to be a rare disease, but it is now believed to affect as many as three million Americans. This disease, which often goes undiagnosed, results from an immune response mounted by cells in the intestinal tract to the common protein gluten, which is found in grains such as wheat, barley and rye. Celiac disease has strong genetic ties. Currently, the only treatment for celiac disease is a gluten-free diet, which results in remission for most affected individuals.

In June 2004, a Consensus Development Conference on Celiac Disease, sponsored by the NIDDK and the Office of Medical Applications of Research, was held at the NIH. The meeting focused on currently available data regarding the awareness, diagnosis, and management of celiac disease. In a Consensus Statement, the conference panel concluded that heightened awareness of this disease was imperative. It recommended that the NIDDK lead an educational campaign for physicians, dietitians, nurses, and the public about celiac disease.

In response to the Consensus Statement, the NIDDK's National Digestive Diseases Information Clearinghouse (NDDIC) is developing a Celiac Disease Awareness Campaign designed to inform and educate healthcare professionals and the public about the disease. An *ad hoc* committee—with representatives from the medical and health care fields, interest groups, and the clearinghouse—held a conference call to discuss the best strategies to raise awareness of the disease.

To supplement these strategies, research was conducted with health care professionals to determine the level

of awareness of celiac disease, to identify barriers to diagnosing celiac disease, and to elicit ideas to heighten awareness of celiac disease. This research included focus groups with a total of 72 primary care physicians in four states. Results from the focus groups indicated that an awareness campaign will need to center on the prevalence of celiac disease, some of the less-well-known, non-gastrointestinal symptoms, and the long-term consequences for patients who are not diagnosed.

As part of the new campaign, an updated version of the Celiac Disease Fact Sheet is now available on the Clearinghouse website *http://digestive.niddk.nih.gov*. Copies of the June 2004 Consensus Development Conference Statement also are available on the site.

FEATURE

Small Cells Yield Big Breakthrough in Hepatitis C Research

The hepatitis C virus (HCV) is one of at least five hepatitis viruses (hepatitis A to E viruses) that cause liver disease in humans. However, HCV stands out as the main cause of chronic hepatitis, cirrhosis, and liver cancer in the United States, and the major reason for liver transplantation in American adults.[1, 2] *Obtaining fundamental knowledge of HCV has been a daunting task for researchers, due to a lack of useful or convenient cell culture or animal models of HCV infection. The challenge of developing better HCV infection models to put in the hands of researchers eager to address persistent questions has been a long-standing focus of Dr. T. Jake Liang and his group in the NIDDK Liver Diseases Branch. Recently, their efforts have come to fruition in the form of a major breakthrough in the field—a cell culture system for HCV that promises to shed light on viral strategies for causing disease, how to counter and prevent them, and to generally accelerate the rate of discovery.*

State of Knowledge on Hepatitis C

Population-based surveys in the U.S. estimate that 2.7 million adults harbor HCV RNA in their blood, indicating a chronic HCV infection.[3] The virus is spread mainly through contact with infected blood due to needle sharing or receipt of contaminated blood products. However, it can also be transmitted through sex with an infected partner, occupational exposure to infectious blood, organ transplantation from an infected donor, unsafe medical practices, or birth to an infected mother. While a therapy for hepatitis C exists—typically a combination of antiviral drugs called peginterferon/interferon and ribavirin—these agents often cause major side effects and are effective in only about half of patients. No vaccine exists to prevent the spread of hepatitis C. While much is known about the serious public health threat posed by HCV, gaps in knowledge persist regarding its means of infecting cells and causing disease—important pieces of the puzzle for developing more effective approaches to treatment and prevention. For other types of viral hepatitis, the availability of good cell and animal models has accelerated the pace of discovery in recent years, leading to such advances as the development of safe and effective vaccines for hepatitis A and B, and the discovery of new drugs to treat hepatitis B. In contrast, the pace of research on HCV has been relatively slower since its identification in 1989. An improved working knowledge of HCV and its infection strategies in basic research models could enable the development of better treatments and the elusive HCV vaccine.

The Inimitable Hepatitis C?

In order to understand the pathogenic impact and uncover therapeutic or preventive weaknesses of the hepatitis C virus, researchers must understand its structural composition and how it infects the liver cells in which it causes disease. The basic components of HCV have been identified; from the outside working in, the virus is made up of two envelope proteins, a core or "nucleocapsid," and a positive-strand RNA genome. The RNA genome of HCV can vary, with at least six known genetic variants or "genotypes" of HCV. HCV genotypes 1-6 appear in different frequencies in infected populations throughout the world, with genotype 1 being the most common in the U.S. and worldwide, as well as the most difficult to treat using standard antiviral therapy. Once a person is exposed to HCV through one of the transmission routes mentioned previously, the full viral life cycle involves: (1) entry of the virus into the host cell (typically a hepatocyte, a liver cell); (2) production of structural proteins and enzymes; (3) replication of its RNA genome; (4) assembly; and (5) release of new

viruses from the infected cell, which then repeat the cycle by infecting neighboring, healthy cells.

HCV infection has proven particularly difficult to mimic in cell culture and in the small animal models typically used by basic researchers, for reasons that are currently unknown. What little is known to date of HCV's structure and life cycle of infection is the result of years of research using available, but sub-optimal cell culture and animal models for studying HCV. The chimpanzee model is widely considered to be the best animal model of HCV infection. However, research using this model instead of the mice or rats used most frequently for laboratory research is more costly, time-consuming, and inconvenient, due to such issues as limited access to research animals. As an alternative to animal models and in order to observe and manipulate viral behavior in a simplified system, researchers struggled for years to create an efficient cell culture model for HCV. An important advance was the development of the HCV "replicon" system, which enabled study of viral replication. Yet, even this model could not recreate the entire viral life cycle to yield mature viruses. The elusive "holy grail" in basic research on HCV was an efficient and reproducible cell culture system capable of supporting all stages of the viral life cycle to produce infectious hepatitis C viruses.

Cracking the Case—A Cell Culture Model for HCV

At the NIDDK, Dr. Liang's group of researchers, including Dr. Theo Heller, Dr. Satoru Saito and Dr. Takanobu Kato, worked for several years to develop an innovative solution to the lack of a useful cell culture model for HCV. They pursued multiple research approaches in parallel to address the problem from several fronts. Their primary focus was on creating a DNA expression "construct" that would produce the complete HCV RNA genome, including its complex end structures that are important for HCV replication and production, within the liver cell. To do this, they obtained HCV RNA from a patient with HCV genotype 1 and "reverse transcribed" the RNA into DNA, to which they added special sequences coding for "ribozymes," enzymes that cut RNA at precise locations. When transferred into human liver cells in culture and transcribed, the construct not only produced the HCV RNA genome, but also added the ribozymes to its ends, which cut the viral RNA to recreate the unique structures at the ends of the genome. After delivering the HCV RNA genome into the liver cells, Dr. Liang's group showed that their HCV-ribozyme cell culture system was capable of producing viral RNA and proteins, as well as assembling and releasing full viral particles. The publication of these findings in the *Proceedings of the National Academy of Sciences* in February 2005 signaled a groundbreaking achievement—the first cell culture system capable of producing high levels of complete HCV particles.[4] Soon after this publication, the group went on to show that the particles of HCV produced by this system are capable of infecting other healthy liver cells, indicating that the system can be used to study the complete life cycle of HCV and to produce infectious virus.

While the HCV-ribozyme cell culture system was in development, Dr. Liang also collaborated with Japanese and German researchers on a different, but complementary research approach to creating a cell culture model for HCV. For these studies, the researchers utilized a unique strain of virus isolated from a Japanese patient infected with a genotype 2 HCV. They introduced the HCV RNA genome directly into human liver cells in culture. In this system, they observed robust replication of the RNA and production of viral particles, which were secreted from the cells and, most importantly, found to be infectious in both healthy liver cells in culture and when injected into healthy chimpanzees. When these findings were published in *Nature Medicine* in July 2005, they represented the first public demonstration of a cell culture system capable of producing HCV particles that were shown to be infectious.[5]

Since the announcement of these two parallel research advances to develop an HCV cell culture

system, Dr. Liang's group has combined the two approaches. They introduced the robust HCV genotype 2 strain from the Japanese patient into the HCV-ribozyme construct system, again demonstrating successful production of HCV particles. The advantage of using the HCV-ribozyme construct system, which uses DNA to deliver the HCV RNA genome to liver cells as opposed to directly delivering viral RNA, is that DNA is more convenient for researchers to handle, assay, and store. Additionally, the DNA-based construct could be used in future experiments to generate continuous cell lines based on DNA's ability to integrate into the cell's genome.

Collaboration and friendly competition within the international research community drove these advances and helped to dispel skepticism that the "holy grail" of an efficient cell culture system for HCV could not be found. Around the time that Dr. Liang and his collaborators in Japan and Germany announced their success in developing a cell culture system using the robust HCV genotype 2 strain, two other American research groups, one of which received partial support from the NIDDK, published similar results using the same HCV strain. Recently, other groups have also demonstrated the success of HCV DNA expression culture systems like the HCV-ribozyme system developed by Dr. Liang's group.

New Research and Clinical Possibilities for Hepatitis C

The number of potential uses for the cell culture systems developed by Dr. Liang's group, and through his collaborations with Japanese and German researchers, is limited only by scientists' imaginations. Planned applications range from basic research on how HCV causes disease to tools for drug development.

Because Dr. Liang's group used a genotype 1 HCV in their initial experiments to test the HCV-ribozyme culture system, these studies have implications for the widest number of people with hepatitis C, many of whom do not respond well to standard antiviral therapy. However, Dr. Liang's group also plans to create HCV-ribozyme constructs of all HCV genotypes, using viruses isolated from patients, in order to study the basis for the unique pathology and response to treatment associated with different HCV genotypes. Plans to use the HCV-ribozyme construct to develop stable cell lines that continuously produce HCV would save researchers from having to reintroduce the virus for each experiment and would facilitate comparison of findings across research groups. The production of high levels of HCV in the HCV-ribozyme cell culture model is even enabling highly detailed views of the 3-dimensional structure of the virus at the level of its individual molecules; this knowledge will inform researchers about how HCV interacts with host cells and antibodies.

Additionally, these cell culture systems can be used to screen new drugs for their effectiveness in treating HCV infection. Dr. Liang's group has already tested a standard treatment for hepatitis C, interferon, on the cell culture system and confirmed that it is effective in the system at fighting the virus. Another attractive research direction for this system is to develop an experimental HCV vaccine that would be effective against the widest variety of HCV genotypes found throughout the world.

While these cell culture models provide researchers with a simplified system in which to study HCV replication and infection, animal models are still key to understanding how HCV behaves within a whole organism. Small animal models of HCV infection are needed to study the viral life cycle, host immune response, natural history, and response to experimental therapy and vaccines. Dr. Liang's group hopes to address this need by using the same HCV-ribozyme construct to create transgenic mouse models that have the construct in their DNA and, therefore, naturally produce HCV. These mouse models would enable researchers to study the effect of specific HCV genotypes and experimental therapies to treat hepatitis C or to prevent its progression to diseases such as liver cancer. This research in animal models would complement ongoing clinical trials on

treatment and prevention of chronic hepatitis C progression such as the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis or "HALT-C" trial supported by the NIDDK.

Dr. Liang is sharing his constructs, cells, and other materials with the research community to enable pursuit of the host of experimental possibilities opened up by the availability of a cell culture system for HCV. He hopes that other researchers—either those already studying HCV or who were reluctant to do so due to a lack of research tools—will also use these small cells to think big…and find answers to many lingering questions about HCV.

[1] *Vong S and Bell BP. Chronic liver disease mortality in the United States, 1990-1998. Hepatology 39: 476-483, 2004.*

[2] *2003 Annual Report of the U.S. Organ Procurement and Transplantation Network (OPTN) and the Scientific Registry of Transplant Recipients (SRTR): Transplant Data 1993-2002. Department of Health and Human Services, Health Resources and Services Administration, Office of Special Programs, Division of Transplantation, Rockville, MD; United Network for Organ Sharing, Richmond, VA; University Renal Research and Education Association, Ann Arbor, MI. Available online at http://www.optn.org/AR2003/default.htm Accession date: December 19, 2005.*

[3] *Centers for Disease Control and Prevention, Division of Viral Hepatitis, "Disease Burden from Viral Hepatitis A, B, and C in the United States," 2003.*

[4] *Heller T, Saito S, Auerbach J, Williams T, Moreen TR, Jazwinski A, Cruz B, Jeurkar N, Sapp R, Luo G, Liang TJ. An in vitro model of hepatitis C virion production. Proc Natl Acad Sci U S A 102: 2579-2583, 2005.*

[5] *Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Krausslich HG, Mizokami M, Bartenschlager R, Liang TJ. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med 11: 791-796, 2005.*

Combating Toxic Iron Overload

Most people know that iron is essential for good health. What people may be surprised to learn is that too much iron—a condition called iron overload—can actually threaten health by damaging tissues and organs. Iron overload occurs in diseases such as hemochromatosis and Cooley's anemia. Although these conditions have different causes, they both involve the accumulation of excess iron, which is stored in the heart and/or liver. This excess iron can damage these organs so that they no longer work properly. Unfortunately, the human body does not have a natural way to rid itself of excess iron. To address these problems, NIH-funded research is providing insights regarding the regulation of iron metabolism and methods for removing toxic excess iron.

In hemochromatosis, genetic mutations alter control mechanisms that would otherwise precisely regulate iron absorption. The standard therapy for treating this condition is blood-letting (phlebotomy), which is equivalent to blood donation and is a relatively simple means of restoring normal iron stores in the body. However, research into the genetic basis of the disease could provide a platform for developing more effective treatments and prevention strategies. For example, a genetic screen to identify those at risk for hemochromatosis could permit early intervention (by phlebotomy) to prevent progression to organ toxicity caused by iron overload.

A decade ago very little was known about the genes that harbor the disease-causing mutations in hemochromatosis and the proteins these genes encode. A major advance occurred when mutations in a gene called *HFE* were discovered to underlie the most common form of the disease in humans.

The discovery of variants of the *HFE* gene that lead to mutant proteins provided an opportunity for early and rapid genetic identification of individuals at risk for development of hereditary hemochromatosis. A majority of patients diagnosed with iron overload due to hereditary hemochromatosis have been found to have two copies of a mutant *HFE* gene referred to as C282Y (one mutant gene copy inherited from each parent); a second *HFE* mutation (H63D) has also been associated with hemochromatosis but only if it occurs in association with C282Y. To further explore the potential for population screening for common *HFE* mutations, NIDDK-supported researchers screened a large number of people for the presence of *HFE* mutations and associated symptoms of hemochromatosis. In the study, among the individuals found to have two copies of the C282Y mutation, many had elevated iron levels. Researchers also observed an increase in liver disorders seen in those with *HFE* mutations, but did not observe greater frequency of most other symptoms characteristically associated with hemochromatosis. In fact, most of the people with mutant *HFE* genes had not developed clinical symptoms and signs of organ toxicity, indicating that having the *HFE* mutations does not guarantee clinical symptoms. This study revealed that additional mutations or environmental factors contribute to hereditary hemochromatosis. In a more recent study of an even larger population, NIH-funded researchers found similar results. Their study also revealed that the C282Y mutation likely does not account for high iron levels seen in non-Caucasian populations; the C282Y mutation had previously been found to be more common in Caucasians than in other groups. These findings will likely spur new research to identify other genetic or environmental factors that influence the development of clinical symptoms of hemochromatosis.

STORY OF DISCOVERY

Other insights into hemochromatosis emerged from studies showing the significance of hepcidin as a regulatory protein that controls iron balance in the body. These findings emerged from a series of seemingly unrelated studies in mice. One group of researchers found that mice fed a diet high in iron express in their livers high levels of *Hepc*, a gene encoding the antimicrobial peptide hepcidin. Other scientists made the fortuitous finding that iron overload develops in mice carrying a genetic mutation (in the upstream stimulatory factor 2 gene, *Usf2*). Additional experiments indicated that these mutant mice have completely lost their ability to express the *Hepc* gene in the liver. A year later, this same group of scientists reported that forced overexpression of the *Hepc* gene in the liver of mice led to offspring with pale skin, anemia, and decreased body iron levels—with death frequently occurring within a few hours of birth. These mice thus displayed characteristics of an experimental form of iron deficiency. Collectively, these studies highlight the important role the liver plays in sensing excessive levels of iron.

But how does hepcidin—the protein product of the *Hepc* gene—regulate the level of iron that is transferred from the intestine into the circulation? NIH-supported scientists hypothesized and demonstrated that, by binding to and destroying an iron exporter (FPN1), hepcidin prevents the transfer of iron from the intestinal cell into the circulation. Hepcidin causes the iron to be retained in the intestine cell, and thus protects other tissues and organs from iron overload. In addition, it is now known that hemochromatosis patients carrying mutations in several different genes each have inappropriately low levels of hepcidin.

Another group of patients who face problems with iron overload are those affected with anemias, such as Cooley's anemia, which require lifelong blood transfusions as a treatment regimen. Transfusions provide anemia patients with desperately needed functional red blood cells that their bodies cannot make in sufficient quantity. However, there is a serious downside to this treatment. Because the transfusions provide iron-rich blood directly into the circulation, they by-pass the normal intestinal control of iron absorption. As a result, toxic levels of iron build up in blood and body tissues—producing symptoms similar to those of untreated hemochromatosis. To combat iron overload, the NIH has supported research to develop agents, known as iron chelators, to remove excess iron from the body. The standard chelator, deferoxamine, generally must be administered under the skin using a pump for 10 to 12 hours per day, 5 days per week. Although this intervention will extend life in chronic severe anemias, it is enormously time-consuming, inconvenient and painful for patients, especially young patients, who find compliance with this regimen extremely difficult and frustrating. A new oral chelator has recently come on the market, but it alone may not remove sufficient iron to prevent lifelong problems.

To improve treatment options for patients with chronic severe anemia, the NIH continues to seek new and better iron chelators. In animal tests, one new agent (HBED) has been shown to remove three times more iron than the standard chelator. Although this new chelator must be injected, researchers hope that treatment may be limited to one short injection two or three times a week, which would be less demanding on patients. Phase I clinical trials of this new chelator are under way, supported by the pharmaceutical industry. The NIH is also supporting the adaptation of magnetic resonance technology for the measurement of body iron. This non-invasive technique would be enormously helpful in monitoring the effectiveness of new iron chelating agents and in the possible refinement of therapeutic approaches aimed at achieving iron homeostasis in patients.

Multiple studies by many research teams have shed new light on the mechanisms by which iron is regulated with respect to absorption and movement throughout the body. These research findings are thus helping to lay the groundwork for more accurate means of screening for genetic hemochromatosis and better and safer treatment strategies for combating iron overload in patients with hemochromatosis or Cooley's anemia. The NIH will continue to propel the translation of these and other discoveries into improvements in patient care.

PATIENT PROFILE

Joe Crossan

Hemochromatosis Means Excess Iron Build-up in the Body, Which Can Spell Trouble

Nearing 58 years of age, Joe Crossan thought the only health concerns he needed to deal with were elevated cholesterol levels, somewhat higher than normal blood pressure and being overweight by about 20 pounds. He had no idea that his body was insidiously harboring excess amounts of iron that, if left untreated, could eventually lead to a wide range of serious health conditions, including liver disease, heart abnormalities, damage to his pancreas and adrenal gland, arthritis, thyroid deficiency, and more.

"I was being treated for my cholesterol and having my blood tested when the results came back and I was told that my iron and red blood cell counts were both extremely high." His physician immediately referred him to a hematologist/oncologist specialist…and Joe began thinking that something was seriously wrong.

After conducting a battery of tests, the specialist told Joe that he had some bad news and some good news. The bad news was that Joe suffers from hemochromatosis, an inherited disorder that causes the body to absorb and store too much iron. The extra iron builds up in organs and damages them. Without treatment, the disease can cause these organs to eventually fail. The good news is that treatment for hemochromatosis is simple, inexpensive, and safe.

Just as Joe was able to control his cholesterol, blood pressure and weight through diet and exercise, he was able to get his iron and red blood cell counts well into the normal range through phlebotomies—

Joe Crossan

therapeutic blood-lettings to remove the excess iron from his circulation. In addition, Joe volunteered to participate in an NIH study to determine what damage, if any, his hemochromatosis may have already done to his heart or other organs. He underwent a variety of tests, including magnetic resonance imaging (MRI) to detect iron deposits in vital organs, such as the liver and pancreas; echocardiograms to visualize the heart and assess its function; a video scan of his liver, pancreas and thyroid; a bone density test; and more. Except for some thickening of the wall in one of the four chambers of his heart, "everything else proved negative," says Joe.

Joe was lucky. The disease had been discovered before the iron build-up had time to seriously damage any of his organs. However, because hemochromatosis is usually hereditary, Joe believes his father

PATIENT PROFILE

and mother may not have been as fortunate. His father died of a heart attack at age 62. His mother, though she lived to be 88, experienced severe arthritic pain (a symptom of the disease) to the point that, in her later years, she was almost completely disabled. "I believe both my parents may have suffered from hemochromatosis that had never been diagnosed," says Joe.

Joe, on the other hand, is now 60 years old, retired, and looking forward to continuing his lifelong passions of fishing and woodworking, and to spending time with his wife and four grown children. "I feel extremely fortunate," he says.

About Hemochromatosis

Genetic or hereditary hemochromatosis is one of the most common genetic disorders in the United States and is primarily associated with a defect in a gene called *HFE*. There are two known, important genetic mutations in *HFE*, termed C282Y and H63D. C282Y is the most important. When C282Y is inherited from both parents, iron is overabsorbed from the diet and hemochromatosis can result. Someone who inherits the mutated gene from only one parent is a carrier for the disease, but usually does not develop it.

Interestingly, recent studies have revealed that not everyone who inherits two copies of C282Y develops clinical symptoms of hemochromatosis. Researchers are still studying why this is so. One hypothesis is that variations in other genes involved in iron absorption and metabolism "modify" the disease manifestation (phenotype) of hereditary hemochromatosis. (See the accompanying "Story of Discovery: Combating Toxic Iron Overload.")

As a middle-aged, white male of Irish/German decent, Joe presented the stereotypical profile for the disease. The genetic defect of hemochromatosis is present at birth, but symptoms rarely appear before adulthood. Also, the disease is less common in African Americans, Asian Americans, Hispanic Americans, and American Indians. And although men and women can inherit the gene defect, men are about five times more likely to be diagnosed with the effects of hereditary hemochromatosis than women.[1]

Excess iron in the body, if not detected early and treated, may eventually lead to serious health problems, such as:

- Arthritis
- Liver disease, including an enlarged liver, cirrhosis, cancer and liver failure
- Damage to the pancreas, possibly causing diabetes
- Heart abnormalities, such as irregular heart rhythms or congestive heart failure
- Impotence
- Early menopause
- Abnormal pigmentation of the skin, making it look gray or bronze
- Thyroid deficiency
- Damage to the adrenal gland

Joint pain is the most common complaint of people with hemochromatosis. Joe, in fact, experienced some minor pain in his knuckles, but because his mother suffered with arthritis, he didn't think much of it. He also had some skin discoloration on his fingers and the lower part of his legs.

Other common symptoms include fatigue, abdominal pain, and loss of sex drive. Symptoms tend to occur in men between the ages of 30 and 50, and in women over age 50. Many people, however, may have no symptoms when they are diagnosed. In fact, because it is considered a rare disorder, and initial symptoms often mimic the symptoms of many other diseases, hemochromatosis often goes undiagnosed. Also, physicians may focus on the conditions caused by the disease—arthritis, liver disease, heart disease, and diabetes—rather than on the underlying iron overload itself.

Treating the Disease

Once diagnosed, the first step in treating hemochromatosis is to rid the body of excess iron. The process is called phlebotomy, which simply means removing blood—which, in turn, removes excess iron from the circulation, allowing total body iron to gradually drop back to normal levels. The blood is drawn the same way it is drawn from donors at blood banks, which means "the treatment is no more painful than giving blood," confirms Joe. Depending on the severity of the iron overload, a pint of blood is taken once or twice a week for several months to a year, occasionally longer, and then on a more intermittent schedule.

When Joe was first diagnosed with the disease, his level of ferritin—a measure for the concentration of iron in the blood, expressed as nanograms per milliliter of serum—was 1650; normal levels are between 12 and 300.[2] It took 35 withdrawals and nearly a year before his ferritin levels returned to within the normal range. During the treatment period, Joe felt fatigued. As his ferritin numbers decreased, the skin discoloration in his fingers and lower legs began to recede, as did the pain in his joints. "They weren't stiff anymore," says Joe. He is currently in the maintenance stage of his treatment, in which blood is withdrawn every few months, instead of nearly every week.

At one point during the treatment, Joe asked whether the blood that was being drawn from him was good. He was told that his blood was "very good," perhaps too rich in iron for someone like Joe, but fine for someone with anemia, who could benefit from healthy, iron rich blood. When Joe learned that his blood was being discarded, he told the physician, "I'd like to do something else with it." Joe's blood is now being infused into a person with anemia caused by sickle cell disease, and Joe is extremely pleased to know that his blood is being put to good use. "It's very satisfying to know that I'm helping someone else with his or her health crisis," says Joe.

Although treating hemochromatosis is relatively easy, there is no simple, inexpensive, and accurate test for routine screening, and the options that do exist have their limitations. DNA testing, for example, provides a definitive diagnosis, but is expensive, and currently cannot be used to accurately predict who will develop disease symptoms. Blood tests for transferrin saturation are widely available and relatively inexpensive, but unless performed carefully and more than once, may not produce a correct diagnosis.

Therefore, health professionals recommend the following:

- Brothers and sisters of people who have hemochromatosis should have their blood tested to see if they have the disease or are carriers.
- Parents, children and other close relatives of people who have been diagnosed with the disease should consider being tested.
- Doctors should consider testing people who have joint disease, severe and continuing fatigue, heart disease, elevated liver enzymes, impotence, and/or diabetes, because these conditions may result from hemochromatosis.

Joe's sister, for example, has the disease. As a result, he is urging others in his family to get tested. "Thirty years ago, when my father died, very little was known about hemochromatosis. Thanks to research, a lot is known today about the disease. People at risk, at the very least, need to have their blood tested, and if their ferritin levels are found to be high, they need to be treated."

---

[1] *http://digestive.niddk.nih.gov/ddiseases/pubs/hemochromatosis/*

[2] *http://www.nlm.nih.gov/medlineplus/ency/article/003490.htm*