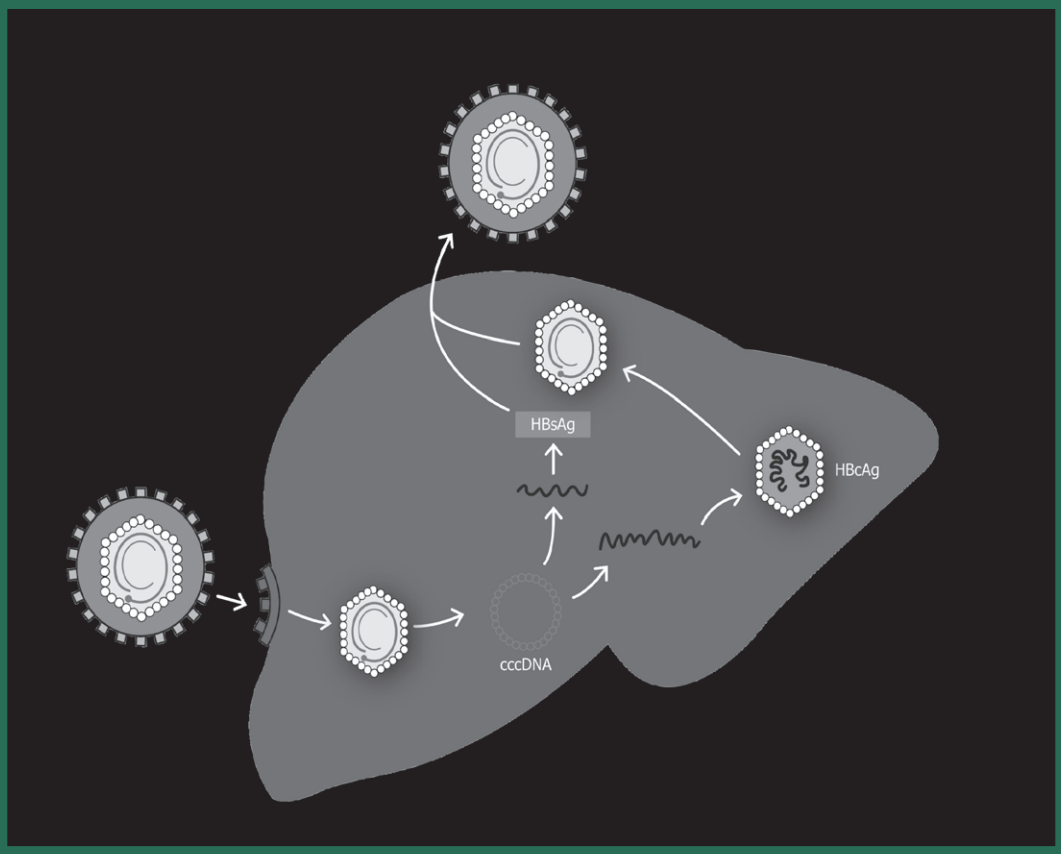

Illustration of the life cycle of the hepatitis B virus in hepatocytes. Knowledge of how hepatitis B infects the liver has aided the development of many new treatments for this disease.

Image courtesy of Dr. Edward Doo, reprinted from the Management of Chronic Hepatitis B: 2006 conference, April 2006 (http://www.niddk.nih.gov/fund/other/hbv2006/index.htm).

Diseases of the Liver and Biliary System

SUMMARY OF RESEARCH GOALS

Major research goals relating to diseases of the liver and biliary system were set forth in the trans-NIH *Action Plan for Liver Disease Research*, which was released in 2004. Taking that effort into consideration, the Commission proposes research goals that are intended to complement and reinforce the comprehensive recommendations made in the *Action Plan*. Understanding normal liver and biliary function and development will provide a solid foundation for new approaches to detect, prevent, and treat liver and biliary diseases. Although the burden of some forms of viral hepatitis has decreased in recent years due to efforts such as the development of vaccines and antiviral therapies, more work is needed to find safe, effective means for prevention and treatment of all forms of acute and chronic viral hepatitis, as well as human immunodeficiency virus (HIV)-associated liver disease. Hepatic steatosis (fatty liver disease) is an increasingly common form of liver disease in the U.S. Research to discover the basic mechanisms underlying steatosis will point to new therapeutic strategies. Similarly, research is needed to uncover the genetic bases and fundamental cellular mechanisms of a range of disorders, including drug-induced liver disease, autoimmune diseases of the liver, childhood syndromes and other hereditary liver diseases, cirrhosis, liver cancers, and gallstones. More knowledge of all of these conditions will accelerate the search for new means of prevention, diagnosis, and treatment, such as improved procedures for liver transplantation, to reduce the burden of liver and biliary diseases in the U.S.

INTRODUCTION AND BACKGROUND

Diseases of the liver and biliary system are major causes of illness and death worldwide and in the U.S. These diseases encompass a wide range of conditions, from viral hepatitis to gallstones, alcoholic hepatitis, fatty liver disease, autoimmune liver and biliary conditions, inherited and congenital disorders, liver conditions caused by toxins or medications, and liver and bile duct cancer. Collectively, diseases of the liver and biliary system rank in the top ten causes of death and accounted for over 55,000 deaths in 2004 (~2.5 percent of all deaths). Importantly, the majority of liver and biliary diseases can be accurately and easily diagnosed, and specific therapies are available for most. This optimistic assessment, however, is a recent change. Fifty years ago, most causes of liver disease were unknown, diagnosis was difficult, and only one or two rare conditions were treatable. This dramatic change in the last 50 years has been brought about by significant advances in our understanding of the liver and its diseases followed by inroads into means of prevention and cure.

Examples of important breakthroughs include: (1) discovery of the hepatitis B virus in 1964, followed by development of means for its detection, prevention, and treatment; (2) discovery of the hepatitis A virus in 1974 with subsequent development of means for diagnosis and an effective hepatitis A vaccine; (3) discovery of the hepatitis C virus in 1989, followed rapidly by means for its detection, prevention of its spread by blood transfusion, and steady improvement in treatment; (4) development of liver transplantation over the period of 1963 to 1983 with its subsequent acceptance as the standard of care for management of end-stage liver disease and liver cancer; (5) improvements in understanding how medications can cause serious liver disease, including the mechanisms by which acetaminophen injures the liver and how aspirin, when given to children during viral illnesses, can lead to fatal liver injury; (6) development of means of early detection of liver cancer at a point when resection or liver transplantation can prevent spread and prolong life; and (7) improved means of diagnosis and management of gallstones and evolution of laparoscopic surgery for cholecystectomy (removal of the gallbladder).

These advances have all been the result of progress in medical research on liver and biliary disease and have had a material effect on the frequency (incidence and prevalence) and impact (mortality and morbidity) of liver disease. Rates of death from cirrhosis and end-stage liver disease have been steadily declining in the last 15 years and are at an all-time low. Rates of acute hepatitis A, B, and C have declined by more than 80 percent in the last 20 years, and these diseases are now at historically low levels.

The changes in understanding and ability to diagnose, prevent, and treat liver disease in the last 25 to 50 years have been profound. However, the burden of liver disease in the U.S. remains an important problem. Deaths from liver and biliary disease account for approximately 2.5 percent of all deaths and have remained constant at approximately 55,000 per year for the last 10 years. Over the last decade, liver cancer incidence has increased in the U.S. at a rate second only to that of adenocarcinoma of the esophagus. Hepatitis C remains a major cause of end-stage liver disease and takes an increasing toll on medical resources. Pediatric liver disease, although rare, remains largely untreatable except with liver transplantation. Gallstone disease remains common, and surgery for gallstones is still a major health cost in the U.S. With the burgeoning epidemic of obesity throughout the world, non-alcoholic fatty liver

disease is emerging as an important new cause of significant liver disease.

These factors are the basis for a call to promote further research on liver and biliary tract structure, function, and disease. Given the recent dramatic advances in genetics and genomics research, with the completion of the Human Genome Project and with very substantial advances in cell and molecular biology, the promise of research directed at specific liver conditions is all the greater.

The preparation of this chapter was greatly aided by the availability of the 2004 comprehensive plan for research in liver and biliary diseases, the trans-NIH *Action Plan for Liver Disease Research* (http://liverplan.niddk.nih.gov). Developed by a series of 16 working groups of researchers, academicians, and lay persons involved in liver disease research, the *Action Plan* proposed a total of 214 research goals in 16 categorical areas of research. The National Commission for Digestive Diseases, in recognizing the major contribution of that plan and its updates, made extensive use of the *Action Plan* as a foundation to synthesize and update goals for research in liver and biliary diseases for this research plan. This chapter extensively cross-references the goals of the *Action Plan*, and the two documents are intended to be used in a complementary fashion by those interested in advancing liver and biliary disease research. The cross-referenced goal in the *Action Plan* is provided after the listing of each research objective in this document (by *Action Plan* chapter and goal number). In addition, the structure of this chapter, including the alignment of recent research advances with individual research goals, is meant to optimize cross-referencing with the *Action Plan* and is not an indication of priority of scientific topics.

GOALS FOR RESEARCH

MOLECULAR AND CELL BIOLOGY OF THE LIVER AND BILIARY SYSTEM (*ACTION PLAN* CHAPTER 1)

Recent Research Advance

Progress in defining molecular pathways controlling liver and biliary cells. There are numerous recent examples of continuing progress in defining the molecular pathways that control liver and biliary cell function. Two striking advances, one on hepatocyte polarity and another on regulation of cholesterol synthesis, are described here. Radixin is a major hepatocyte protein that has been shown to tether proteins such as MRP2 to the canalicular membrane; siRNA suppression of radixin results in loss of polarity, structure, and function of apical hepatocyte membranes. Studies in cell culture have further strengthened the "convergent mechanism" for feedback control of cholesterol synthesis and uptake by hepatocytes; this control is mediated by sterol-regulatory element binding proteins (SREBPs) in the endoplasmic reticulum (ER). In the presence of low levels of cholesterol, SREBPs move to the Golgi and release a transcription factor portion that up-regulates cholesterol synthetic and transporter pathways, but in the presence of high levels of cholesterol, SREBPs are bound to Scap and Insig-1 and retained in the ER, resulting in decreased transcription of target genes.

Research Goal 11.1: Define the molecules, processes, and pathways that underlie normal liver cell function, which can then be applied to understanding the cellular and molecular basis of disease processes.

The liver is the largest solid organ in the body and serves essential functions in regulating metabolism and homeostasis. The major cell of the liver is the hepatocyte, one of the body's most versatile cells, which serves to produce proteins, lipids, and complex carbohydrates, as well as to regulate energy balance, glucose and cholesterol metabolism, and to excrete foreign substances and breakdown products of metabolism, drugs, or toxins by excreting them directly into the bile or converting them to forms that can be excreted in the urine. No single test can measure the overall function of the liver and its hepatocytes, and the full range of their activity has yet to be completely described. Research on molecular and cell biology of the liver and biliary system is essential to understanding the functions of the liver, how they are altered in disease, and how they can be corrected or improved.

Objectives:

- Define how molecules are transported across membranes and to specific sites in the liver cell (cell trafficking). (Chapter 1: A3, C1)
- Fully understand the signaling mechanisms that control the activity of liver cells (signal transduction) and how these signaling mechanisms interact. (Chapter 1: A1)
- Elucidate the steps of regulation of cholesterol and lipid synthesis, transport, and excretion. (Chapter 1: A2)
- Develop a comprehensive knowledge base of the normal liver proteome, comprising an analysis of the proteins in the hepatocytes, their amino acid sequences, carbohydrate and lipid modifications, secondary and tertiary structure, interactions, and functions. A better understanding of the normal liver proteome would advance all components of knowledge about the liver and its functioning. (Chapter 1: C3)

LIVER CELL INJURY, INFLAMMATION, FIBROSIS, AND REPAIR (*ACTION PLAN* CHAPTER 2)

Recent Research Advance

Mechanisms of fibrosis. An important research advance has been the recognition that activation of hepatic stellate cells is a major factor in hepatic fibrosis. Further dissection of the pathways and cells involved in this process may provide the basis for therapeutic interventions to prevent or reverse fibrosis in the liver.

Research Goal 11.2: Understand the cellular mechanisms of liver injury, inflammation, repair, and fibrosis and develop effective means for monitoring and treating diseases caused by these processes.

Tissue injury and inflammation, repair, and fibrosis are fundamental components of all forms of acute and chronic liver diseases. Understanding the mechanisms of liver cell injury and the resulting inflammation, fibrosis, and repair will help to develop ways of preventing liver injury and reversing its effects. Liver cells die in response to inflammation or immune attack in an orderly, predetermined fashion, known as programmed cell death (apoptosis). The cellular pathways involved in apoptosis are complex, but their delineation would help in developing means to treat virtually all liver diseases. Liver cell injury is usually accompanied by inflammation and immune reactivity. Cell injury, particularly if severe and accompanied by inflammation, can lead to abnormal healing and progressive hepatic fibrosis. Understanding fibrosis and the processes that lead to cirrhosis, including how to detect fibrosis in its early stages, are major goals for research in this area.

GOALS FOR RESEARCH

Objectives:

- Delineate the steps in the process of hepatocyte apoptosis. (Chapter 2: A2a)
- Understand how liver cells produce and respond to inflammatory mediators and cytotoxic signaling mechanisms. (Chapter 2: A1a, B1)
- Develop better small animal models for liver cell injury, inflammation, repair, and fibrosis. (Chapter 2: C1)
- Develop noninvasive means of assessing liver injury and fibrosis. (Chapter 2: A3; Chapter 16: C1b)
- Translate findings about cell injury, inflammation, repair, and fibrosis to clinical diseases. Identify small molecules that might alter these processes and form the basis for translational research. (Chapter 2: B3, C2a, C3)

DEVELOPMENTAL BIOLOGY AND REGENERATION OF THE LIVER (*ACTION PLAN* CHAPTER 3)

Recent Research Advance

Identification and characterization of hepatic stem cells in fetal and adult liver. Multipotent progenitor cells have been isolated from human fetal liver that can differentiate into hepatocytes and cholangiocytes. In mice, embryonic stem cells can be induced to differentiate into hepatic lineages and can contribute to hepatic repair when transplanted into immune-deficient mice.

Research Goal 11.3: Define the molecular and cellular mechanisms underlying liver development and regeneration in health and disease and apply these findings to developing improved therapies for liver disease.

The liver has a marked ability to regenerate. Resection of half of the human liver is followed within 2-4 weeks by full restoration of the liver structure, size, and function. The process of regeneration recapitulates, in many respects, the development of the liver *in utero*. Understanding the developmental biology of the liver, how embryonic stem cells differentiate into mature and functional hepatocytes and cholangiocytes, and what processes and cellular signals initiate, promote, and conclude regeneration would aid understanding of liver disease and how the liver recovers from cell injury. Identifying signals that promote regeneration may well lead to therapies that would aid in recovery from liver injury or surgery. Such research is also likely to help identify biomarkers for regeneration and for failure of regeneration in situations such as acute liver failure or liver transplantation.

Objectives:

- Define the cellular and molecular events that underlie liver development and what processes are shared with liver regeneration. (Chapter 3: A1b, B3).
- Identify and characterize the stem cells of the liver and determine how to use such cells in gene transfer and transplant studies to correct genetic mutations that lead to inherited diseases, such as hemophilia, porphyria, alpha-1-antitrypsin deficiency, Crigler-Najjar syndrome, and other devastating diseases. (Chapter 3: A1a, C3a).
- Improve gene transfer techniques, gene transfer vectors, and means of supporting cell viability as they are directed to home to the liver (Chapter 3: B1a, C1).

BILE, BILIRUBIN, AND CHOLESTASIS (*ACTION PLAN* CHAPTER 4)

Recent Research Advance

Identification of physical and molecular regulators of bile flow. Cilia on cholangiocytes have been shown to respond to bile flow and induce increases in intracellular calcium and decreases in cyclic AMP mediated through flow receptors (polycystin-1), calcium channels (polycystin-2), and adenylyl cyclase on the cilia, providing a new model for regulation of ductal bile secretion. Fibroblast growth factor (FGF)-15 has been identified as playing an important role in gallbladder filling. FGF-15 is induced in the terminal ileum as a result of bile acid signaling through the farnesoid X receptor (FXR) and circulates in a hormonal fashion to the liver and gallbladder where it leads to gallbladder filling by acting on cyclic AMP-linked receptors on biliary smooth muscle cells. Thus, intra- and inter-cellular signals coordinate to control gallbladder filling and emptying.

Research Goal 11.4: Delineate the normal pathways of uptake, metabolism, and secretion of bile salts, bilirubin, and other biliary lipids and solutes; characterize the alterations in these pathways that participate in the pathogenesis of liver diseases; and develop means for diagnosis, treatment, and prevention of cholestatic liver disease and disorders of bilirubin metabolism.

A major and distinctive function of the liver is to make and excrete bile. Bile facilitates digestion and absorption of lipids and is also the major means of elimination of cholesterol and the breakdown products of metabolism, such as bilirubin. Persons with severe liver disease usually have altered bile formation and retention of the products of bile and bilirubin, which results in jaundice. Disruptions in the steps of bilirubin elimination and bile formation are responsible for many of the severe inherited forms of liver disease in children. Understanding the processes that lead to bile formation and excretion will provide necessary insights into diseases of the liver and how to correct them. Regulation of these pathways in health and disease might well improve liver function and aid in recovery from liver injury. Furthermore, mutations in the genes that regulate the steps in bile and cholesterol metabolism account for several severe forms of inherited liver disease that might be alleviated by altering these pathways using small molecules or gene therapy.

Objectives:

- Fully define the normal physiology and regulation of bile formation, including cholesterol synthesis and catabolism. (Chapter 4: B2a, B2b)
- Understand the pathophysiology of acquired forms of cholestatic liver disease, which disturbed processes account for the retention of bile, and how to alleviate the consequences of bile retention. (Chapter 4: A2, C1, C2)

VIRAL HEPATITIS (*ACTION PLAN* CHAPTER 5)

Recent Research Advances

New treatments for hepatitis B. In the past 5 years, new drugs that block hepatitis B virus (HBV) replication have been developed and tested, such that there are now six licensed options. The effectiveness of each of these has been characterized, and guidelines for their use have been updated. Antiviral therapy of hepatitis B has been shown to prevent disease progression.

Understanding the life cycle of hepatitis C virus (HCV). A landmark breakthrough in HCV research has been the recent growth of this virus in cell culture, which will permit more detailed studies of the entire viral life cycle, including initial infection, replication, packaging, and release of virus. Detailed

knowledge of these steps will permit identification of potential new approaches to treating the infection.

Demonstration of the efficacy and safety of a hepatitis E virus (HEV) vaccine. A landmark study conducted in Nepal has shown protective immunity against HEV infection with a three-dose schedule of a recombinant HEV vaccine.

The research goal in the area of viral hepatitis is to develop practical, safe, and effective means of prevention, treatment, and control of all forms of the disease. Viral hepatitis can be caused by any one of five viruses that infect the liver in humans, appropriately named hepatitis A, B, C, D, and E virus. Hepatitis A and E are forms of infectious hepatitis that are very contagious, occur in outbreaks of typically acute clinical courses, and are associated with poor sanitation and fecal-oral spread. Both viruses can cause severe hepatitis, but they do not cause chronic liver injury or cirrhosis. Hepatitis B, C, and D are forms of serum hepatitis that are less infectious, can cause chronic infection, are blood-borne, and are spread largely by parenteral or sexual routes. All five viruses have been identified in the last 30 years, and tests to identify infections and means of prevention with immune globulin or vaccines are available for four of them (A, B, D, and E). The incidence of new cases of viral hepatitis has been decreasing steadily in the U.S. for the last 20 years. Nevertheless, cases still occur and cases of chronic hepatitis B and C remain common causes of cirrhosis and end-stage liver disease. Full control and elimination of viral hepatitis will require several more breakthroughs in hepatitis research.

Research Goal 11.5: Develop safe and effective means to prevent and treat hepatitis C.

Progress in developing a hepatitis C vaccine has been impeded by the lack of apparent immunity from re-infection and lack of cell culture and small animal systems to study infectivity. The recent description of a cell culture propagation method for a strain of hepatitis C may help overcome these difficulties. Current therapies are effective in only half of patients and are poorly effective or too toxic for patients with advanced liver disease, patients with a solid organ transplant, or those who have other serious co-morbidities, such as renal, cardiac, or cerebrovascular disease.

Objectives:

- Develop a vaccine or specific means of prevention of hepatitis C. (Chapter 5: C3a)
- Develop safer and more effective means of treating chronic hepatitis C that can be applied to all categories of patients. (Chapter 5: B2a, B1b, C2a, C2b)
- Understand the factors underlying the differential disease burden and poorer response to therapy in African American patients with hepatitis C.
- Better understand the structure and replication cycle of the hepatitis C virus to help develop new therapeutic targets and better small molecular therapies for this disease. (Chapter 5: A3, B1a, B3a)
- Better understand the host and HCV interactions that determine viral clearance versus persistence. (Chapter 5: A2, B1a, B3a)

Research Goal 11.6: Improve strategies for use of current therapies of hepatitis B and develop new, improved treatment regimens.

Advances in hepatitis B research in the last 40 years have been impressive, but further efforts are needed for the complete control of this disease, including defining how to best use available therapies in practice. There are currently at least six licensed therapies for hepatitis B, all of which lead to improvements in the disease when given for 1-2 years; however, the optimal therapy or combinations of therapies, how they are to be

given, and for how long have yet to be identified. In addition, new classes of therapies that target other viral replication steps or elicit favorable immune responses would enhance treatment options for hepatitis B.

Objectives:

- Develop a better understanding of the pathogenesis of hepatitis B in all its forms (acute, chronic, active, inactive). (Chapter 5: B1a, B2b, B3b)
- Better define the optimal means for treatment of chronic hepatitis B. (Chapter 5: B2a, B2b, C1a, C1b, C3b)
- Understand the factors responsible for the ethnic and racial disparities in hepatitis B infection, which disproportionately affects Asian Americans in the U.S.
- Conduct clinical trials to compare multimodality therapies for hepatitis B. (Chapter 5: C1b)

Research Goal 11.7: Develop improved means to prevent and manage acute viral hepatitis.

At present, only emergency liver transplantation has been shown to be effective in management of acute liver failure due to viral hepatitis. New medical therapies are needed, as these diseases can be severe, protracted, and even fatal.

Objective:

- Develop and evaluate new approaches to therapy in all five forms of acute viral hepatitis. (Chapter 5: B2a, C1a)

HIV INFECTION AND THE LIVER (*ACTION PLAN* CHAPTER 6)

Recent Research Advance

Regimens to treat HIV/HBV co-infection. The response to therapies for chronic viral hepatitis in patients with concurrent treatment for HIV infection is different from patients with mono-infection, and there is considerably less evidence for optimal treatment regimens. Recently, tenofovir has been shown to have more potent antiviral activity against HBV than adefovir in HIV/HBV co-infected persons. The combination of tenofovir and emtricitabine is now recommended as standard of therapy for HBV/HIV co-infection.

Research Goal 11.8: Define the causes of liver disease associated with HIV and develop means to prevent and treat liver disease in HIV-infected persons.

Patients with HIV infection are susceptible to a wide range of liver diseases, and the interactions of these diseases with HIV infection and its therapies are complex and challenging. As patients with HIV infection are surviving longer as a result of effective anti-retroviral therapy, liver disease has become an increasingly important cause of morbidity and mortality. Liver disease among persons with HIV infection represents a major challenge to research. The spectrum of liver conditions among HIV-infected persons includes acute and chronic viral hepatitis; alcoholic and non-alcoholic steatohepatitis; drug-induced liver injury; autoimmune conditions of the liver; bacterial, fungal, and protozoal infections of the liver and biliary system; and liver and bile duct cancers, including lymphomas of the liver. Viral hepatitis is particularly problematic among HIV-infected persons, many of whom are in risk groups with a high rate of hepatitis B and C. The optimal approach to therapy of hepatitis C among HIV-infected persons remains unclear, and better therapies are needed.

Objectives:

- Define the prevalence and incidence of liver disease in HIV-infected persons and identify the causes of liver disease associated with HIV, as well as their means of diagnosis, prognosis, and management. (Chapter 6: B1a, B2a, B2b, B3, C3b)

- Identify the optimal approach to therapy of HCV in HIV-co-infected individuals and apply new agents active against HCV as soon as possible to this important cohort of patients. (Chapter 6: A1b, A2, C1b)

FATTY LIVER DISEASE (*ACTION PLAN* CHAPTER 7)

Recent Research Advance

Treatment of non-alcoholic steatohepatitis (NASH). In a proof-of-concept study, the administration of pioglitazone led to metabolic and histologic improvement in subjects with NASH, justifying the need for larger clinical trials, which are ongoing.

Research Goal 11.9: Understand the basic mechanisms of injury and develop means to prevent and treat non-alcoholic and alcoholic fatty liver disease.

Fatty liver disease occurs in two major forms: alcoholic and non-alcoholic, both of which are marked by accumulation of fat in the liver followed by inflammation, liver cell injury, fibrosis, and cirrhosis. Alcoholic liver disease is a major cause of illness and accounts for up to half of deaths from liver disease. Non-alcoholic fatty liver disease is a somewhat newly described problem, but appears to be the major reason for liver test abnormalities among Americans and is an increasingly common cause of significant liver disease and the need for liver transplantation. The cause of non-alcoholic fatty liver disease is unknown, but it is closely linked to obesity, diabetes, and high triglyceride levels and, thus, appears to be a part of the metabolic syndrome. There are no current means of treatment of either form of fatty liver disease, other than abstinence for alcoholic forms and attempts at weight loss for the metabolic forms of fatty liver disease.

Objectives:

- Identify the underlying pathogenesis of non-alcoholic fatty liver disease. (Chapter 7: A3, B2a)
- Elucidate the basic mechanisms of pathogenesis of alcoholic liver disease. (Chapter 7: B1a, B2a)
- Develop noninvasive means to distinguish steatosis and steatohepatitis. (Chapter 7: B2b; Chapter 16, C1b)
- Identify and test safe and effective means of treatment of both forms of fatty liver disease. (Chapter 7: B1b, B3a, B3b, C1a, C1b)

DRUG-INDUCED LIVER DISEASE (*ACTION PLAN* CHAPTER 8)

Recent Research Advance

Development of a diagnostic assay for acetaminophen toxicity. An assay for acetaminophen adducts has been developed and shown to identify 90-100 percent of cases of acetaminophen-induced acute liver failure in adults and children. These adducts are not present in cases of acute liver failure of other known causes, but are present in 12-19 percent of cases of unknown cause. Thus, some idiopathic cases of acute liver failure may be caused by unrecognized or unacknowledged acetaminophen overdose—a finding that has major therapeutic implications. The test requires further modification to become clinically useful, and its sensitivity for milder forms of acetaminophen injury requires elucidation.

Research Goal 11.10: Establish means to predict, prevent, diagnose, and treat hepatotoxicity due to drugs, herbal medications, and environmental toxicants.

Drug-induced liver disease has become an increasingly important health problem in the U.S. Liver injury from medications is rare, but can be protracted, severe, and even fatal. Indeed,

drug-induced liver disease is now the leading cause of death from acute liver failure. While medications rarely cause liver injury, the increasing use of greater numbers of medications by the American public has raised the importance of this problem. In addition, the American public has also been increasingly using herbal medications and combinations of minerals, vitamins, and natural products, many of which can cause liver injury. At the same time, there are few insights into why medications damage the liver and why hundreds or thousands of people can take a medication safely, while a rare individual gets serious liver disease. Finally, drug-induced liver disease is challenging to the physician, has many diverse manifestations, and diagnosis is often delayed or missed. For all these reasons, new approaches are needed to broaden the understanding of drug-induced liver injury and how to prevent or manage it properly.

Objectives:

- Develop reliable animal models or laboratory systems to study different forms of drug-induced liver injury. (Chapter 8: A3a, B3b)
- Elucidate the genetic basis of drug- and toxicant-induced liver injury. (Chapter 8: C1)
- Develop standardized methods to accurately diagnose drug-induced liver toxicity. (Chapter 8: A1)
- Develop predictive tests for risk of drug-induced liver injury that may allow for prevention of serious liver disease. (Chapter 8: C2b, C3)
- Develop effective means of treating drug-induced liver injury. (Chapter 8: C2a)

AUTOIMMUNE LIVER DISEASES (*ACTION PLAN* CHAPTER 9)

Recent Research Advance

Animal models of autoimmune liver diseases. Rapid research progress in autoimmune liver diseases has been significantly hindered by the absence of animal models. In the last year, three promising models for primary biliary cirrhosis (PBC) have been described, including the *Nod.c3c4* congenic mouse, the TGF-β receptor II dominant-negative mouse, and an IL-2 receptor α knock-out (-/-) mouse. Each of these mouse models develops liver disease and anti-mitochondrial antibody reactivity with specificity for PDC-E2, typical of the human autoantibody. Further work in the *Mdr2* knock-out (-/-) mouse model for primary sclerosing cholangitis (PSC) indicates that side-chain modification of ursodiol yields a bile acid with greater therapeutic activity against PSC than standard ursodiol. These and additional models under development will provide new opportunities for research into disease mechanisms that cannot be accomplished in human research.

Research Goal 11.11: Determine the etiology, pathogenesis, and potential new targets for therapy of the three major forms of autoimmune liver disease: autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC).

The three major forms of autoimmune liver disease—AIH, PBC, and PSC—are distinctly different forms of liver diseases that are characterized by inflammatory destruction of hepatocytes or the biliary system and clinical features of autoimmunity. While uncommon, all are important causes of chronic liver disease that may progress to cirrhosis, liver failure, need for transplantation, or liver or biliary cancer. Current approaches to medical management are not curative and are sometimes completely ineffective. AIH has many common features of prototypic autoimmune diseases, including female predominance, typical autoantibodies, and responsiveness to corticosteroids and immunosuppressive therapies. PBC also has female predominance, typical autoantibodies, and seems to be unresponsive to standard anti-inflammatory or immunosuppressive regimens, but does respond to treatment with ursodiol. In contrast, PSC has

male predominance, is commonly associated with inflammatory bowel diseases (IBD), and is refractory to all forms of conventional therapy, including treatment of associated IBD. Syndromes of AIH and PSC may occur in early childhood, but have features that distinguish them from their adult counterparts with the same names. Many hurdles have impeded research progress in these diseases, including among others their slow, indolent clinical course, low prevalence, absence of animal models, and the great difficulty of conducting immunopathogenesis studies in human liver.

Objectives:

- Develop robust animal models to study mechanisms of liver injury associated with autoimmunity and conduct preclinical studies of novel treatments. (Chapter 9: B3b)
- Define the genetic risk alleles for each of the three major forms of autoimmune liver disease, followed by identification of physiologic pathways associated with these alleles that may contribute to understanding the pathogenesis or reveal new opportunities for therapy. (Chapter 9: B3a)
- Identify novel biomarkers and define surrogate endpoints to assist in diagnosis, assess disease activity, or predict natural history or response to different treatments. (Chapter 9: C2)
- Conduct natural history studies in childhood onset autoimmune liver diseases to refine phenotype definitions, identify genetic risk alleles, and search for potential environmental triggers. (Chapter 9: A2)

PEDIATRIC LIVER DISEASES (*ACTION PLAN* CHAPTER 10)

Recent Research Advances

Molecular basis of Alagille syndrome. Using a variety of molecular approaches, Jagged1 (*JAG1*) mutations can be identified in 94 percent of children with Alagille syndrome, and a proportion of the remaining children have mutations in the gene encoding NOTCH2, the receptor for Jagged1.

Genetic basis of diseases causing intrahepatic cholestasis in children. Studies of a number of cholestatic diseases of children have led to the identification of genetic defects in bile synthesis, formation, and secretion and in development of the biliary tree. These discoveries have also led to the development of new murine models that provide new research opportunities.

Research Goal 11.12: Determine the molecular and genetic pathways responsible for the major forms of inherited and early-onset, severe liver diseases of childhood, including biliary atresia, neonatal hepatitis, progressive familial intrahepatic cholestasis, Alagille syndrome, alpha-1-antitrypsin deficiency, neonatal hemochromatosis, and mitochondrial hepatopathies in order to devise potential new targets for therapy.

A number of liver diseases of early childhood, listed above, are often severe and lead to progressive liver failure and need for transplantation. While all of these diseases are rare, the most common—biliary atresia—is also probably the least understood. A surgical procedure, the Kasai procedure, may ameliorate the disease when performed early in life, but the majority of patients continue to have progressive liver failure, ultimately requiring transplantation. While the genetic basis is known for some of the other forms of familial diseases, current knowledge of the genes and their molecular pathways has yet to be translated into therapies for these diseases, and liver transplantation remains the only form of therapy for many patients.

Objectives:

- Define the etiology and pathogenesis of biliary atresia and identify new pathways for development of potential therapies. (Chapter 10: C3a)

- Optimize current approaches to medical and surgical therapy of biliary atresia. (Chapter 10: C1a)
- For familial childhood liver diseases, determine their genetic basis, including primary alleles and modifying alleles, which will permit development of standardized genetic tests and identification of potential pathways for development of translational therapies. (Chapter 10: A1b, B2a, C2)

Research Goal 11.13: Evaluate and improve existing adult medical and surgical therapies for treatment of children with liver diseases.

In addition to familial and congenital diseases of children that often have a severe course and present early in life, children may also be affected by diseases that are found in adults, including viral hepatitis, NASH, drug-induced liver disease, fulminant hepatitis, autoimmune liver diseases, and other genetic diseases, such as Wilson disease. While medical therapies may exist for some of these conditions, such as viral hepatitis, typically they have not been carefully validated and optimized for treatment of children. Furthermore, while liver transplantation is often successful and may be life-saving for end-stage liver diseases in children, long-term outcomes, including effects of transplantation regimens on growth, development, and cognitive function, are unknown.

Objectives:

- Conduct clinical studies to validate use of medical regimens and liver transplantation for treatment of liver diseases in children. (Chapter 10: B1b)
- Identify biomarkers and surrogate markers for assessment of children with chronic liver diseases. (Chapter 10: A1a, B3)

GENETIC LIVER DISEASES (*ACTION PLAN* CHAPTER 11)

Recent Research Advance

Unraveling the pathogenesis of hemochromatosis and iron metabolism. In the past decade, numerous research discoveries have elucidated the pathogenesis of hereditary hemochromatosis and the pathways of iron metabolism that are altered in this disease. In 1996, mutations in the *HFE* gene were shown to account for the majority of cases of hemochromatosis, which was recapitulated in murine models having deletions of this gene. Subsequently, additional proteins regulating iron metabolism were identified, and their roles in the disease were elucidated, including divalent metal transporter 1, transferrin receptor-2, hephaestin, hemojuvelin, ferroportin-1, and most recently hepcidin—a protein produced by hepatocytes that is the key regulator of iron absorption in the gut. Commercial testing for hemochromatosis is now available, and further advances in this field are likely to fully elucidate the molecular mechanisms responsible for this disease and its various manifestations.

Research Goal 11.14: Elucidate the molecular pathways responsible for hereditary forms of liver disease, including hereditary hemochromatosis, Wilson disease, the porphyrias, cystic fibrosis, polycystic liver disease, and congenital hepatic fibrosis; use knowledge of these pathways to devise novel approaches to treatment.

Hereditary hemochromatosis is the most common inherited liver disease in Caucasians, affecting approximately 1 in 200 individuals. In this disease, excess iron absorption leads to serious damage to multiple organs, including the liver, heart, pancreas, and other organs, and liver cancer. Early diagnosis and treatment with phlebotomy prevents all of the complications of this disease. Wilson disease is an uncommon cause of liver disease

due to abnormalities of copper transport and, like hemochromatosis, early recognition and treatment of the disease can prevent complications. There are five main forms of porphyria that can affect the liver, and these rare diseases can cause severe, disabling disease. Some, but not all, forms have treatments of varying efficacy. With longer survival of cystic fibrosis patients, liver disease is emerging as an important long-term complication that can lead to liver failure. Severe polycystic liver disease is an autosomal dominant condition that can lead to liver failure and is caused by genes distinct from those causing polycystic kidney disease. Collectively, for many of these diseases there has been rapid progress in identifying the molecular mechanisms responsible; however, progress has been variable in development of clinical genetic testing and development of novel, effective therapies.

Objectives:

- For each of the genetic liver diseases, define the primary genes and modifying genes and their molecular pathways that lead to disease. (Chapter 11: A1a, A3, B2a, B3, C2a)
- Accelerate translational research to identify new target pathways for treatment, including development of animal models for preclinical testing. (Chapter 11: C2b)
- For diseases having no target for drug treatment or alternative approaches to treatment, develop approaches to gene therapy to correct the underlying defect. (Chapter 11: C3b)
- Develop clinically applicable noninvasive tests to accurately measure metabolic consequences of these diseases, such as iron or copper overload. (Chapter 11: C1, C3a; Chapter 16: C1b)

LIVER TRANSPLANTATION (*ACTION PLAN* CHAPTER 12)

Recent Research Advance

Benefits and risks of living donor liver transplantation. More than 2,000 adult-to-adult living donor liver transplants (LDLT) have been performed in the U.S., yet the potential benefit to liver transplant candidates of undergoing LDLT compared to waiting for deceased donor liver transplant (DDLT) until recently was unknown. A recent large cohort study demonstrated that LDLT was associated with lower mortality than the alternative of waiting for DDLT, although this reduction in mortality of the recipient must be balanced against risks to the living donors.

Research Goal 11.15: Refine current procedures in liver transplantation, including assessment of potential transplant recipients, immunosuppressive regimens, and management of donors and recipients for living donor transplantation, and improve management of recurrent liver diseases in transplanted patients.

Liver transplantation is the standard of care for patients with end-stage liver disease or acute liver failure. The most common procedure used is deceased donor orthotopic liver transplantation, but living donor transplantation is available, particularly for children and to a lesser extent for adult transplantation. The major reason for transplantation is end-stage liver disease from any cause; other indications include acute liver failure, liver cancer, and, rarely, metabolic diseases that can be corrected with transplantation. Despite significant and continuing improvements in liver transplantation with respect to organ procurement and distribution, surgical techniques, and medical management of the transplant patient since the 1960s, a number of challenges remain in this field. Among the problems are the continuing shortage of donor organs, premature death of transplant recipients due to complications of procedures or immunosuppression, and recurrence of underlying liver disease after transplant, such as viral hepatitis or cancer. Ultimately, improvements in preventing the progression of the multiple different forms of chronic liver diseases to end-stage liver disease offers the best solution to the problems of

transplantation, but for the foreseeable future liver transplantation will continue to be an essential form of therapy that requires further improvements through research.

Objectives:

- Further refine the Model for End-Stage Liver Disease (MELD) and Pediatric End-Stage Liver Disease (PELD) systems to optimize allocation of livers for transplantation. (Chapter 12: A1)
- Identify biomarkers for adequate immunosuppression, active rejection, and immune tolerance. (Chapter 12: A2, C2b)
- Develop approaches to improve long-term tolerance for allografts in order to minimize the need for immunosuppressive drugs. (Chapter 12: C2a, C1a)
- Improve treatment for recurrence of underlying liver diseases, such as viral hepatitis, in transplant recipients. (Chapter 12: B2b, C3a)

COMPLICATIONS OF LIVER DISEASE (*ACTION PLAN* CHAPTER 13)

Recent Research Advance

Pathophysiology of portal hypertension. Patients with cirrhosis have a hyperdynamic state in the splanchnic bed that is caused, at least in part, by nitric oxide (NO). Vasodilation induced by NO may be mediated by vascular endothelial growth factor (VEGF). In the liver, the vasodilator response to NO is blunted, which in a rat model appears to be due to up-regulation of endothelial phosphodiesterase-5, the enzyme responsible for degrading NO. Therefore, both phosphodiesterase-5 and VEGF may be targets for therapy of portal hypertension.

Research Goal 11.16: Identify ways to prevent or ameliorate the complications of portal hypertension and cirrhosis.

The majority of patients who die of cirrhosis ultimately succumb to complications of portal hypertension, which include variceal hemorrhage, ascites, spontaneous bacterial peritonitis (SBP), hepatic encephalopathy, and hepatorenal and hepatopulmonary syndromes. Currently available techniques to diagnose and treat these complications have significant limitations and often do not significantly prolong life or eliminate the need for liver transplantation. In addition, patients with advanced liver disease may suffer from intractable fatigue and pruritus.

Objectives:

- Define in detail the pathophysiologic mechanisms that cause portal hypertension. (Chapter 13: A3a)
- Identify small molecular targets for interventions to reduce portal hypertension. (Chapter 13: B3a)
- Develop reliable, non- or minimally invasive methods to measure portal pressure and screen for esophageal varices. (Chapter 13: B3b, C3b)
- Better characterize the cause of increased susceptibility to bacterial infections in cirrhosis, particularly SBP, and determine how to manage these infections. (Chapter 13: A3b, B1)

Research Goal 11.17: Develop better means of prevention, management, and treatment of acute liver failure.

Acute liver failure is defined as the sudden onset of severe liver injury with signs of hepatic failure in a person without previous liver disease. Acute liver failure accounts for up to 2,000 deaths each year in the U.S. and typically strikes previously healthy individuals, including children and adolescents. The major cause of acute liver failure in the U.S. today is drug-induced liver injury, either due to acetaminophen or to idiosyncratic injury due to a medication or herbal preparation. Other causes include hepatitis A and B and autoimmune liver diseases. Strikingly, at least half of acute liver

failure in children and approximately one-quarter of cases in adults are due to unknown causes (idiopathic). While viruses are suspected to be the cause of idiopathic acute liver failure, the actual cause has so far eluded research investigation. Nevertheless, acute liver injury results in death or need for liver transplantation in approximately three-quarters of patients, and there are currently no therapies or means of temporary support for this dire complication of acute liver disease.

Objectives:

- Identify the cause(s) of idiopathic acute liver failure. (Chapter 10: A3)
- Develop biomarkers that more accurately reflect hepatic regeneration and reserve in acute liver failure. (Chapter 13: C2a)
- Develop non-specific, hepatoprotective therapies that improve survival or allow time for liver transplantation in acute liver failure. (Chapter 13: A1b, B2a)
- Develop and evaluate bioartificial liver support devices that improve survival in acute liver failure or allow for temporary support until a liver becomes available for transplantation. (Chapter 13: C3a)

LIVER AND BILIARY CANCER (*ACTION PLAN* CHAPTERS 14 AND 15)

Recent Research Advances

Genetic profiling of hepatocellular carcinoma (HCC). An important research advance in HCC has been the description of the molecular signatures of this form of cancer. Although in an early stage, these discoveries have diagnostic, prognostic, and therapeutic implications, in that they may eventually permit identification of new targets for therapy and stratification of patients based on their type of cancer and individualized therapies. More research is needed to fully realize the value of this advance.

Animal models of cholangiocarcinoma. Advances in diagnosis and treatment of the form of human biliary cancer known as cholangiocarcinoma have been hampered by the absence of animal models for detailed studies that are not possible in humans. Three animal models for cholangiocarcinoma have now been described that should help advance the science and therapy of this disease.

Research Goal 11.18: Develop effective strategies for early detection and treatment of hepatocellular carcinoma and cholangiocarcinoma in high-risk groups.

Malignant diseases of the liver and biliary tree can be either primary or secondary to other forms of cancer that metastasize to the liver. Primary liver cancers include the most common form, HCC, and others, including cholangiocarcinoma, hepatoblastoma, fibrolamellar carcinoma, angiosarcoma, and other rare forms. Most cases of HCC arise in the setting of other chronic liver diseases and cirrhosis; thus, patients at risk include those with many common forms of chronic liver disease, such as viral hepatitis, alcoholic and non-alcoholic fatty liver disease, and essentially any other cause of chronic liver injury. HCC is a highly lethal disease, and current diagnostic and medical treatments have limited efficacy. Liver transplantation is an option for cases having early diagnosis. Other forms of liver cancer, notably cholangiocarcinoma, have other distinctly different risk factors and clinical behaviors and, most notably, this form of cancer is a complication of long-standing primary sclerosing cholangitis.

Objectives:

- Identify new biomarkers for early detection of primary liver cancers, particularly HCC and cholangiocarcinoma. (Chapter 14: A2a; Chapter 15 C2b)

- Develop new imaging techniques that detect primary liver and biliary cancers in the setting of underlying chronic liver disease. (Chapter 14: A3; Chapter 15, A3; Chapter 16, C1b)
- Identify the cellular and molecular pathways leading to primary liver cancer in order to identify potential new targets for therapy. (Chapter 14: A2b, C2, C3)
- Identify strategies to prevent HCC and cholangiocarcinoma in high-risk populations. (Chapter 14: C1; Chapter 15: C1)

GALLBLADDER AND BILIARY DISEASE (*ACTION PLAN* CHAPTER 15)

Recent Research Advances

New approaches to prevent cholesterol gallstones. Human trials on prevention of gallstones have not yet been initiated, but several studies in animal models have suggested potential novel approaches. Fibroblast growth factor (FGF)-15, which is produced in the ileum in response to bile acid signaling through FXR, plays an important role in gallbladder filling. Lack of gallbladder filling may predispose to gallstones, which perhaps explains the link between diseases of the terminal ileum and gallstone formation. Furthermore, agonists of FGF-19 (the human homologue to mouse FGF-15) might play a role in prevention of gallstones. In another study in mice, targeted deletion of *Gpbar1*, a gene involved in regulation of cholesterol secretion, led to resistance to gallstone formation in response to a high-fat diet. Thus, inhibitors of this cell-surface receptor for bile acids may be a means of decreasing the likelihood of gallstones.

Genetic risk for gallstone disease. Researchers have identified variations in the gene encoding hepatic cholesterol transporter ABCG5/G8 that are associated with gallstone disease in human patients. Finding ways to modulate this transporter could lead to important new therapies to prevent or treat the formation of gallstones.

Research Goal 11.19: Develop better means to prevent and treat gallstones.

There are multiple diseases of the gallbladder and biliary tree, including gallstones, acute cholecystitis, acalculous cholecystitis, primary sclerosing cholangitis, biliary atresia, choledochal cysts, gallbladder cancer, and cholangiocarcinoma. However, gallstone disease is by far the most common of these conditions, affecting about 12 percent of the adult U.S. population. Gallstone disease leads to approximately 700,000 cholecystectomies per year and is one of the most costly digestive diseases for the healthcare system.

Objectives:

- Determine the genetic basis for increased risk and protection from gallstone disease. (Chapter 15: A1, C2a)
- Better define the pathophysiologic basis of gallstone formation, including the role of bacterial factors. (Chapter 15: B2)
- Identify biomarkers for gallstone formation. (Chapter 15: B3)
- Design approaches to prevent gallstone formation in high-risk groups. (Chapter 15: C3)

MAJOR CHALLENGES AND STEPS TO ACHIEVE THE RESEARCH GOALS

Basic mechanisms of liver diseases: The mechanisms that trigger damage to the liver are partially known. Development of multidisciplinary and interdisciplinary collaborations between investigators interested in liver diseases and basic scientists in areas such as immunology, genetics, virology, oncology, molecular and cell biology, and other disciplines would be greatly facilitated by creating the necessary resources for collaborative studies, such as gene, liver, and serum repositories of samples from well-defined patients.

Translational research: The absence of robust animal models for many liver diseases has greatly hampered progress in understanding these diseases or preclinical testing of novel therapies. Identification of new models may allow for more rapid progress, and further studies of animal models are needed. Findings from these models need to be rapidly applied to clinical research.

Clinical research: Clinical research in liver diseases is hampered by limitations of standard criteria for case definition, need for and lack of precision in disease assessment by liver biopsy, absence of biomarkers and surrogate markers that are particularly needed for indolent diseases, and absence of a pipeline of potential new therapeutic interventions that might emerge from basic and translational research. When new approaches to treatment become available, clinical trials will often require a substantial number of research centers because of low disease prevalence. In partnership with the U.S. Food and Drug Administration, best practices for clinical trial design could be developed, along with the formation of public-private partnerships with the pharmaceutical industry to generate interest in drug development for uncommon liver and biliary diseases.