Digestive Disease Interagency Coordinating Committee
June 16, 2003

Translational Research in Primary Biliary Cirrhosis

The DDICC held a half day meeting on June 15, 2003 that focused on the current understanding of the etiology of primary biliary cirrhosis (PBC) and status of both standard and experimental therapies. The aims of the workshop were to help define future needs for basic and translational research in PBC.

Dr. Jay Hoofnagle, director of the Liver Disease Research Branch, NIDDK, provided an overview and background to the topic. PBC is an uncommon, although not rare, disease of the liver that is estimated to affect between 2 and 40 persons per 100,000 population. It typically occurs in middle aged women with an average clinical onset of 50 years. PBC has not been described in children and is rarely diagnosed before the age of 30. Women are affected more often than men (in a ratio of 9:1), and PBC is believed to be most common among persons of northern European descent and less frequent among Asians and Africans. The typical clinical onset is with nonspecific, mild and intermittent symptoms of fatigue and itching. At present, however, PBC is often diagnosed before the onset of symptoms on the basis of the findings of abnormal liver tests on routine blood testing. The natural history of PBC is characterized by the slow progression of disease. Ultimately, signs and symptoms of advanced intrahepatic cholestasis and cirrhosis appear: such as jaundice, weakness, wasting, hyperpigmentation, xanthomas, ascites, and esophageal varices.

The typical laboratory abnormalities found in PBC are elevations in serum alkaline phosphatase and gamma glutamyl transpeptidase (GGTP). Serum aminotransferases are also raised, but to a lesser degree. Other abnormalities include increases in serum IgM and cholesterol levels. Most characteristic, however, of PBC is the presence of antimitochondrial antibody (AMA), which is found in high titer in 90-95 % of patients. AMA is rarely associated with other diseases and it arises early during the course of PBC, being present long before the onset of symptoms and even before the onset of abnormal serum liver tests.

The hallmark and diagnostic findings of PBC, however, are histological changes of non-suppurative destructive cholangitis, marked by lymphocyte injury to small bile ducts and granulomatous inflammation in the portal and periportal areas. The chronic injury to bile ducts eventually leads to their loss ("ductopenia") as well as progressive cholestasis and hepatic fibrosis. Generally, by the time cirrhosis is present, few or no bile ducts can be found.

Of course, the central question in etiology of PBC is what causes the injury to bile ducts. Ultimately, therapies that control or prevent this disease will have to be aimed at prevention or reversal of this bile duct injury.

At present, the only licensed, approved therapy of PBC is ursodiol, a hydrophilic bile acid. Several randomized controlled trials of ursodiol in PBC have showed that therapy with ursodiol in doses of 12-15 mg/kg/day markedly improves liver test abnormalities and may slow the progression of disease. Combined analyses of these trials suggest that ursodiol therapy prolongs survival in PBC, but this conclusion remains somewhat controversial. What is clear is that ursodiol does not cure or fully reverse this disease and its major effect appears to be the prevention of injury caused by retention of harmful bile acids that accumulate due to cholestasis in this disease. Interestingly, it has not been clearly shown whether ursodiol reverses bile duct injury in PBC or whether it prevents the progressive loss of bile ducts that underlies the pathogenesis and defines the natural history of this disease.

PBC is believed to be a "model" autoimmune disease. Unlike other autoimmune diseases, however, PBC does not respond to typical immunosuppressive therapy in a consistent manner. Thus, randomized controlled trials of azathioprine, d-pencillamine, chlorambucil and corticosteroids have shown either no benefit or slight benefit but unacceptable toxicity. Colchicine has been evaluated in several small trials and may have partial effect on this disease but its effects have not been critically evaluated in a properly controlled clinical trial.

Trials of other immunosuppressive regimens and of newer cytokines and anticytokines have not been done. Meanwhile, results of small trials of newer approaches and of a large trial of methotrexate are just now being reported.

Dr. Eric Gershwin of the University of California Davis summarized current concepts of pathogeneses of Primary Biliary Cirrhosis.

The serological hallmark of PBC is the presence of high titers of AMA, a non-species and non-organ specific autoantibody first identified in the early 1960's using indirect immunofluorescence. This autoantibody was found to interact with antigens on the inner membranes of mitochondria. An important breakthrough came with the cloning of autoantigen in PBC, which was subsequently shown to be the E2 component of the mitochondrial 2-oxo-dehydrogenases, most commonly the pyruvate dehydrogenase (PDH) complex. These enzymes are large complexes that are highly conserved in evolution, such that human AMA reacts well even with eukaryotic mitochondrial antigens. The mapping of the E2 component of PDH revealed that AMA reacted specifically with the lipoic domain of E2. Furthermore, these domains also have CD4 and CD8 epitopes that are recognized by T cells from patients with PBC. These domains contain the amino acid lysine to which lipoic acid binds. The highest numbers of E2 reactive T-cells are found in the liver rather than lymph nodes or blood. These features all point to the importance of the autoantigen in the pathogenesis of PBC.

Thus, the bile duct injury in PBC is most likely due to highly reactive CTL (both CD8+ and CD4+) lymphocytes that interact with the lipoic domain of E2 in oxodehydrogenases

(as well as other lipoic acid containing complex antigens). At issue then is why these lymphocytes react only with bile duct cells (and perhaps other similar ductal cells in salivary glands, lacrimal ducts and perhaps pancreas) and not with all cells that have mitochondria. Furthermore, how do these reactive immunocytes recognize E2 inside of mitochondria within cells, when immune recognition requires presentation of the antigen on the surface of the cell, in association with MHC molecules and other co-signaling molecules? Finally, even if the E2 specific CTL's are the effectors of this disease injury, what triggers and sustains their production?

A working hypothesis is that PBC is induced by an environmental exposure (infection, drug, or chemical) in late childhood or early adulthood. After a latency of months to years an autoimmune process is induced caused by an aberrant injury to bile duct cells causing them to present E2 antigen associated with MHC complex on their cell surface. The initial exposure may be limited ("hit-and-run") or persistent (as with a chromic infection or exposure). Similar disease examples would be rheumatic heart disease where the streptococcal infection is self limited and induces a protracted autoimmune injury to cardiac valves (hit-and-run). In contrast, cryoglobulinemia is an autoimmune disease induced by chronic infection with hepatitis C virus ("persistent exposure") that resolves when hepatitis C is treated. In both instances, the chronic injury appears to be due to an overactive immune response.

Pursuit of these hypotheses has been challenging. Thus, immunization of laboratory animals with purified or recombinant PDH can induce AMA activity similar to the human antibody in specificity and ability to inhibit enzymatic activity. Furthermore, several organic chemicals that are molecular mimics of lipoic acid bound to E2 can also induce AMA activity. However, the immunized animals do not develop typical liver or bile duct injury and titers of antibody are often not sustained.

Thus, the etiology of PBC has yet to be definitively elucidated. A limitation on current research into PBC is the lack of a small animal model and the fact that the disease has not been identified in species other than humans.

Dr. Burton Combes of the University of Texas Southwestern described results of randomized, double blind controlled trial of methotrexate in patients with PBC.

In the early 1990's, several case reports and uncontrolled case series suggested that methotrexate might be effective in improving laboratory test abnormalities and liver histology in PBC. The effects of methotrexate on survival or progression of disease was believed to be worthy of prospective controlled analysis. The Primary Biliary Cirrhosis Ursodiol Methotrexate-Placebo Study (PUMPS) was conducted at 11 clinical centers in the United States. A total of 535 patients with PBC were screened. Inclusion criteria were age between 20-69 years, known chronic cholestatic liver disease, the presence of AMA, elevations in alkaline phosphatase levels, and compensated liver disease. Patients were first monitored while receiving standard doses of ursodiol (12-15 mg/day) for at least 6 months. They then underwent baseline liver biopsy and were randomized to receive either methotrexate (15 mg/week) or placebo.

The pre-established endpoint of therapy was the time to treatment failure, which was defined as death, liver transplantation, or evidence for hepatic decompensation as shown by variceal hemorrhage, development of ascites, rise of bilirubin to above 3 mg/dl, or intractable symptoms of endstage liver disease (itching).

A total of 132 patients were randomized to receive methotrexate and 133 placebo. The two groups were well matched in regards to age (50.4 vs. 52.2 yrs.), gender (92% women in both groups), duration of disease and disease stage by liver biopsy.

This trial was continued for 9 years at which time it was halted on the advice of the Data Safety and Monitoring Board, because of lack of effect on primary outcomes and futility of continuing the trial. All patients had been followed for at least 5 years and some for as long as 9 years. At the point the study was stopped 14 of the methotrexate and 16 of the placebo-treated patients had reached an endpoint. The Kaplan Meyer plots of time to treatment failure for the two groups were identical. Similar findings were evident when individual endpoints in the overall assessment of treatment failure were analyzed (such as time to death or liver transplantation). These results indicate that long-term therapy with methotrexate does not prolong survival in patients with PBC receiving ursodiol. More detailed analyses of changes in laboratory tests, symptoms, and liver histology are now underway.

Dr. Keith Lindor of the Mayo Clinic Foundation (Rochester, MN) presented an overview of new approaches in therapy of PBC.

Small usually uncontrolled and short-term trials of innovative approaches to treatment of PBC have been carried out in recent years. Most of these studies have been based on hypotheses regarding the pathogenesis of PBC but few have included intensive analyses of the underpinnings of these therapies.

Induction of oral tolerance is an approach to therapy has been evaluated in several autoimmune diseases, largely with negative results. In a pilot study, purified beef heart pyruvate dehydrogenase (PDH) complex was given to patients with well documented PBC. The dose of PDH was approximately 50 times that present in a typical American diet. Patients were treated for six months and monitored for symptoms, serum biochemical tests and AMA titers. There were no changes in alkaline phosphatase or ALT levels or in AMA titers in blood. Therapy was stopped early in one patient because of rash. Thus, attempts at induction of oral tolerance using this autoantigen preparation appeared to have no effect on disease manifestations or AMA reactivity.

The search for infectious etiologies for PBC has yielded promising but inconclusive results. Supportive of an infectious etiology would be a clinical or serological response to an appropriate anti-infective therapy. Retroviral sequences have been identified in liver biliary tissue from some patients with PBC. In a trial of lamivudine therapy among 12 patients with PBC, a one-year course of treatment led to no consistent changes in liver enzymes or AMA levels. A subsequent study was initiated using the combination of

lamivudine and AZT (Combivir). Among 14 patients treated for up to 1 year, serum aminotransferases and alkaline phosphatase levels improved in the majority and became normal in almost half. This study is still in progress.

Chlamydia pneumonia infection has been linked to several chronic diseases and recently immunohistochemical staining for chlamydia was found in a high proportion of patients with PBC. A pilot study of a 3-week course of tetracycline (500 mg 4 times daily) was conducted in 15 patients. No patient demonstrated clinical or serum biochemical improvements with therapy.

Bezafibrate is a cholesterol lowering agent that has been shown to increase hepatic expression of MDR-2, an important canalicular transporter for phospholipids. As such, bezafibrate may increase bile flow. In a study of 24 patients with PBC treated with ursodiol (600 mg/day) or bezafibrate (400 mg/day), improvements in liver biochemical tests occurred in both groups, most strikingly with bezafibrate. The dose of ursodiol in this study was lower than generally recommended (8-10 mg/kg/day as opposed to 12-15 mg/kg/day).

Silymarin or milk thistle is an herbal antioxidant product that is widely used in Europe for liver disease. Silymarin is effective in preventing liver injury in a wide variety of in vitro and in vivo small animal models. Its role in human liver disease is controversial. In a pilot, open label trial, 27 patients with PBC who were receiving ursodiol were treated with a silymarin (140 mg three times daily). During the year of the study there were no significant changes in alkaline phosphastase or ALT levels or in Mayo risk score.

Budesonide is an oral corticosteroid that has an extensive first pass metabolism (85%). The potential advantage of budesonide over other corticosteroids is the lower risk for systemic side effects which can be particularly difficult in patients with PBC (osteoporosis, muscle wasting, skin changes). In a pilot study, 22 patients who had a suboptimal response to ursodiol (alkaline phosphatase levels greater than twice the upper limit of normal) were treated with budesonide in a does of 3 mg thrice daily for 1 year. There were minimal improvements in liver biochemical test results. Importantly, several patients had worsening of bone mineral density.

Mycophenylate mofetil is a purine analogue and broad immunosuppressive agent that is increasingly used after solid organ transplantation. It appears to be more potent and better tolerated than azathioprine. In a pilot study, 25 patients with a suboptimal response to ursodiol were treated with escalating doses of mycophenylate mofetil to as high as 3 gm/d. Dose reductions were required in 8 patients and three withdrew because of severe adverse events. There were overall improvements in serum alkaline phosphatase and AST levels. Interestingly improvements were greater in those with more advanced disease. There were no changes in Mayo Risk Scores and liver histology was not assessed.

Thus, uncontrolled pilot studies have been conducted in several agents of potential benefit in PBC. Most promising at present is combination antiretroviral therapy,

bezafibrate and mycophenylate mofetil. More rigorous studies with objective assessment of liver histology and disease progression are needed.

Dr. Jeffrey N. Siegel, the Center for Biologics Evaluation and Review of the Food and Drug Administration provided an overview of the use of immunodolatory biologic agents for autoimmune disease.

In recent years, a number of potent biologic agents have been introduced into clinical medicine many of which have profound effects on the course and outcome of autoimmune diseases, such as rheumatoid arthritis and Crohn's disease. None of these agents had undergone formal assessment in PBC. These biologic agents fall into several categories including pro-inflammatory cytokine blockers, anti-inflammatory cytokines, inhibitors of T cell function and inhibitors of B-cell function.

Tumor necrosis factor alpha (TNF) is a major pro-inflammatory cytokine which has been shown to be an important mediator of injury in several autoimmune diseases. Three TNF blocking agents have been developed and approved by the FDA for treatment of autoimmune forms of arthritis and Crohn's disease.

Etanercept (Embrel) is a TNF receptor fusion protein that blocks the actions of TNF in vitro and in vivo and has potent activity in active rheumatoid arthritis, juvenile rheumatoid arthritis, and psoriatic arthritis. It is given by i.m. injection twice weekly. Infliximab is a chimeric humanized mouse monoclonal antibody to TNF that has been shown to be effective in both rheumatoid arthritis (when used in combination with methotrexate) and Crohn's disease. It is given by i.v. infusion and 2 to 6 courses of treatment have been found to induce remissions of Crohn's disease. Adalimusmab is a human monoclonal antibody to TNF recently licensed for treatment of rheumatoid arthritis. It is also given by i.v. infusions. Other pro-inflammatory cytokines that are potential targets for blocking agents are IL1, IL2, IL6, IL12, IL15 and IL18. Early stage trials are ongoing with products targeting these cytokines, but at present only IL-1 receptor antagonist has been proven effective in clinical trials.

While the TNF blockers have potent activity against inflammatory diseases, they also have the potential for severe adverse events, the most important being infectious diseases including reactivation of tuberculosis. Rare adverse events include demyelinating disease and congestive heart failure. The possibility of development of neutralizing antibodies with associated loss of efficacy with long-term infusions of antibodies to TNF has also been a concern.

Anti-inflammatory cytokine therapy has also been proposed as potential therapy of autoimmune diseases. Products in development include IL4, IL10, IL11, and IL18 but to date none have been proven to have beneficial effects.

Inhibition of T- or B-cell function is another experimental approach to therapy of autoimmune disease. T cell inhibition can be accomplished with CTLA-4Ig, a fusion protein between the CTLA4 receptor and the Fc portion of human immunoglobulin that

blocks the interaction of co-stimulatory molecules on T cells (CD28) and their ligands on antigen presenting cells (CD80/86). A soluble form of LFA-3 (alefacept) blocks CD2 on T cells and depletes both CD4 and CD8+ cells and has been shown to be safe and effective in the treatment of psoriasis. Another anti-T cell approach is anti-CD11a monoclonal antibody that also has been demonstrated to be safe and effective in treatment of psoriasis. Other initially promising anti-T cell approaches have been associated with unexpected significant adverse events (anti-CD40L).

Finally, B cell depletion using anti-CD20 monoclonal antibody (rituximab) has been shown to be effective in low-grade B cell lymphoma. Infusions result in marked depletion of B cells that persists for several months. Preliminary results suggest benefit of B cell depletion in cryoglobulinemia and possibly rheumatoid arthritis.

While none of these newer biological immunomodulating agents have been studied in PBC, their track record for efficacy and relative safety in autoimmune arthritides and Crohn's disease suggests that they should be evaluated in PBC.

Dr. Henry Bodenheimer from the Beth Israel Hospital, NY led a group discussion of the challenges and future needs in clinical research in PBC.

PBC presents several challenges to progress in its clinical management. First, its underlying pathogenesis is still unclear and there are no suitable animal models in which to evaluate potential therapies. The intermediate pathways of inflammatory response and injury are less well established than in diseases such as rheumatoid arthritis or inflammatory bowel disease.

Second PBC is largely a silent disease that has a slow and unpredictable natural history. Thus, studies require a long-duration to show any benefit.

Third, the appropriate endpoints of treatment for PBC are not well defined. Endpoints used in pilot studies have been changes in alkaline phosphatase or ALT levels, the significance of which is unclear. Certainly no natural history study has shown an association of degree of enzyme elevations and disease outcome or survival. Similarly, AMA titers may be assessed in clinical trials, particularly of immunomodulatory agents but presence or level of AMA has not correlated with disease stage or outcome. Finally, symptoms and signs, which are the typical endpoints used in studies of rheumatoid arthritis and Crohn's disease, are subjective and difficult to quantify in PBC.

The endpoints used in large prospective randomized controlled trials in PBC have been death or transplantation and, more recently, hepatic decompensation. These endpoints are challenging as they represent the end-stage of PBC that occur only after years or decades. Use of these hard endpoints in therapeutic trials in PBC dictate that large numbers of patients be followed for many years. The limitations of these endpoints are shown most strikingly in the recently released results from the PUMPS trial, a study that required a study of 265 patients followed for 5 to 9 years to demonstrate a lack of effect.

Another difficulty in the PUMPS trial as well as the many pilot studies is that ursodiol is now approved as therapy for PBC and has striking effects on serum biochemical tests and may improve histology and prolong survival. The availability of ursodiol makes it impossible to conduct studies without its use, but potentially obscures effects of the other agents.
An endpoint that is less well defined and not always used in clinical trials of PBC is liver histology. Liver histology is only minimally improved if at all by ursodiol treatment and no therapy has been found to reliably reverse or ameliorate histological changes. Yet, if any treatment improves survival in PBC, it must have some effect on liver histology. Furthermore, if the primary lesion in PBC is bile duct damage, then an effective therapy should reverse or ameliorate this damage. While liver biopsy is an invasive and expensive procedure it seems clear that liver histology should be a primary endpoint in any study of therapy of PBC. Thus, liver histology should be an adequate surrogate marker for disease progression in this disease.

In this regard, the staging and grading systems in PBC deserve reassessment. In chronic hepatitis an important advance was the standardization of a histological grading and staging system that separated disease activity (inflammation and cell necrosis) and disease stage (fibrosis). The current systems in PBC mix these two elements and do not quantify bile duct injury or loss, clearly an important factor in the progression of this disease. Finally, the current grading systems have never been adequately assessed as a means of evaluating the course and progression of disease.

A final issue of great importance is that the majority of patients with PBC have few if any symptoms and the disease is usually only slowly progressive. Any therapy of early or intermediate stages of PBC has to be well tolerated and acceptable to asymptomatic or minimally symptomatic patients. Potent immunosuppressive agents with major toxicities are clearly not suitable for the average patient with early disease.

Thus, the challenges to clinical research in PBC are many. Current recommended therapy using ursodiol is effective in improving biochemical laboratory in the majority of patients and treatment may prevent disease progression and prolong survival. Nevertheless, more effective therapies are needed. While ultimately a better understanding of the pathogenesis of PBC is needed, translation of the research findings to practical means of treatment is needed now.

Summary Recommendations

- Fundamental studies of the pathogenesis and etiology of PBC should be expanded. The roles of immunological as well as infectious, genetic and environmental factors need to be pursued.
- Analyses of mediators of bile duct cell and hepatocyte damage in PBC would potentially help advance therapy of this disease by directing treatment modalities to key pathways in the inflammatory cascade.

- A small animal model of PBC is a major priority. Such a model should help advance understanding of the pathogenesis of this disease and potential therapies.
- A reassessment of the histological scoring of PBC is needed. Any system should be assessed in liver biopsy collections presenting the entire spectrum of disease as well as the stages of disease progression.
- Small pilot studies of promising therapeutics are of value in PBC but they should be designed to assess rigorous and objective endpoints and control for ursodiol effects.
- Any further large randomized controlled trials of promising therapy for PBC should have adequate rigorous design and sufficient power to demonstrate clinically important effects. Such studies should only be initiated once there is adequate preliminary results documenting appropriate dose and dosing of the medication and standardization of histological scoring systems and primary endpoints of treatment.