PRIMARY BILIARY CIRRHOSIS
WITH EMPHASIS ON
IMMUNOPATHOGENESIS AND THERAPY

MEDICAL GRAND ROUNDS

September 4, 1997

Burton Combes, M.D.

and

Marlyn J. Mayo, M.D.

Name: Burton Combes, M.D.

Rank: Professor of Internal Medicine

Division: Hepatology

Areas of Interest:

1. Eclectic in Hepatology
2. Primary Biliary Cirrhosis
3. Autoimmune Hepatitis

Name: Marlyn J. Mayo, M.D.

Rank: Assistant Instructor in Internal Medicine

Division: Hepatology

Areas of Interest:

1. Immune-mediated Liver Disease
2. Primary Biliary Cirrhosis

Primary Biliary Cirrhosis (PBC) is a disease in which the interlobular and septal bile ducts within the liver undergo inflammation and destruction. Once initiated, the disease persists and progresses, but at varying rates. Neither the initiating nor the perpetuating events are well understood. Nevertheless, current best guesses are that immunologically-mediated injury accounts for progressive duct damage.

It is now clear that the disease may exist for relatively long periods of time in an asymptomatic state (1-3). Prolonged follow-up reveals that many if not most of these asymptomatic persons will eventually develop symptoms and signs of their disease (4-8). Duct destruction leads to impaired transport of compounds into bile (cholestasis) with its consequences, pruritus and hyperbilirubinemia. Cytotoxic aspects of bile components likely to be present in increased concentrations in obstructed areas of liver presumably cause hepatocyte injury and death. The dihydroxy bile acids, deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA), and the monohydroxy bile acid lithocholic acid (LCA) undoubtedly contribute to this and have the additional potential to impair biliary transport in non-damaged hepatocytes. Portal triads usually contain a prominent mononuclear infiltrate, with a variable small number of polymorphonuclear cells. Such cells are able to generate cytokines that may themselves have cytotoxic potential and stimulate fibrogenesis. Thus, interaction between components of bile and products of inflammatory cells appear likely to contribute to scarring and hepatocyte injury, resulting in progressive fibrosis and cirrhosis with the attendant complications of portal hypertension, esophageal varices, ascites and liver failure. Progression of the disease, intuitively related in a major way to the rate of bile duct destruction, would also depend then secondarily on local concentrations of factors that themselves may induce cell damage and fibrosis.

1. Sherlock S, Scheuer PJ. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. New Engl J Med 289:674-678, 1973.
2. James A. Macklon AF, Watson AJ. Primary biliary cirrhosis - a revised clinical spectrum. Lancet i:1278-1281, 1981.
3. Metcalf JV, Mitchison HC, Palmer JM et al. Natural history of early primary biliary cirrhosis. Lancet 348:1399-1402, 1996.
4. Long RG, Scheuer PJ, Sherlock S. Presentation and course of asymptomatic primary biliary cirrhosis. Gastroenterology 72:1204-1207, 1977.
5. Beswick DR, Klatskin G, Boyer JL. Asymptomatic primary biliary cirrhosis. A progress report in long-term follow-up and natural history. Gastroenterology 89:267-271, 1985.
6. Balasubramaniam K, Grambsch PM, Wiesner RH et al. Diminished survival in asymptomatic primary biliary cirrhosis. Gastroenterology 98:1567-1571, 1990.
7. Nyberg A, Loof L. Primary biliary cirrhosis. Clinical features and outcome, with special reference to asymptomatic cases. Scand J Gastroenterol 24:57-64, 1989.
8. Mahl TC, Shockor W, Boyer JL. Primary biliary cirrhosis: survival of a large cohort of symptomatic and asymptomatic patients followed for 24 years. J Hepatol 20:707-713, 1994.

IMMUNE PATHOGENESIS OF PRIMARY BILIARY CIRRHOSIS

Several lines of evidence support the theory that primary biliary cirrhosis (PBC) is an autoimmune disease. Clinically, it shares features with other autoimmune diseases such as a predominance in middle-aged females and frequent coincidence with other autoimmune disorders. Prevalence studies have failed to reveal a clear mode of transmission that could be compatible with an infectious etiology, such as parenteral exposure or fecal-oral contamination. Microscopically, bile duct destruction is associated with T cell infiltrates and expression of biliary epithelial cell surface markers of immune-mediated destruction such as adhesion molecules and HLA class II molecules. In addition, virtually 100% of PBC patients have circulating autoantibodies, often to mitochondrial or nuclear antigens.

PATHOGENICITY OF ANTI-MITOCHONDRIAL ANTIBODIES

Whether or not these autoantibodies play an important role in the pathogenesis of bile duct destruction has been the subject of considerable discussion throughout the last two decades. PBC patients have been reported to possess a number of different circulating autoantibodies (9-14) (see Table 1), most commonly anti-mitochondrial and antinuclear antibodies. Among these, the anti-mitochondrial antibodies are the most likely candidates for pathogenic antibodies because of their relatively high sensitivity and specificity as a marker for the disease, as well as reports of biliary epithelial cell surface expression of mitochondrial antigens.

Table 1

Incidence of autoantibodies found in patients with primary biliary cirrhosis. Percentages vary according to method of detection (immunofluorescence or ELISA)

AUTOANTIBODY	INCIDENCE
Anti-mitochondrial	90%-95%
Anti-nuclear	48%
centromere	24%
speckled	10%
homogeneous	10%
nuclear dot	4%
Anti-SS DNA	71%
Anti-nuclear pore gp 210	21%-28%
Anti-histone	81%
Anti-Ro-60 (SS-A)	30%
Anti-Ro-52 (SS-A)	25%
Anti-Sm/RNP	24%
Anti-topoisomerase-1	24%
Anti-La 48 (SS-A)	21%
Anti-DS DNA	10%
Anti-carbonic anhydrase	8%-45%
Anti-platelet	40%

Anti-mitochondrial antibodies (AMA) have been identified as serologic markers of primary biliary cirrhosis since 1965. Then, as now, this laboratory test is performed using indirect immunofluorescence. Modern laboratories can purchase ready-made slides with small sections of rat stomach, kidney, and liver tissue. These slides are incubated with the patient's serum followed by a fluorescently labeled anti-human IgG antibody. The tissue and cellular localization of fluorescence identifies anti-mitochondrial, anti-smooth muscle, anti-liver/kidney/microsomal, and anti-parietal cell antibodies. Using immunofluorescence, approximately 90-97% of PBC patients will be AMA positive. The true sensitivity of antimitochondrial antibodies is difficult to quantify because no gold standard exists for the diagnosis of PBC. Even liver biopsy is not pathognostic. Detection of anti-mitochondrial antibodies, particularly those that are low in titer, may be transient. In one series of 218 PBC patients (15), nine were AMA negative by immunofluorescence, but seven of these were subsequently found to be AMA positive when additional serum samples were examined. Causes of AMAs by immunofluorescence other than PBC are rare, but include syphilis, myocarditis, tuberculosis, collagen vascular disease, and iproniazid.

In 1987, Gershwin and colleagues identified the primary structure of the major target antigen of PBC anti-mitochondrial antibodies (16-18). The availability of a recombinant form of this mitochondrial antigen has greatly facilitated our ability to examine the potential pathogenicity of these autoantibodies. The major target antigen in PBC was identified as the human mitochondrial enzyme, dihydrolipoamide acetyl transferase, also known as the E2 component of the pyruvate dehydrogenase complex (PDC). PDC belongs to a family of multi-enzyme complexes involved in cellular respiration called the 2-oxo-acid dehydrogenase complexes. PDC catalyzes the oxidative decarboxylation of pyruvate to acetyl Co-A and NADH. Other members of the 2-OADC family include the branched chain 2-oxo-acid dehydrogenase complex (BCOADC) and 2-oxoglutarate dehydrogenase complex (2-OGDC). Each complex consists of multiple copies of functionally related enzymes. The PDC complex is composed of a central core with approximately 60 copies of the E2 enzyme (dihydrolipoamide acetyl transferase), encased by 20-30 copies of E1 (pyruvate decarboxylase), approximately 6 copies of E3 (dihydrolipoamide dehydrogenase), and 12 copies of component X. Component X is the least well described of these enzymes, but appears to share significant structural and functional homology with E2 and is capable of substituting for E2 in its absence. Component X has recently emerged as an important target autoantigen in PBC that was not previously appreciated.

Knowledge of the components of these enzyme complexes and their molecular weights has permitted us to examine patients' reactivity against mitochondrial antigens by immunoblotting. Although it is primarily a research tool and is not generally available in community laboratories, some patients who are repeatedly AMA negative by immunofluorescence will have detectable anti-mitochondrial antibodies by immunoblotting. In this procedure, purified enzymes or enzyme complexes are first denatured and separated by length on a polyacrylamide gel into their individual components. The proteins are transferred to a membrane which is incubated with the patients' serum and the hybridized with a labelled anti-human IgG antibody. Individual bands detected represent antibodies to a specific mitochondrial enzyme.

PBC patients may carry antibodies to any of the 2-OAD complexes. Although PDC-E2 has been identified as the major target autoantigen in PBC, some PBC patients have additional antibodies to other 2-OAD enzymes or lack antibodies to PDC-E2. Forty PBC patients were examined by immunoblotting in one series from England (Table 2). In this study (19), 95% of PBC patients recognized one of the PDC enzymes (E2, X, or E1). Sera from 50% of patients recognized the E2 components of all three 2-OAD complexes. Interestingly, sera from 5% of PBC patients recognized the E2 components of BCOADC or 2-OAGDC, but not PDC; and only 3% of PBC patients reacted to none of the antigens tested.

TABLE 2

Reactivity to 2-OAD-E2 enzymes in PBC patients

PDC-E2/X	BCOADC-E2	OGDC-E2	No. PBC patients (%) N = 40
+	+	+	21 (52.5)
+	+	-	2 (5)
+	-	+	6 (15)
+	-	-	9 (22.5)
-	+	+	2 (5)

Therefore, there clearly exists a small group of patients that conform to all clinical and histologic descriptions of PBC that do not have detectable anti-mitochondrial antibodies despite repeated testing using different techniques. These patients can be considered as "living proof" that anti-mitochondrial antibodies are not pathogenic. Long-term follow up of these AMA negative patients has shown that their clinical course is no less aggressive than that of AMA positive patients (20). In AMA positive patients, AMA titers do not correlate well with histologic severity.

Experimental models have also failed to demonstrate that anti-mitochondrial antibodies are responsible for targeting destruction of bile duct epithelial cells in PBC. Rats, guinea pigs, rabbits, rhesus monkeys have all been immunized with the recombinant form of PDC-E2. Antibodies to PDC-E2 were readily induced and detected in all species. In humans, the anti-PDC-E2 antibody interferes with the functional activity of the enzyme, and in rabbits and guinea pigs, the AMAs generated were also specifically reactive with the enzyme's functional site. Despite generation of high titer AMAs, none of the animals developed abnormal liver tests or abnormal liver histology after eight months of follow-up (21).

Perhaps equally convincing as the lack of experimental evidence that anti-mitochondrial antibodies are pathogenic is the intuitive conception that PDC-E2 is an unlikely candidate for target antigen in biliary epithelial cells. PDC-E2 is a mitochondrial enzyme which is present in all aerobic cells and exists deep inside the intracellular compartment, away from immune recognition. Tissues with high energy requirements, such as muscle, contain much higher concentrations of PDC than biliary epithelia. Even hepatocytes contain more mitochondria per cell than cholangiocytes. It is difficult to reconcile the organ specificity of PBC with the ubiquitous presence of mitochondrial antigens unless we propose that cholangiocytes are first injured by another process. This would allow release of intracellular antigens which could then be recognized by the immune system, resulting in the production of AMAs as a byproduct of bile duct destruction.

This logic was questioned in 1991 when Neuberger and colleagues reported surface staining of intact bile duct epithelial cells with antibodies to PDC-E2 in PBC liver (22). In this experiment, rabbit antibodies were raised against the purified subunits (E1, E2 and E3) of PDC and affinity purified. They were used to stain frozen hepatectomy specimens obtained at the time of orthotopic liver transplantation (Figure 1). Staining for E2, but not E1 or E3, of bile ducts of PBC patients did not parallel the reported distribution of mitochondria. In addition to the expected diffuse granular pattern of cytoplasmic staining consistent with mitochondrial PDC-E2, intense membrane associated staining was also seen in PBC bile ducts. Similar aberrant staining was not seen in normal liver sections or liver from patients with other transplant indications.

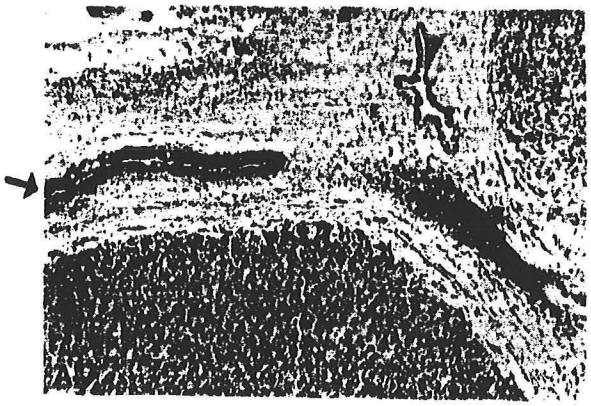

Figure 1

Cell surface staining of bile duct epithelial cells in duct on left (arrow) but not in the duct on right

These results have been confirmed by other investigators using antibodies derived using several different techniques, including mouse monoclonal antibodies generated against recombinant PDC-E2 and human combinatorial antibodies derived from lymph nodes of PBC patients; and using several different tissue sources, including formalin-fixed liver and isolated bile duct epithelial cells (22-29, see Table 3). Electron microscopy studies demonstrate that the staining is associated with the luminal side of the apical membrane.

TABLE 3

Staining of bile duct epithelial cell surface with antibodies to the pyruvate dehydrogenase complex.

	ANTIBODY	TISSUE
Joplin et al, 1991	rabbit vs denatured bovine PDC-E2	frozen liver and lymph node
Tsuneyama et al, 1994	mouse monoclonal vs recombinant E2 (C355.1) and human combinatorial from lymph node (LC5)	formalin fixed liver
Joplin et al, 1995	C355.1 and human combinatorial from lymph node (SP4)	formalin fixed liver and salivary gland
Nakanuma et al, 1995	rabbit vs denatured bovine PDC E-2 and human affinity purified	fixed and non-fixed isolated biliary epithelial cells
Nakanuma et al, 1995	C355.1	frozen liver
Tsuneyama et al, 1995	C355.1 and SP4	formalin-fixed liver

Reactivity to PDC-E2 on the surface of bile duct epithelial cells in PBC suggested that biliary epithelial cells may be abnormally expressing PDC-E2 on their surface, leading to early recognition by the immune system and induction of cholangiocyte death. Alternative hypotheses to explain the positive staining include the surface expression of an abnormal or partial length PDC-E2, or a molecule which is cross-reactive with antibodies generated against PDC-E2. The fact that only a subset of monoclonal antibodies generated against PDC-E2 react with PBC bile ducts supports these theories. A cross-reactive molecule could, in fact, originate from an infectious organism and generate antibodies that also react to human PDC-E2, a process referred to as "molecular mimicry". PDC enzymes are well conserved across species, making this an attractive hypothesis.

Recent data suggest that the molecule recognized by PDC-E2 antibodies on the external membrane of cholangiocytes in PBC may not be PDC-E2. In situ hybridization of PDC-E2 mRNA failed to demonstrate increased production of PDC-E2 in PBC biliary epithelial cells (30). Unpublished data from Neuberger and colleagues which have recently been presented in abstract form demonstrate that antibodies which are specific to the X component of PDC and do not react with the E2 component react strongly with PBC bile duct epithelia. Immunoblotting of PBC cholangiocyte membrane antigens concurs that this molecule is likely to be component X (31). E2 and X share significant structural homology and co-purify (32). The antibodies which have been used in previous immunohistochemical studies of biliary epithelia, including the mouse monoclonal antibody C355.1 generated against recombinant PDC-E2 and human combinatorial antibody LC5 derived from lymph nodes of a PBC patient, as well as the rabbit antibodies used in the initial studies by Neuberger himself, are all known to react with both PDC-E2 and PDC-X. Thus, the molecule on the surface of bile duct epithelial cells of PBC patients may not be a foreign antigen but may be human PDC component X. Unfortunately, the original questions of why a mitochondrial antigen is expressed on the external membrane of these cells, and what role this has in the pathogenesis of PBC, remain unanswered.

The early appearance of both expression of this antigen and development of anti-mitochondrial antibodies is consistent with the hypothesis that anti-mitochondrial antibodies are pathogenic in PBC. One study has compared biliary cell surface staining with anti-PDC-E2 antibodies in needle biopsy specimens obtained from patients with early (histologic stages I and II) versus late (stages III and IV) disease (29). Abnormal expression of the PDC-E2-like molecule on the luminal surface of biliary epithelium occurred early in Stage I disease before other markers of immune destruction were present. Clinically, anti-mitochondrial antibodies also arise early in the disease process, often prior to development of symptoms or abnormal alkaline phosphatase. Mitchison and colleagues examined 29 patients who were AMA positive without symptoms or abnormal liver enzymes. Twenty-seven of 29 had histologic features compatible with PBC on liver biopsy. Sixteen of the patients were followed for a mean of 8.7 years, and 11/16 developed an abnormal alkaline phosphatase and 5/16 developed symptoms of fatigue or pruritus (33). After extended follow-up for a mean of 17.8 years, five had died of non-liver causes and 76% had developed symptoms and 83% had developed abnormal liver tests consistent with PBC (3).

Despite their early appearance, anti-mitochondrial antibodies are not likely to be sufficient to cause disease in a short period of time. After liver transplantation for PBC, AMAs commonly persist without eventual development of PBC liver disease. In addition, there is no evidence that complement-dependent cytotoxicity is occurring near the small bile ducts in PBC liver. Deposits of C3d (derived from the terminal complex of complement) are not found near bile ducts (34), and immune complexes are not consistently identified in PBC patients (35). Although Fc receptor mediated clearance is defective in patients with PBC, it is not related to disease severity (36).

9. Chou M, Lee S, Chen TY et al. Specificity of anti-nuclear antibodies in primary biliary cirrhosis. Ann Rheum Dis 54:148-151, 1995.
10. Wesierska-Gadek J, Hohenauer H, Hitchman E et al. Autoantibodies from patients with primary biliary cirrhosis preferentially react with the amino-terminal domain of nuclear pore complex glycoprotein gp210. J Exp Med 182:1159-1162, 1995.
11. Nickowitz R, Wozniak R. Schaffner F et al. Autoantibodies against integral membrane proteins of the nuclear envelope in patients with primary biliary cirrhosis. Gastroenterology 106:193-199, 1994.
12. Gordon SC, Quattrociocchi-Longe TM, Khan BA et al. Antibodies to carbonic anhydrase in patients with immune cholangiopathies. Gastroenterology 108:1802-1809, 1995.
13. Muratori P, Muratori L, Lenzi M et al. Antibodies to carbonic anhydrase in autoimmune cholangiopathy. Gastroenterology 112:1053-1054, 1997.
14. Feistauer S, Penner E, Mayr W et al. Target platelet antigens of autoantibodies in patients with primary biliary cirrhosis. Hepatology 25:1343-1345, 1997.
15. Munoz LE, Thomas HC, Scheuer PJ et al. Is mitochondrial antibody diagnostic of primary biliary cirrhosis? Gut 22:136-140, 1981.
16. Gershwin ME, Mackay IR, Sturgess A et al. Identification and specificity of a cDNA encoding the 70kD mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol 138:3525-3531, 1987.
17. Fussey SPM, Guest R, James OFW et al. Identification and analysis of the major M2 autoantigen in PBC. Proc Natl Acad Sci USA 85:8654-8658, 1988.
18. Coppel RL, McNeilage J, Surh CD et al. Primary structure of the human M2 mitochondrial autoantigen of PBC: dihydrolipoamide acetyltransferase. Proc Natl Acad Sci USA 85:7317-7321, 1988.
19. Bassendine MF, Fussey SPM, Mutimer DJ et al, Identification and characterization of four M2 mitochondrial antoantigens in primary biliary cirrhosis. Sem Liver Dis 9:124-131, 1989.
20. Invernizzi P, Crosignani A, Battezzati M et al. Comparison of the clinical features and clinical course of anti-mitochondrial antibody-positive and-negative primary biliary cirrhosis. Hepatology 25:1090-1095, 1997.
21. Krams SM, Surh CD, Coppel RL et al. Immunization of experimental animals with dihydrolipoamide acetyltransferase, as a purified recombinant polypeptide, generates mitochondrial antibodies but not primary biliary cirrhosis. Hepatology 9:411-416, 1989.
22. Joplin R. Lindsay JG, Hubscher SG et al. Distribution of dihydrolipoamide acetyltransferase (E2) in the liver and portal lymph nodes of patients with primary biliary cirrhosis: an immunohistochemical study. Hepatology 14:442-447, 1991.
23. Van der Water J, Turchany J, Leung PSC et al. Molecular mimicry in primary biliary cirrhosis: Evidence for biliary epithelial cell expression of a molecule cross-reactive with pyruvate dehydrogenase complex-E2. J Clin Invest 91:2653-2664, 1993.
24. Tsuneyama K, Van der Water J, Nakanuma Y et al. Human combinatorial autoantibodies and mouse monoclonal antibodies to PCD-E2 produce abnormal apical staining of salivary glands in patients with coexistent primary biliary cirrhosis and Sjogren's syndrome. Hepatology 20:893-898, 1994.
25. Joplin R, Wallace LL, Johnson GD et al. Subcellular localization of pyruvate dehydrogenase dehydrolipolyamide acetyl transferase in human intrahepatic biliary epithelial cells. J Pathol 176:381-390, 1995.

26. Nakanuma Y, Tsuneyama K, Kono N et al. Biliary epithelial expression of the pyruvate dehydrogenase complex in primary biliary cirrhosis: an immunohistochemical and immunoelectron microscopic study. Hum Pathol 26:92-98, 1995.
27. Joplin RE, Johnson GD, Matthews JB et al. Distribution of pyruvate dehydrogenase dihydrolipoamide acetyl transferase (PDC-E2) and another mitochondrial marker in salivary gland and biliary epithelium from patients with primary biliary cirrhosis. Hepatology 19:1375-1380, 1994.
28. Joplin RE, Lindsay JG, Johnson GD et al. Membrane dihydrolipoamide acetyltransferase (E2) on human biliary epithelial cells in primary biliary cirrhosis. Lancet 339:93-94, 1992.
29. Tsuneyama K, Van der Water J, Leung PCS et al. Abnormal expression of PDC-E2 on the luminal surface of biliary epithelium occurs before MHC class II and BB1/B7 expression. Hepatology 21:1031-1037, 1995.
30. Harada K, Van der Water J, Leung P et al. In situ nucleic acid hybridization of pyruvate dehydrogenase complex-E2 in primary biliary cirrhosis: pyruvate dehydrogenase complex-E2 messenger RNA is expressed in hepatocytes but not in biliary epithelium. Hepatology 25:27-32, 1997.
31. Joplin R, Wallace LL, Johnson GD et al. Distribution of component X of pyruvate dehydrogenase complex (PDC-X) in liver and salivary gland of patients with primary biliary cirrhosis (PBC) and controls. Hepatology 20:150A, 1994 (Abstract).
32. Neagle J, de Marcucci O, Dumbar B et al. Component X of mammalian pyruvate dehydrogenase complex: structural and functional relationship to the lipamide acetyltransferase (E2) component. FEBS Lett 253:11-15, 1989.
33. Mitchison HC, Bassendine MF, Hendrick A et al. Positive antimitochondrial antibody but normal liver function tests: is this primary biliary cirrhosis? Hepatology 6:1279-1284, 1986.
34. Garred P, Lyon H, Christofferesen P et al. Deposition of C3, the terminal complement complex and vibronectin in primary biliary cirrhosis and primary sclerosing cholangitis. Liver 13:305, 1993.
35. Goldberg MJ, Kaplan MM, Mitamura T et al. Evidence against an immune complex pathogenesis of primary biliary cirrhosis. Gastroenterology 83:677, 1982.
36. Ekdahl KN, Loof L, Nyberg A et al. Fc receptor-mediated clearance in patients with primary biliary cirrhosis. Gastroenterology 101:1076, 1991.

T LYMPHOCYTE-MEDIATED DESTRUCTION OF BILIARY EPITHELIAL CELLS

There is greater evidence that bile duct destruction occurs through T cell-mediated mechanisms in PBC. This is not surprising considering the fact that the vast majority of cell types in PBC portal tract infiltrates are T lymphocytes. In contrast to antimitochondrial antibodies, T lymphocytes isolated from patients with primary biliary cirrhosis are capable of inducing a PBC-like disease in experimental animals. Gershwin et al injected T cells from PBC patients into severe combined immunodeficient (SCID) mice and observed the development of biliary lesions similar to PBC (37).

Exactly how and why T cells may be activated to induce death of cholangiocytes in PBC is not well understood. Classically, T cells are activated after recognition of an antigen in the context of MHC and co-stimulatory molecules on the surface of a professional antigen presenting cell, such as a macrophage. In PBC, T cells may be capable of recognizing antigen directly on the surface of bile duct epithelial cells. The ability of cholangiocytes to present antigen to T cells has not been directly demonstrated (38). However, indirect evidence suggests that this may be occurring. Immunohistochemistry clearly demonstrates that biliary epithelial cells of PBC patients exhibit increased expression of HLA class I, HLA class II, and intracellular adhesion molecule 1 (ICAM-1) (39-42). Isolated cholangiocytes from PBC patients are functionally capable of binding lymphocytes via their ICAM-1 (43). The presence of the co-stimulatory molecule B7 has been demonstrated by some investigators (29), but not by others (42,43). Collectively, these cell surface proteins create the appropriate environment in which T cells could make direct contact with cholangiocytes and recognize antigens.

Both CD4+ and CD8+ T cells have been implicated in the pathogenesis of PBC. In vitro, cytotoxic CD8+ cells are the major lymphocyte subset that binds to cholangiocytes (43). When combined in vitro, T cells from PBC patients are directly cytotoxic to autologous biliary epithelial cells, inferring that CD8+ cytotoxic T cells may be active in bile duct destruction (44, see Figure 2).

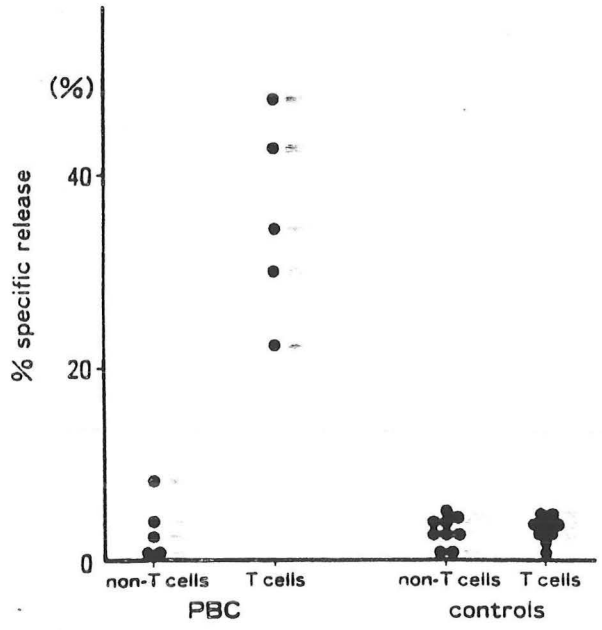

Figure 2

PBC T cells are cytotoxic for autologous bile duct cells

Aberrant staining for HLA class II on biliary epithelium, however, suggests that CD4+ T cell recognition of antigen on cholangiocytes may be important. Clinical studies examining HLA associations with PBC are difficult to interpret, given their contradictory observations and use of different technologies. The most frequently observed association across studies is a weak link with HLA-DR8. In large studies from Japan, England, the United States, and Germany, up to 36% of Caucasian females with PBC carried the HLA-DR8 antigen (45-49, see Table 4). Immunohistochemical analysis of T cell portal infiltrates and of T cell clones isolated from PBC liver tissue has been similarly inconclusive, as both CD4+ and CD8+ T cells are abundant.

TABLE 4

Incidence of HLA DR8 in PBC patients

Country	PBC patients	Normals
United States	30.1%	4.7%
England	11%	4%
England	18.5%	9.2%
Germany	36%	3.6%
Japan	76%	23%

In an effort to define the role of mitochondrial antigens in T cell-mediated damage of bile ducts in PBC, several investigators have looked for pyruvate dehydrogenase complex-reactive T cells in blood and liver of PBC patients (50-52, see Table 5). All have detected PDC-specific T cells, albeit with varying frequency. Between 5% and 73% of PBC patients have peripheral blood T lymphocytes that proliferate in response to purified bovine pyruvate dehydrogenase complex, and 53-58% of PBC patients have circulating T cells reactive to the purified E2/X component of PDC. Between 3-8% of liver-derived T cell clones from PBC patients are also specific for PDC. The majority of these PDC-reactive T cells are CD4+ cells, consistent with the hypothesis that these T cells are recognizing mitochondrial antigens on the surface of biliary epithelial cells along with MHC class II molecules. Not surprisingly, the exact epitopes of the dihydrolipoamide acetyl transferase enzyme that are recognized by T cells differ slightly from those recognized by B cells. The predominant autoantibody response in PBC is directed against the inner lipoyl domain of PDC-E2, whereas T cell responses appear to be directed at both inner and outer domains. In one study of 11 patients with PDC-E2 reactive T cells, 54% reacted to the outer lipoyl domain region, compared with 36% to the inner domain. Only 2% of patients reacted to both.

TABLE 5

Reactivity of T cells to whole or E2/X components of pyruvate dehydrogenase isolated from blood or liver of PBC patients

	Antigen	Blood*		Liver**	
		PBC patients	Controls	PBC patients	Controls
Lohr et al, 1993	PDC	11/15 (73%)	0/25	9/115 (8%)	
Jones et al, 1995	PDC	12/24 (50%)	24/48 (50%)		
	E2/X	14/24 58%)	6/48 13%)		
Van der Water et al, 1995	PDC	1/19 (5%)	0/12		
	E2/X	10/19 (53%)	0/12	14/450 (3%)	0/156

Patients with pre-cirrhotic disease are more likely to have T cell responses to PDC-E2/X than those with cirrhosis (80% vs 22%). This may reflect the early appearance of mitochondrial antigens on biliary epithelial cell surfaces and/or the relative paucity of remaining bile ducts that may be seen in late histologic disease.

The mere presence of PDC-reactive T cells does not confirm their role in bile duct destruction. In fact, peripheral blood T cell responses to PDC are not unique to PBC. Some normal controls and patients with other chronic active liver diseases have been found to have T cells reactive to PDC. Reactivity to PDC-E2/X is more specific, occurring significantly more often in PBC patients than in normals and non-PBC patients.

Antigens other than mitochondrial antigens may be primarily responsible for T cell activation in the liver in PBC. Few additional candidate antigens have actually been identified (53). However, there is substantial evidence that T cells within the liver of PBC patients are oligoclonal populations, in agreement with the notion that they are antigen-specific T cells. Analyses of the T cell receptor repertoire in the liver have consistently identified preferential utilization of certain T cell receptor beta gene chain rearrangements (54-57). These results contrast with studies examining the T cell receptor repertoire in chronic viral hepatitis, where T cell activation occurs polyclonally. Van der Water et al have reported that the T cell receptor genes utilized by PDC-reactive

clones infiltrating the liver in PBC are remarkably heterogeneous (52). Thus, PDC-reactive T cells alone are probably not responsible for the clonal populations of T cells found in PBC liver, and other candidate antigens must be examined.

37. Krams SM, Dorshkind K, Gershwin ME. Generation of biliary lesions after transfer of human lymphocytes into severe combined immunodeficiency (SCID) mice. J Exp Med 170:1919-1927, 1989.
38. Leon MP, Bassendine MF, Wilson JL et al. Immunogenicity of biliary epithelium: investigation of antigen presentation to CD4+ T cells. Hepatology 24:561-567, 1996.
39. Adams DH, Hubscher SG, Shaw J et al. Increased expression of intercellular adhesion molecule I (ICAM-1) on bile ducts in primary biliary cirrhosis and sclerosing cholangitis. Hepatology 14:426-431, 1991.
40. Van den Ord JJ, Sciot R, Desmet VJ. Expression of MHC products by normal and abnormal bile duct epithelium. J Hepatol 3:310-317, 1987.
41. Ballardini G, Mirakian R, Bianchi FB et al. Aberrant expression of HLA-DR antigens on bile duct epithelium in primary biliary cirrhosis: relevance to pathogenesis. Lancet 2:1009-1013, 1984.
42. Spengler U, Leifield L, Braunschweiger I et al. Anomalous expression of costimulatory molecules B7-1, B7-2 and CD28 in primary biliary cirrhosis. J Hepatol 26:31-36, 1997.
43. Leon M, Bassendine M, Gibbs P et al. Immunogenicity of biliary epithelium: study of the adhesive interaction with lymphocytes. Gastroenterology 112:968-977, 1997.
44. Onishi S, Saibara T, Nakata S et al. Cytotoxicity of spleen-derived lymphocytes against autologous biliary epithelial cells in autopsy patients with primary biliary cirrhosis. Liver 13:188-192, 1993.
45. Maeda T, Onishi S, Tanaka H et al. The association of primary biliary cirrhosis with HLA DRw8 in Japan. Int Hepatol Commun 1:104-108, 1993.
46. Gores RJ, Moore SB, Fisher LD et al. Primary biliary cirrhosis: associations with class II major histocompatibility complex antigens. Hepatology 7:889-892, 1987.
47. Manns MP, Bremm A, Schneider PM et al. HLA DRw8 and complement C4 deficiency as risk factors in primary biliary cirrhosis. Gastroenterology 101:1367-1373, 1991.
48. Underhill J, Donaldson P, Bray G et al. Susceptibility to primary biliary cirrhosis is associated with the HLAS-DR8-DQB1*0402 haplotype. Hepatology 16:1404-1408, 1992.
49. Gregory WI, Mehal W, Dunn AN et al. Primary biliary cirrhosis: contribution of HLA class II allele DR8. Quart J Med 86:393-399, 1993.
50. Lohr H, Fleischer B, Gerken G et al. Autoreactive liver-infiltrating T cells in primary biliary cirrhosis recognize inner mitochondrial epitopes and the pyruvate dehydrogenase complex. J Hepatol 18:322-327, 1993.
51. Jones DEJ, Palmer JM, James OFW et al. T cell responses to the components of the pyruvate dehydrogenase complex in primary biliary cirrhosis. Hepatology 21:995-1002, 1995.
52. Van der Water J, Ansari A, Prindiville T et al. Heterogeneity of autoreactive T cell clones specific for the E2 component of pyruvate dehydrogenase complex in primary biliary cirrhosis. J Exp Med 181:723-733, 1995.

53. Miyamoto T, Maida T, Onishi S et al. T cell autoreactivity against a 38 kD biliary protein (B1-p28) in primary biliary cirrhosis. J Hepatol 22:423-430, 1995.
54. Moebius U, Manns M, Hess G et al. T cell receptor gene rearrangements of T lymphocytes infiltrating the liver in chronic active hepatitis B and primary biliary cirrhosis (PBC): oligoclonality of PBC-derived T cell clones. Eur J Immunol 20:889-896, 1990.
55. Diu A, Moebius U, Ferradini L et al. Limited T-cell diversity in liver-infiltrating lymphocytes from patients with primary biliary cirrhosis. J Autoimm 6:611-619, 1993.
56. Mayo M, Combes B, Jenkins R. T-cell receptor Vβ gene utilization in primary biliary cirrhosis. Hepatology 24:1148-1155, 1996.
57. Tsai SL, Lai MY, Chen S. Analysis of rearranged T cell receptor (TCR) Vβ transcripts in livers of primary biliary cirrhosis: preferential Vβ usage suggests antigen-driven selection. Clin Exp Immunol 103:99-104, 1996.

CYTOKINES IN PRIMARY BILIARY CIRRHOSIS

Immune reactivity to intracellular and extracellular antigens is regulated by the cytokine environment. When reviewing the important cytokines implicated in the pathogenesis of PBC, it is helpful to categorize them into groups of cytokines that are typically associated with one of the T helper subsets, Th1 (producing mainly IFN-gamma, IL-2, TNFβ, and lymphotoxin) or Th2 (producing IL-4, IL-5, IL-6, IL-10 and IL-13). This polarized characterization of CD4+ T helper cells is well described in the mouse and defines functionally distinct classes of T cell responses. Th1 cells support macrophage activation, delayed type hypersensitivity responses, cytotoxic functions, and immunoglobulin switching to IgG1. Th1 cells constitute the predominant T cell response in several other organ specific human autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, juvenile diabetes, and autoimmune thyroid disease. Th2 cells, on the other hand, promote B cell activation, immunoglobulin switching to IgG2a, and are functionally important in allergic diseases. Both subsets possess features of theoretical importance in PBC.

The dichotomy between Th1 and Th2 cells may not be as distinct in humans as was previously assumed. It is important to remember that the Th1/Th2 classification may be used to characterize T cell populations, but that individual T cells may produce cytokines characteristic of both responses. Other cell lineages, such as CD8+ cells, NK+ cells, B cells, epithelial cells, and monocytes/macrophages may also secrete cytokines that influence the immune response. In addition, in vitro effects of cytokines do not necessarily occur in vivo. With these caveats in mind, it is still helpful to determine the overall pattern of cytokine secretion because the functional predominance of Th1 or Th2 cytokines directs the final outcome of immune responses.

Examining spontaneous cytokine expression at the site of inflammation probably best reflects the in vivo cytokine milieu that regulates tissue specific inflammation. Several investigators have measured cytokine secretion from peripheral blood T cells from PBC patients both before and after in vitro stimulation (58-63). The results are often contradictory, and the relationship between these levels and those encountered in the liver is not known.

Relatively few investigators have examined cytokine production in PBC liver. In studies examining cytokine RNA in PBC liver (64-66a, see Table 6), the most consistent finding was the presence of gamma interferon. Gamma interferon expression was greater in PBC liver than in control livers from patients with autoimmune hepatitis. Normal liver tissue from transplant donors did not express gamma interferon. Increased expression of gamma interferon in the liver of PBC patients is particularly significant because peripheral blood T cells from PBC patients that are stimulated in vitro have repeatedly demonstrated subnormal production of gamma interferon (66a,67,68). Increased expression of gamma interferon in the liver of PBC patients, therefore, represents more than just a systemic upregulation of pro-inflammatory cytokines due to chronic inflammatory disease. Moreover, gamma interferon has been shown to promote expression of MHC class II molecules and may be partly responsible for the aberrant HLA expression that is seen on PBC cholangiocytes. Although resting biliary epithelial cells constitutively express ICAM-1, gamma interferon substantially upregulates its expression and increases lymphocyte adhesion to biliary epithelial cells (69).

TABLE 6

Cytokine RNA expression in PBC liver

	γ-IFN	IL-2	IL-1	IL-4	IL-5	IL-6	IL-10
Martinez et al 1995	+	+			+	+	
Shindo et al 1996	+	-	-	-	-	-	
Dumoulin et al 1997	+	+		-			+
Harada et al 1997	+			+			

In the single study (65) that has examined cytokine RNA by PCR in liver biopsy specimens from PBC patients, no IL-2, IL-4, IL-5 or IL-6 was detected. Other studies using liver explants from end-stage PBC patients have been able to detect expression of both Th1 and Th2-type cytokines. Expression of IL-5, a Th2 associated cytokine, was detected in PBC liver but not autoimmune hepatitis or normal liver.

Liver T cells that are isolated from PBC liver and stimulated in vitro also produce gamma interferon, and they secrete increased amounts of IL-4 and IL-10 (Th2 cytokines) as compared to T cells from normal liver or liver infected with viral hepatitis (70). Characterization of T helper subsets may be possible on the basis of cell surface markers instead of measuring cytokine production. Specifically, high or low levels of the CD45RB isoform have been correlated with Th1 and Th2 cells, respectively. Liver infiltrating T cells from explanted livers of PBC patients more frequently have low levels of CD45RB expression (Th2 phenotype) than T cells from normal donor livers (71).

Thus, there appears to be a trend of increased Th2 influences in PBC liver as compared to other liver diseases, but these changes may not be present in early stages of PBC. More data from patients without end-stage PBC are needed to clarify this issue. Gamma interferon, on the other hand, is present in PBC liver even at earlier stages and dominates the overall cytokine profile.

Production of these cytokines is presumed to originate mostly from the large infiltrate of T cells in the portal tracts. As mentioned previously, other cell types in the liver such as Kupffer cells, natural killer cells, and B cells may also contribute to the cytokine profile, although this has not been specifically studied in PBC. Exciting recent reports suggest that biliary epithelial cells may also be capable of influencing their own environment by cytokine secretion. Reverse transcriptase PCR of a cholangiocarcinoma cell line demonstrates expression of several pro-inflammatory cytokines such as TNF-α, IL-1, IL-6 and IL-8 (72,73, see Table 7).

TABLE 7

Cytokine expression of cultured cholangiocytes

IFN-γ	GM-CSF	TNF-α	TGF-β	IL-1	IL-2	IL-4	IL-5	IL-6	IL-8	IL-10	IL-12
-	-	++	+	++	-	+	+	++	++	+	-

58. Tilg H, Wilmer A, Vogel W et al. Serum levels of cytokines in chronic liver diseases. Gastroenterology 103:264-274, 1992.
59. Muller C, Zielinski CC. Interleukin-6 production by peripheral blood monocytes in patients with chronic liver disease and acute viral hepatitis. J Hepatol 15:372-377, 1992.
60. Miller LC, Kaplan MM. Serum interleukin-2 and tumor necrosis factor-α in primary biliary cirrhosis: decrease by colchicine and relationship to HLA-DR4. Am J Gastroenterol 87:465-470, 1992.
61. Broome U, Eriksson LS, Sundin U et al. Decreased in vitro production of tumor necrosis factor in primary biliary cirrhosis patients. Scand J Gastroenterol 27:124-128, 1992.
62. Miller LC, Sharma A, McKusick AF et al. Synthesis of interleukin-1 in primary biliary cirrhosis: relationship to treatment with methotrexate or colchicine and disease progression. Hepatology 22:518-524, 1995.
63. Klein R, Leuscher U, Berg PA. Cytokines in primary biliary cirrhosis and primary sclerosing cholangitis. In: Berg PA, Leuscher U (eds): Bile Acids and Immunology. Dordrecht/Boston/London: Kluwer Academic Publishers, 1996, pp.195-215.
64. Martinez OM, Villaneuva JC, Gershwin ME et al. Cytokine patterns and cytotoxic mediators in primary biliary cirrhosis. Hepatology 21:113-119, 1995.
65. Shindo M. Mullin GE, Braun-Elwert L et al. Cytokine mRNA expression in the liver of patients with primary biliary cirrhosis (PBC) and chronic hepatitis B (CHB). Clin Exp Immunol 105:254-259, 1996.
66. Dumoulin FL, Bach A. Leifeld L et al. Semiquantitative analysis of intrahepatic cytokine mRNAs in chronic hepatitis C. J Infect Dis 175:681-685, 1997.
66a. Harada K, Van der Water J, Leung P et al. In situ nucleic acid hybridization of cytokines in rpimary biliary cirrhosis: predominance of the Th1 subset. Hepatology 25:791-796, 1997.
67. Spengler U, Moller A, Jung MC et al. T-lymphocytes from patients with primary biliary cirrhosis produce reduced amounts of lymphotoxin, tumor necrosis factor, and interferon gamma upon mitogen stimulation. J Hepatol 15:1299-1335, 1992.
68. Jones DE, Palmer JM, Leon MP, Yeaman SJ et al. T cell responses to tuberculin purified protein derivative in primary biliary cirrhosis: evidence for defective T cell function. Gut 40:277-283, 1997.
69. Ayres RCS, Neuberger JM, Shaw J et al. Intercellular adhesion molecule-1 and MHC antigens on human intrahepatic bile duct cells: effect of pro-inflammatory cytokines. Gut 34:1245-1249, 1993.
70. Lohr HF, Schlaak JF, Gerken G et al. Phenotypical analysis and cytokine release of liver-infiltrating and peripheral blood lymphocytes from patients with chronic hepatitis of different etiology. Liver 14:161-166, 1994.
71. Leon MP, Spickett G, Jones DEJ et al. CD4+ T cell subsets defined by isoforms of CD45 in primary biliary cirrhosis. Clin Exp Immunol 99:233-239, 1995.
72. Phillips JO. Bile duct epithelial cell immunobiology. In: Diseases of the Bile Ducts: Pathogenesis, Pathology, and Practice. AASLD Postgraduate Course, 1996.

73. Morland CM, Fear J, Joplin R et al. Human biliary epithelial cells express chemokines IL-8 and monocyte chemotactic protein-1 in response to inflammatory cytokine stimulation. Hepatology 24:332A, 1996 (Abstract).

SUMMARY OF CURRENT VIEWPOINTS

1. Destruction of biliary epithelial cells in primary biliary cirrhosis is most likely T-cell mediated.

2. Mitochondrial antigens on the surface of bile ducts may be targets for some T cells in the liver, but other important target antigens probably exist and should be sought.

3. Anti-mitochondrial antibodies themselves are not responsible for initiating the immune attack on cholangiocytes.

4. Gamma interferon production in the liver is important in modulating inflammation in PBC patients.

5. Biliary epithelial cells may play an active role in their own eventual demise by participating in lymphocyte adhesion, antigen presentation and cytokine secretion.

THERAPY OF PRIMARY BILIARY CIRRHOSIS

Therapies for PBC have to be judged in the context of the natural history of the disease (2,74,75). Far advanced PBC, uniformly fatal until recent years, can now be salvaged with high survival and rehabilitation rates in those who have access to liver transplantation (76,77). Transplantation is very expensive, however, and on this basis alone not yet available to many suitable candidates. Moreover, organ availability is also an important limiting factor in access to transplantation.

74. Christensen E, Crowe J, Doniach D et al. Clinical pattern and course of disease in primary biliary cirrhosis based on an analysis of 236 patients. Gastroenterology 78:236-246, 1980.
75. Dickson EP, Grambsch PM, Fleming TR et al. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 10:1-7, 1989.
76. Pasha TM, Dickson ER. Survival algorithms and outcome analysis in primary biliary cirrhosis. Sem Liver Dis 17:147-158, 1997.
77. Neuberger J. Transplantation for primary biliary cirrhosis. Sem Liver Dis 17:137-146, 1997.

The aims of medical therapy are to arrest and, if possible, to reverse progression of the disease. In the process, one would seek through improvement and/or reversal in symptoms to improve the quality of life, and through prevention of complications to prolong life and prevent the need for liver transplantation.

Medical therapies that have been and are currently being assessed fall into two major categories:

1. Immunosuppressive - antiinflammatory
   - Azathioprine
   - Chlorambucil
   - Colchicine
   - Cyclosporine
   - Methotrexate
   - Prednisolone

2. Bile acid therapy
   - Ursodeoxycholic acid

Past reviews of medical therapy (78,79) included extensive information on trials of d-penicillamine, an agent which is judged to be ineffective in altering the course of PBC. D-penicillamine, which has immunologic and decoppering effects, will not be considered further, therefore, in the following discussion of the treatment modalities listed above.

78. Wiesner RH, Grambsch PM, Lindor DK et al. Clinical and statistical analyses of new and evolving therapies for primary biliary cirrhosis. hepatology 8:668-676, 1988.
79. Kaplan MM. Medical treatment of primary biliary cirrhosis. Sem Liver Dis 9:138-143, 1989.

IMMUNOSUPPRESSIVE - ANTIINFLAMMATORY AGENTS

Azathioprine

Azathioprine has been evaluated in two controlled clinical trials (80-82). A total of 45 patients was randomized in the first (80), 22 to azathioprine (2 mg/kg/day), 23 to no therapy. Asymptomatic, cirrhotic and patients with evidence of liver failure were excluded from the study. Subjective improvement in pruritus was noted in azathioprine-treated patients. Liver tests were not improved. Histologic progression to cirrhosis occurred in 62% of azathioprine-treated patients as compared to 50% of controls during the first 2 years of study. A trend towards improved survival in azathioprine-treated patients was noted after 3 years. The investigators concluded that azathioprine treatment was probably not beneficial for symptomatic precirrhotic patients.

The second multicenter trial (81,82) involved 248 patients, 127 on azathioprine (0.5-1.5 mg/kg/day), 121 on placebo. Incapacitation as measured by an incapacitation index was delayed in azathioprine-treated patients. Survival was not significantly different in the two treatment groups. When data were adjusted for slight imbalances in levels of serum bilirubin, however, the azathioprine group was shown to have a significant improvement in survival by 20 months in the average patient. Relatively few side effects were observed. Despite this conclusion, this therapy has not attracted much attention and has largely been abandoned. It should be pointed out that almost 60% of the patient were jaundiced at the time of entry into this trial. Moreover, the initial mean serum bilirubin was close to 4 mg% in the study of Heathcote et al (80). Thus, both series dealt largely with more advanced disease that may not be amenable to therapy that might arrest or slow down ongoing bile duct destruction. An adequate assessment of azathioprine in milder disease has not yet been carried out.

80. Heathcote J Ross A, Sherlock SA. A prospective controlled trial of azathioprine in primary biliary cirrhosis. Gastroenterology 70:656-660, 1976.
81. Crowe J, Christensen E, Smith M et al. Azathioprine in primary biliary cirrhosis: a preliminary report of an international trial. Gastroenterology 78:1005-1010, 1980.
82. Christensen E, Neuberger J, Crowe J et al. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Gastroenterology 98:1084-1091, 1985.

Chlorambucil

This alkylating agent was evaluated in a randomized, controlled, non-blinded trial (83) in which 13 patients received chlorambucil and 11 received no therapy. Patients were followed for a mean period of 4.1 years. Symptoms were not evaluated. Serum bilirubin fell modestly in treated patients compared to a progressive rise in untreated controls. IgM decreased to normal and IgG fell. Inflammation decreased in liver biopsies, but no effect was seen on fibrosis or histologic stage. Insufficient information is available to assess effects on survival. The treatment appeared to arrest progression of the disease. A downside of therapy was that some degree of bone marrow depression was seen in all patients. In 4, it was severe enough to lead to discontinuation of treatment. The concern with bone marrow suppression, and the potential increased risk of developing malignancies such as lymphoma and acute myelogenous leukemia, will limit the long-term use of chlorambucil in PBC.

83. Hoofnagle DH, Davis GL, Schafer DF et al. Randomized trial of chlorambucil for primary biliary cirrhosis. Gastroenterology 91:1327-1334, 1986.

Colchicine

Data are now available in definitive publications from three controlled trials (84-86). The studies agree in most respects. Improvement in laboratory tests, when documented, was modest. Symptoms, signs and histologic progression were not improved. There was a trend toward improved survival but the number of patients and controls followed for prolonged periods was small. In general, colchicine is safe. Some patients withdrew from therapy because of diarrhea. Longer observation in a larger number of patients will be required before treatment can be considered to be efficacious.

84. Kaplan MM, Alling DW, Zimmerman HJ et al. A prospective trial of colchicine for primary biliary cirrhosis. New Engl J Med 315:1448-1454, 1986.
85. Warnes TW, Smith A, Lee FI et al. A controlled trial of colchicine in primary biliary cirrhosis. Trial design and preliminary report. J Hepatol 5:1-7, 1987.
86. Bodenheimer H, Schaffner F, Pezzullo J. Evaluation of colchicine therapy in primary biliary cirrhosis. Gastroenterology 95:124-129, 1988.

Cyclosporine

Data are available from 5 groups on a total of 45 patients with PBC treated for 2 (87), 6 (89,89) and 12 months (90,91), respectively. Longer clinical experiences have now been reported by Wiesner et al (92), and by Lombard et al (93). In the study of Wiesner et al (92) dealing with treatment of 29 precirrhotic patients, 19 received cyclosporine and 10 a placebo. Symptoms improved, and significant decreases were observed in serum levels of bilirubin, alkaline phosphatase and ALT. After 2 years of therapy, liver histology progressed in only 1 of 13 patients in the cyclosporine group as compared to 5 of 7 in the placebo group. Signs of nephrotoxicity developed in 12, and increased blood pressure in 9 of the 19 cyclosporine group.

In a much larger multicenter trial (93), 349 patients with PBC were randomized to receive cyclosporine or placebo and followed for up to six years. Cyclosporine had a positive effect on pruritus but not fatigue. Favorable cyclosporine effects were observed for serum bilirubin, alkaline phosphatase, aminotransferases and serum albumin. Liver histology was not affected. The number of deaths and transplants was not favorably affected. However, time from entry to death or transplantation was prolonged by approximately 16 months in the average patient. Hypertension and renal impairment were significant complications of prolonged therapy. Coupled with only a modest effect on transplantation and death, it is unlikely that cyclosporine will play a major role in the therapy of PBC.

87. Routhier G, Epstein O, Janozsy G et al. Effects of cyclosporin A on suppressor and inducer T lymphocytes in primary biliary cirrhosis. Lancet 2: 1223-1226, 1980.

88. Karlson-Parra A, Totterman TH, Nyberg A et al. Immunological effects of cyclosporin in primary biliary cirrhosis: suppression of activated T cells and autoantibody levels. Int Arch Allergy Appl Immunol 83:256-264, 1987.
89. Beukers R and Schalm SW. Effect of cyclosporine and cyclosporine plus prednisone in primary biliary cirrhosis. Transplant Proc 20:340-343, 1988.
90. Wiesner RH, Dickson ER, Lindor DK et al. A controlled trial evaluating cyclosporin in the treatment of primary biliary cirrhosis: a preliminary report. Hepatology 7:1025, 1987 (Abstract).
91. Minuk GY, Bohme CE, Pzurgess E et al. Pilot study of cyclosporin A in patients with symptomatic primary biliary cirrhosis. Gastroenterology 95:1365-1363, 1988.
92. Wiesner RH, Ludwig J, Lindor KD et al. A controlled trial of cyclosporine in the treatment of primary biliary cirrhosis. New Engl J Med 322:1419-1424, 1990.
93. Lombard M, Portmann B, Neuberger J et al. Cyclosporin treatment in primary biliary cirrhosis: results of a long-term placebo controlled trial and effect on survival. Gastroenterology 104:519-526, 1993.

Corticosteroids

Until recently, available data on long-term effects of corticosteroids on the course of PBC have been limited largely to uncontrolled and limited experiences (94,95). Mitchison and colleagues (96) reported results of a pilot double-blind, controlled 1 year trial of prednisolone in symptomatic patients who did not have ascites, encephalopathy or variceal bleeding, Prednisolone started at 30 mg/day was decreased by 5 mg every 2 weeks to a dose of 10 mg/day at 8 weeks, where it was maintained for the balance of the year. Pruritus and/or fatigue improved in 15 of the 19 prednisolone-treated patients but none of the 17 placebo-treated controls. As the prednisolone dose was decreased, symptoms recurred in 9 patients. Statistically significant improvements in laboratory tests were noted for alkaline phosphatase at 2 and 23 months (approximate fall of 35%); for AST at 12 months from a mean initial value of 99 to 86 units, whereas mean AST rose from 107 to 119 units in placebo controls, and for total protein at 2 and 23 months due to a fall in immunoglobulins (the only significant fall was for IgG at 12 months). Improvement trends that were not significant statistically included a transient fall in serum bilirubin that returned to pretreatment values by 1 year compared to a small increase in serum bilirubin in placebo-treated controls, a slight rise in serum albumin and decreases in serum IgG and IgM. Thus, mean values of blood tests improved; the magnitude of change was impressive only for alkaline phosphatase and significant statistically for only a few others. Liver histology did not change in most patients. Worsening was detected primarily in placebo-treated patients. Assessments of bone showed a fall in femoral photon absorptiometry and a greater fall in iliac crest trabecular bone volume in prednisolone-treated patients than in placebo-treated controls. Findings after prolongation of the trial on 10 mg prednisolone per day for the next 2 years for a total of 3 years on prednisolone (97) suggested greater progression of liver disease in patients receiving placebo, and more bone loss in patients receiving prednisolone. Concern about long-term deleterious effects of corticosteroids, particularly on bone, will require additional data before corticosteroids are deemed ready for broader testing (98).

94. Howat HT, Ralston AJ, Varley H et al. The late results of long term treatment of primary biliary cirrhosis by corticosteroids. Rev Int Hepatol 16:227-238, 1966.
95. Geubel AP, Baggenstoss AH and Summerskill WHJ. Response to treatment can differentiate chronic active liver disease with cholangitic features from the primary biliary cirrhosis syndrome. Gastroenterology 71:444-449, 1976.
96. Mitchison HC, Bassendine MF, Malcolm AJ et al. A pilot, double-blind, controlled 1-year trial of prednisolone treatment in primary biliary cirrhosis: Hepatic improvement but greater bone loss. Hepatology 10:420-442, 1989.
97. Mitchison HC, Palmer JM, Bassendine MF et al. A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three year results. J Hepatol 15:336-344, 1992.
98. Combes B. Prednisolone for primary biliary cirrhosis - good news, bad news. Hepatology 10:511-513, 1989 (Editorial).

Methotrexate (MTX)

Evidence that oral pulse MTX may be effective in management of precirrhotic PBC has been provided by Kaplan and associates (99-101). Oral pulse MTX, 15 mg per week, resulted in improvement in symptoms, and statistically significant improvement in alkaline phosphatase (mean decrease 61%), ALT (decrease 34%), and bilirubin (decrease 46% from a mean value of 1.24 to 0.67 mg/dl) in 9 women with symptomatic precirrhotic PBC (3 stage I, 2 stage II, 4 stage III). Liver histology improved in 4 and did not worsen in the other 5 patients.

By contrast, of 7 patients with cirrhosis (stage IV) and more advanced disease biochemically, 3 worsened, 2 did not improve, but 2 without liver failure have had improvement in symptoms and liver tests. In a more recent report (100), Kaplan and associates focused on 5 of 19 patients with precirrhotic PBC who had dramatic responses to low-dose methotrexate therapy. An additional 5 demonstrated biochemical and histologic improvement; the other 9 did not respond to methotrexate. The drug was well tolerated in most patients. The finding of a serious interstitial pneumonitis in 15 percent of the patients (101), even though reversible, points out the potential hazard associated with use of methotrexate.

99. Kaplan MM, Knox TA and Arora S. Primary biliary cirrhosis treated with low-dose oral pulse methotrexate. Ann Intern Med 109:429-431, 1988.
100. Kaplan MM. Methotrexate treatment of chronic cholestatic liver disease: friend or foe. Quart J Med 268:757-761, 1989.
101. Kaplan MM and Knox TA. Treatment of primary biliary cirrhosis with low-dose weekly methotrexate. Gastroenterology 101:1332-1338, 1991.

Summary of Immunosuppressive - Antiinflammatory Agents

Most of the immunosuppressive - antiinflammatory agents referred to above exert some positive effects. Liver tests are improved to variable extents by most of these compounds. Inflammation in liver is improved by chlorambucil, cyclosporine and methotrexate. Colchicine is safe. It has no positive effects on symptoms or histology. It's impact on the disease seems small. Some of the investigators involved in the initial controlled studies of the drug appear to feel that it has limited use as a sole therapeutic agent. Chlorambucil and cyclosporine have an interesting impact on the disease. Concern exists about their long-term use, however, because of significant toxicities. Methotrexate in weekly pulse therapy, has been accompanied by significant improvement in a small number of precirrhotic patients. It has not been effective in most patients who are already cirrhotic.

BILE ACID THERAPY

Ursodeoxycholic acid (ursodiol) is undoubtedly the most commonly used medication for treatment of primary biliary cirrhosis because (a) it rapidly induces improvements in laboratory markers of cholestasis (i.e. alkaline phosphatase, gamma glutamyl transpeptidase (GGT), serum bilirubin) and inflammation (AST, ALT) which characterize the disease; and (b) it is well tolerated and safe. The important beneficial uncontrolled initial observations of Poupon (102), Leuschner (103) and their respective associates have now been amply confirmed in many clinical trials but only four (104-107) have dealt with relatively large numbers of patients assessed in a randomized, placebo-controlled, double-blind manner.

Features of the four major randomized trials are presented in Table 8. The trial of Poupon and associates (104) compared ursodiol to placebo, each given for two years. Subsequently, placebo-treated patients were given ursodiol. Heathcote and associates (105) also compared ursodiol and placebo in patients treated for two years. Ursodiol was then offered to the placebo-treated group and approximately 50 percent accepted open label ursodiol (108). Lindor and associates (106) randomized 180 patients over a 50 month period. The study was stopped two years after the 132nd patient was entered, when all patients were switched to ursodiol (109). Thus, 48 randomized patients would have been followed for less than 2 years, 132 for up to 2 years, and an unspecified number for a longer period. Combes and associates (107) compared ursodiol and placebo, each given for two years. Placebo-treated patients were then given open label ursodiol. Thus, the only trial in which initially randomized patients were compared for longer than 2 years is the one of Lindor and associates (106). The number of such patients is uncertain.

The most important end points of beneficial medical therapy are the prevention of liver transplantation and prolongation of survival without transplantation. For the trials with data for only 2 years (104,105,107) there were 35 transplants and deaths without transplantation in the placebo groups and 29 in ursodiol-treated groups. When only liver-related deaths are tallied, these figures become 32 for placebo and 28 for ursodiol-treated patients. In Lindor's patients compared for 3.3 years, 12 deaths or transplants were reported in the placebo

TABLE 8

FEATURES OF THE FOUR MAJOR RANDOMIZED, DOUBLE-BLIND, CONTROLLED TRIALS OF URSODIOL VERSUS PLACEBO IN THE TREATMENT OF PRIMARY BILIARY CIRRHOSIS

	Poupon et al[104]		Heathcote et al[105]		Lindor et al[106]		Combes et al[107]	
	Placebo	Ursodiol	Placebo	Ursodiol	Placebo	Ursodiol	Placebo	Ursodiol
No. of patients	73	73	111	111	91	89	74	77
Percent women	89	95	95	91	87	91	92	86
Mean age (years)	57	55	55	57	52	54	49	49
Histologic stage:								
I, II (%)	58	50	44	47	29	35	28	36
III, IV (%)	42	50	56	53	71	65	72	64
Mayo risk score	4.8	4.9	-	-	5.1	5.2	4.7	4.7
Duration of controlled trial	24 months		24 months		Up to 50 months Mean follow-up 24 months		24 months	
Daily dose (mg/kg)	13-15 in 2 doses		14 with evening meal		13-15 with meals and at bedtime		10-12 at bedtime	
Withdrawals	6 (8%)	5 (7%)	15 (14%)	10 (9%)	13 (14%)	5 (6%)	3 (4%)	2 (3%)
Completed 2-year trial	54 (74%)	62 (85%)	77 (69%)	89 (80%)	Uncertain		60 (80%)	63 (82%)
End point failures at 2 years:								
Death/transplantation	5[a] (7%)	5[a] (7%)	19[b] (17%)	12[b] (11%)	12[c,d]	7[c]	11 (15%)	12 (16%)
Developed cirrhosis	Not stated		Not stated		No difference		No difference	
Developed varices	Not stated		Not stated		No difference		No difference	
Ursodiol effects on:								
Symptoms	Not stated		No treatment effect		No treatment effect		No treatment effect on overall mean values, but significant decrease in development of severe fatigue/ pruritus	
Laboratory tests	Improvements in bilirubin, alkaline phosphatase, GGT, AST, ALT, IgM, cholesterol		Improvements in bilirubin, alkaline phosphatase, GGT, AST, ALT, IgM, cholesterol		Improvements in bilirubin, alkaline phosophatase, AST		Improvements in bilirubin, alkaline phosphatase, GGT, AST, ALT, IgM, albumin, particularly in patients with entry serum bilirubin <2 mg/dl	
Histology	Better for piecemeal necrosis, parenchymal necrosis, portal and lobular inflammation, cholestasis, bile duct paucity and proliferation		Better for periportal ballooning and bile duct paucity		No effect on stage. Other features not yet reported.		Better for piecemeal necrosis, portal inflammation in stratum 1 and fibrosis and cholestasis in stratum 2.	

Abbreviations: GGT, gamma-glutamyltransferase; AST, aspartate transaminase; ALT, alanine transaminase; IgM, immunoglobulin M.

[a] Estimated from Reference 104, Figure 2.
[b] 3 placebo and one ursodiol deaths were not liver-related.
[c] For randomized patients followed 3.3 years. Modified from reference 106.
[d] 4 deaths in the placebo group were not related to liver disease.

group and 7 in the ursodiol group. Again, when only liver-related deaths are taken into account, these values become 8 for placebo, and 7 for ursodiol-treated patients. None of these differences are significant statistically, whether all deaths or only liver-related deaths are counted.

Poupon and associates (110) observed fewer treatment failures including death and transplantation in patients initially randomized to ursodiol for 2 years, then continued on ursodiol for 2 more years; compared to patients initially randomized to placebo for 2 years, then switched to ursodiol for 2 years. In the Canadian trial (108), survival was not significantly different at 6 years in patients initially randomized to ursodiol compared to those randomized to placebo, some of whom then switched to ursodiol at 2 years. In the U.S. Multicenter Trial (107,111), death and transplantation were noted with comparable frequency at 5 years in patients initially randomized to ursodiol, and in those initially randomized to placebo for 2 years then switched to ursodiol. A combined analysis (112) of three of the trials (104-106) including patients initially on placebo then switched to ursodiol, reported extension of survival free of transplantation and a decrease in the risk of dying or being transplanted in patients originally randomized to ursodiol.

Short of survival and transplantation, all trials demonstrate rapid improvement in many laboratory tests. Significant decreases in alkaline phos-phatase, GGT, AST and ALT are frequently noted by six weeks, clearly by 3 months, and usually reach a nadir by 6 months. Responses are more impressive in patients with earlier stages of the disease, particularly in those whose serum bilirubin is less than 2 mg per dl at initiation of therapy (107). Serum bilirubin tends to plateau, contrasting with a rising bilirubin over time in patients on placebo. Doubling of bilirubin occurred much more frequently in placebo than in ursodiol-treated patients, accounting for much of the treatment failures in all of the trials. Improvements in histologic features is reported in three of the trials (104,105,107). In the fourth, histologic stage did not change on ursodiol, but other features have not yet been reported.

Daily dosage of ursodiol was 13-15 mg/kg in three of the trials (104-106) and 10-12 mg/dl in the other (107). Enrichment of the bile acid pool with ursodiol as reflected in analyses of bile sampled at the completion of the trial and subsequently measured in a single laboratory were comparable in two of the studies (106,107). Ursodiol accounted for approximately 40 percent of the bile acids in bile in the patients of Combes et al (107) and 39.5 percent in Lindor's patients. Of interest, the comparable degree of enrichment was achieved even though the patients of Combes et al ingested a lower dose (10 to 12 versus 13 to 15 mg per kg) and took it once at bedtime rather than in divided doses.

Despite striking improvements in results of laboratory tests, improvement in some histologic features with delay in progression in early stages of PBC, and slower development of treatment failures in ursodiol treatment patients, we are still left with uncertainty as to whether ursodiol will impact heavily on the need for transplantation and on transplant-free survival. All of the investigator groups impressed with their favorable results ended the randomized controlled phases of their trials too soon to unequivocally provide an answer. Patients with more advanced disease are unlikely to benefit from ursodiol treatment (107). Patients with less severe PBC randomized to either ursodiol or placebo would have to be

followed for many years to sort this problem out. It is unlikely that such a trial will be carried out. Unfortunately, accurate surrogate markers of efficacy short of transplantation and death are not yet available.

CONCLUSIONS

Ursodiol is well tolerated and safe. Because of the good things that accompany its use including the trend to slower development of various treatment failures, it is likely to be the treatment of choice for PBC. The mechanisms by which ursodiol exerts its favorable effects are still uncertain. Protection against cytotoxic effects of increased concentrations of dihydroxy bile acids that accumulate in PBC is one important effect (113). A decrease in immunologically mediated injury via modulation of expression of HLA antigens on cell surfaces is postulated to be another (114). There is as yet no evidence that ursodiol prevents ongoing bile duct injury. Indeed, florid duct lesions continue to be seen even after a few years of ursodiol treatment. The cause of bile duct lesions is still uncertain although immunologically-mediated injury is felt to be most important in perpetuating the disease. For the immediate future, we are likely to see new therapeutic regimens assessed in which ursodiol is used as baseline therapy, and agents with antiimmunologic-antiinflammatory activities added as second medications.

102. Poupon R, Chretien Y, Poupon RE, Ballet F, Calmus Y, Darrus F. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? Lancet 1987;i:834-836.
103. David R, Kurtz W, Strohm WD, Leuschner U. Die Wirkung von Ursodeoxycholsäure bei chronischen Lebererkrankungen. Eine Pilotstudie. Z. Gastroenterologie 1985;23:420.
104. Poupon RE, Balkau B, Eschwege E, Poupon R. The UDCA-PBC Study Group. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. N Engl J Med 1991;324:1548-1554.
105. Heathcote EJ, Cauch-Dudek K, Walter V, Bailey RJ, Blendis LM, Ghent CA, Michieletti P, et al. The Canadian multicenter double-blind randomized controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-1156.
106. Lindor DL, Dickson ER, Baldus WP, Jorgensen RA, Ludwig J, Murtaugh PA, Harrison JM, et al. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;106:1284-1290.
107. Combes B, Carithers RL, Jr., Maddrey WC, Lin D, McDonald MF, Wheeler DE, et al. A randomized, double-blind, placebo-controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1995;22:759-766.
108. Kilmurry MR, Cauch-Dudek K, O'Rourke K, Heathcote EJ, and the Canadian PBC Trial Group. Is the Mayo Model useful for predicting survival following the introduction of ursodeoxycholic acid treatment for primary biliary cirrhosis? Gastroenterology 1995;108:A1097 (Abstract).

109. Lindor DL, Therneau TM, Jorgensen RA, Malinchoc M, and Dickson ER. Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. Gastroenterology 1996;110:1515-1518.
110. Poupon RE, Poupon R, Balkau B, The UDCA-PBC Study Group. Ursodiol for the long-term treatment of primary biliary cirrhosis. N Engl J Med 1994; 330:1342-1347.
111. Carithers RL, Luketic VA, Peters M, et al. Extended follow-up of patients in the US multicenter trial of ursodeoxycholic acid for primary biliary cirrhosis. Gastroenterology 1996;110:A1163 (Abstract).
112. Heathcote EJ, Lindor KD, Poupon R, et al. Combined analysis of French, American and Canadian randomized controlled trials of ursodeoxycholic acid therapy in primary biliary cirrhosis. Gastroenterology 1995;108:A1082 (Abstract).
113. Heuman DM. Hepatoprotective properties of ursodeoxycholic acid. Gastroenterology 1993;104:1865-1870.
114. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: Effect of ursodeoxycholic acid. Hepatology 1990;11:12-15.