

Primary Biliary Cirrhosis: A Mount Sinai Perspective

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Abstract

Individuals afflicted with primary biliary cirrhosis (PBC) first undergo chronic, nonsuppurative destruction of their intrahepatic bile ducts, eventually leading to cirrhosis. Over nearly 50 years, many faculty members at the Mount Sinai School of Medicine, including Dr. Hans Popper and Dr. Fenton Schaffner, have made important contributions to our understanding of the natural history and histopathologic evolution of PBC. And today, many patients with PBC continue to be cared for at Mount Sinai. In the absence of a cure for the disease, these patients continue to be enrolled in clinical trials and, when necessary, in the Mount Sinai liver transplant program. The establishment of the Center for the Study of Primary Biliary Cirrhosis at Mount Sinai, supported by the Artzt Family Foundation Trust, has enabled the faculty to expand both clinical and basic science initiatives related to primary biliary cirrhosis. Several of these new initiatives are described below and placed in the context of our current understanding of the immunopathogenesis of PBC.

Key Words: Primary biliary cirrhosis, methotrexate, celiac disease, apoptosis, cholangiocytes, autoantibody, protein oxidation, pyruvate dehydrogenase, genetics.

Introduction

PRIMARY BILIARY CIRRHOSIS (PBC) is a potentially fatal chronic liver disease primarily affecting women and is typically diagnosed during the fifth decade of the person's life. PBC is characterized by the chronic, nonsuppurative destruction of intrahepatic bile duct epithelial cells (cholangiocytes) and by high titers of antimitochondrial antibodies (AMA) (1). The disease is often detected incidentally in its asymptomatic stage when elevated serum alkaline phosphatase levels are noted. Fatigue and pruritus are the most common presenting symp-

toms. Although it is an uncommon disease, PBC is a frequent indication for liver transplantation among women. Approximately 70% of patients also have salivary gland involvement (2).

Prior to the era of orthotopic liver transplantation, PBC was often fatal. In recent years, treatment with ursodeoxycholic acid (UDCA) has been shown to slow progression of the disease and delay or obviate the need for liver transplantation. A number of research avenues are being pursued in the hope of further slowing down the progression of the disease or curing it, thereby eliminating any need for liver transplantation. A number of present and past investigators at the Mount Sinai School of Medicine have contributed to our understanding of PBC. In this article, we will briefly outline the current state of knowledge regarding PBC, while focusing on a number of ongoing PBC-related research efforts at Mount Sinai.

Disease Course

The course of PBC from its often long asymptomatic phase to possible liver failure is somewhat predictable. For a complete review, see the landmark paper by Shapiro et al. (3)

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Supported by the Artzt Family PBC Foundation, a Schering/AASLD Advanced Hepatology Fellowship Award, and an NIH K08 Award (NIDDK).

Adapted from a Grand Rounds presentation to the Department of Medicine, Mount Sinai School of Medicine, New York, NY, on November 14, 2000, and updated as of December 2002.

studying the natural history of this disease. In their study of the disease course of PBC, patients were divided into a stable phase and an accelerated phase, based on the level of bilirubin. Though the Mayo Risk Score (4) has somewhat supplanted the bilirubin level as the sole determinant of prognosis, the observations made several decades ago still remain valid. Shapiro et al. noted that most people with PBC remain in the so-called stable phase of disease, during which the bilirubin remains in the normal range, for a prolonged period of time. Patients then enter into an accelerated phase of disease, at which time the bilirubin rises. When two consecutive bilirubin levels are in excess of 2 mg/dL, average survival time is four years. Since this original study (3), the time spent by most patients in the stable phase of the disease has increased to more than 20 years. Part of this increase may be due to earlier recognition and diagnosis of the disease.

Current Medical Treatment

The ultimate goal of therapy in PBC, naturally, is to cure the disease. While there is as yet no cure for PBC, current treatments do slow the disease's progression. Ursodeoxycholic acid is now the mainstay of therapy for PBC. Several large, randomized controlled trials using UDCA have found its use to be beneficial (5–8). With treatment failure and/or bilirubin levels as end points, UDCA had a statistically significant beneficial effect on survival. In the study by Combes et al. (5), this benefit was seen only for those patients whose pretreatment serum bilirubin levels were less than 2 mg/dL. Two studies addressed the optimal dosing of UDCA in PBC. Both studies found that 13–15 mg/kg/day provided the greatest benefit in terms of UDCA enrichment of bile acids and improvement in Mayo Risk Score, total bilirubin, alkaline phosphatase and gamma-glutamyl-transferase (GGT) (9, 10).

In a recent paper by Poupon and the UDCA-PBC study group, an analysis of 10-year survival found that survival rates of non-cirrhotic PBC patients were comparable to those of an age- and sex-matched control population of persons without PBC (11). However, for the group of patients who had PBC with cirrhosis on biopsy, survival was decreased. Treatment with UDCA improved survival rates in both groups (11). A number of drugs including azathioprine, chlorambucil, cyclosporine, penicillamine, and prednisolone have been tested and found to be

either too toxic or ineffective in treating the disease (12).

Effect of Methotrexate on the Course of PBC

In the late 1980s, preliminary studies (13) suggested that methotrexate might be of benefit in treating PBC. In the late 1980s, 110 patients initiated treatment with weekly methotrexate. Accordingly, with IRB approval, we reviewed the data recorded in the charts to assess the effect of methotrexate on survival without liver transplant, Mayo Risk Score and various biochemical and histologic parameters (14). We also assessed how well this drug was tolerated by individuals with PBC. Patients were treated with 15 mg of methotrexate weekly. As the benefits of UDCA became more evident in the early 1990s, UDCA was either initiated or resumed for most patients. Pruritus and osteoporosis were treated according to the individual needs of the patients.

Of the original 110 patients who began therapy, only 57 were still taking methotrexate regularly at 5 years. Of the 53 patients who discontinued treatment prematurely, 27 patients did so because of adverse drug effect which included nausea, chronic cough or shortness of breath, ulcerative stomatitis, alopecia and accelerated bone loss. Thirteen of these 53 patients discontinued the methotrexate and received a liver transplant. MTX was discontinued in 6 individuals who moved out of the area and were no longer under our care. Four patients were diagnosed as having a non-hepatic carcinoma. One patient was reported to have become pregnant after starting MTX. No further follow-up information is available except that she no longer continued on therapy at this institution. One patient had esophageal varices. One patient was noncompliant.

Of the 57 patients who completed at least 5 years of treatment, the effect of methotrexate on several prognostic markers and laboratory was evaluated. Table 1 shows the median slope values and 95% confidence intervals. Because age is a variable used in calculating the Mayo Risk Score, age alone would cause an increase in Mayo Risk Score, even if all other parameters were to remain the same. Therefore, a separate calculation for Mayo Risk Score was performed, excluding age. A positive slope reflects an increase in a value with time, and a negative slope indicates a decrease in a value.

Whether calculated with or without age, Mayo Risk Score increased during treatment with MTX and UDCA. While not statistically sig-

TABLE 1
Comparison of Treatment with Methotrexate Alone or with Methotrexate and Ursodeoxycholate

Endpoint	MTX			MTX/UDCA		
	Median Slope	[95% CI]	p	Median Slope	[95% CI]	p
MRS	0.06	[-0.007, 0.11]	0.07	0.09	[0.06, 0.11]	0.0001
MRS (w/o age)	0.02	[-0.05, 0.09]	0.54	0.05	[0.03, 0.07]	0.0001
Total bilirubin	0.01	[-0.02, 0.04]	0.31	0.01	[-0.004, 0.02]	0.26
Alk. Phos.	-0.07	[-0.44, -0.001]	0.03	-0.07	[-0.13, -0.05]	0.0001
GGT	-0.08	[-0.21, 0.15]	0.41	-0.10	[-0.22, -0.04]	0.0001

* A positive slope represents an increase in the endpoint's value. ** A negative slope represents a decrease. MRS = Mayo Risk Score.

nificant, a rising trend was also noted for Mayo Risk Score and bilirubin during the time the patients received methotrexate alone. Because the study was carried out for only five years, a change in bilirubin would not have been expected. Interestingly, there was a statistically significant decrease in alkaline phosphatase during treatment with methotrexate alone and in combination with UDCA, and for GGT during combination therapy. During treatment with methotrexate alone, GGT activity decreased, though the decrease was not statistically significant.

Forty-nine of the 57 patients had biopsies performed before and after five years of treatment. In 63% of the cases, methotrexate did not alter the extent of portal fibrosis and portal inflammation. Portal fibrosis progressed in 25% of the cases and diminished in 12%. Lobular inflammation remained unchanged in 61% and bile duct loss remained unchanged in 53%. These changes may have been the result of sampling differences.

The second part of our study analyzed survival. Through collaboration with the Mayo Clinic, we were able to construct a historical control group based on data from a previously published placebo-controlled trial of UDCA. These data allowed us to determine if methotrexate had a beneficial effect on survival in those patients who did not receive a transplant, despite our findings that the disease had progressed with treatment. Using a Cox proportional hazard model controlling for Mayo Risk Score and the use of ursodeoxycholic acid, treatment failure (defined as death or liver transplantation) was assessed based on Mayo Risk Score and ursodeoxycholic acid and methotrexate use. As expected, a rising Mayo Risk Score was associated with an increased risk of death or the need for liver transplant ($p=0.0001$). The use of UDCA significantly decreased the likelihood of death or transplant by 16% ($p=0.006$) (14). Methotrexate use did not

cause a statistically significant alteration in outcome, though the trend was toward an adverse outcome. From our study, we therefore concluded that PBC progresses despite therapy with methotrexate. Methotrexate did not improve survival or prevent complications or the need for transplant, and long-term treatment with methotrexate was not well tolerated.

Immunopathogenesis of Autoantibody Production in PBC

Gershwin and others (15–17) have determined that more than 90% of patients with PBC produce autoantibodies against specific regions of the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2), a ubiquitously expressed mitochondrial matrix protein associated with the inner mitochondrial membrane. Auto-reactive T cells specific for PDC-E2 self-peptides from the same region recognized by PBC patient autoantibodies have also been isolated from patients with PBC (18, 19). There is no direct evidence that these autoantibodies or auto-reactive T cells are pathogenic. Indeed, the auto-antibodies and autoreactive T cells cross-react to varying extents with xenobiotic sources of pyruvate dehydrogenase and peptides respectively, including bacterial sources (20, 21). Mason et al. (22) have even identified a virus that may be a source of cross-reactive antigen. In a proposed mouse model of PBC, immunization with bovine pyruvate dehydrogenase complex (PDC) eventually leads to loss of tolerance to self (mouse)-PDC and biliary inflammation (23). Consequently, uncertainty exists with regard to the source of the immunogen responsible for the activation of autoreactive B and T cells in patients with PBC. The results of ongoing studies at Mount Sinai investigating apoptotic cholangiocytes as a potential source of immunogenic PDC-E2 are discussed below.

Antibodies produced in response to a specific immunogen may be capable of recognizing several antigens that share an epitope in common with the original immunogen. Epitope analysis and autoantibody titration experiments have helped define the potential immunogenicity of different sources and forms of PDC-E2. The PDC-E2 epitope recognized by anti-PDC-E2 autoantibodies from patients with PBC is a very specific region within the inner lipoyl domain. The epitope appears to be conformational in nature, since no short peptide sequence completely inhibits binding to the inner lipoyl domain (17). The outer lipoyl domain is also recognized, but this may represent cross-reactivity, since the sequences are similar and both contain lipoyllysine residues (lipoic acid covalently bound to a lysine side-chain), which are important in antibody recognition (24). Other common PBC antigens also contain these uncommon lipoyllysine residues. Lipoic acid contains a dithiol group, and early studies of purified PDC and PDC-E2 indicated that PBC patient sera preferentially recognized PDC-E2 to which sulfhydryl reducing agents had been added (25).

The most tantalizing finding with regard to the source and identity of the immunogenic form of PDC-E2 was obtained by immunohistochemical staining of liver biopsy specimens from patients with PBC autoantibodies. Increased staining by PBC patient autoantibodies was specifically noted in the cholangiocytes of patients with PBC (26, 27). Most of the additional staining was near the apical border of the cell and significantly was non-mitochondrial. *In situ* hybridization of PDC-E2 mRNA in PBC patient cholangiocytes actually showed decreased levels of message in the cholangiocytes of patients with PBC (28). There was no evidence of decreased PDC-E2 protein degradation, which typically accounts for increased levels of the autoreactive epitope. The cause of this abnormal pattern of staining remains unresolved.

Effect of Apoptosis on the Major PBC Autoantigen

In other autoimmune diseases, cells that have undergone apoptosis (programmed cell death) have been proposed as a source immunogen responsible for autoantibody production (29, 30), since unique forms of many self-proteins are present in apoptotic cells (31, 32). A high percentage of autoantigens, unlike non-autoantigens, are specifically cleaved by caspases and become concentrated in cytoplasmic

surface blebs and apoptotic bodies during apoptosis (33). Other autoantigens are phosphorylated during apoptosis (30). The immune response to unique proteins within apoptotic cells appears to vary depending on the local environment. Apoptosis of cholangiocytes is increased in patients with PBC (34–36). However, the presence of distinct forms of PBC autoantigens in apoptotic cells has not been reported. Therefore, we examined the effect of apoptosis on PDC-E2 in cholangiocytes and non-cholangiocytes to determine if cholangiocyte apoptosis might have a unique effect on PDC-E2.

Staining of untreated control cells (Fig. 1) with PBC patient antisera mono-specific for PDC-E2 resulted in a mitochondrial pattern (Fig. 1I, 1K, 1M, and 1O) that was identical to that of mitochondrial cytochrome C oxidase subunit 1 (Cox 1) (data not shown). Following irradiation with ultraviolet B light (UV-B), staining of PDC-E2 in apoptotic normal rat cholangiocytes (NRC) and salivary gland epithelial cells (HSG) (Figs. 1J & 1L, respectively) remained punctate, peri-nuclear, and colocalized with Cox 1 (data not shown). Surprisingly, staining of PDC-E2 in apoptotic HeLa, Jurkat, and Caco-2 cells (Fig. 1N, 1P, data not shown; apoptotic cells are labeled with a white star) was undetectable, though Cox 1 staining persisted (data not shown). In contrast, UV-B irradiated HeLa, Jurkat, and Caco-2 cells that were not yet apoptotic (unlabeled cells) stained strongly for PDC-E2 (Fig. 1N, 1P, data not shown). Loss of PDC-E2 staining in apoptotic cells was also observed after staurosporine treatment of HeLa and Caco-2 cells, and Fas ligation of Jurkat T cells (data not shown). In conjunction with persistent staining of PDC-E2 in apoptotic normal rat cholangiocytes (NRC) after treatment with staurosporine (data not shown), these results indicate that loss of PDC-E2 staining following apoptosis is a common, but not universal, occurrence.

We investigated the cause of the specificity of PDC-E2 recognition of this cell type in apoptotic cells. Decreases in the cellular sulfhydryl redox potential during apoptosis have been reported for several cell types. Since the antigenic epitope of PDC-E2 contains sulfhydryl groups, we analyzed the effect of sulfhydryl-reducing agents on autoantibody recognition of PDC-E2 in apoptotic cell lysates. In the absence of dithiothreitol (DTT) addition, blotting of PDC-E2 was substantially decreased in lysates of apoptotic HeLa (Fig. 2), but not in apoptotic NRC or human salivary gland (HSG) (Fig. 2).

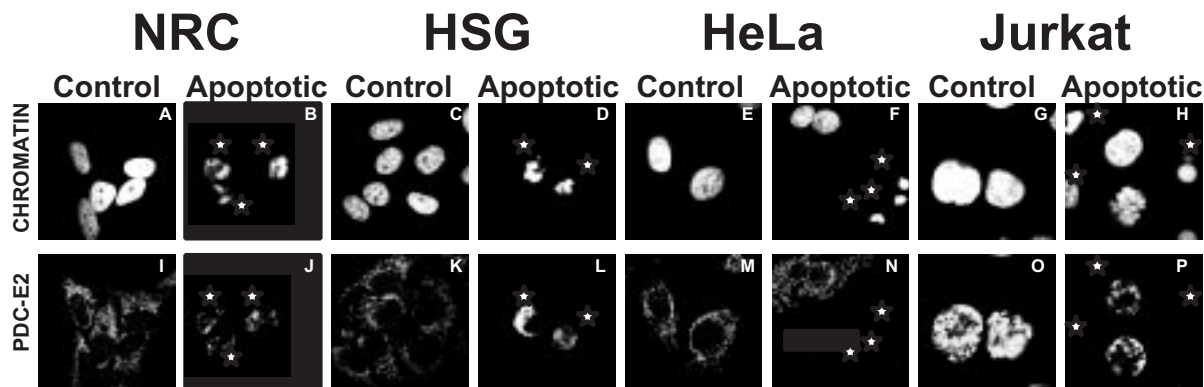


Fig. 1. A–H. Confocal images of control and apoptotic cells stained with DAPI to detect the condensed nuclear chromatin characteristic of apoptotic cells and PBC patient antiserum mono-specific for pyruvate dehydrogenase complex-E2 (PDC-E2) (I–P). A fluorescein isothiocyanate (FITC)-conjugated anti-human immunoglobulin G (IgG) secondary was used for detection of autoantibody binding. Apoptotic cells are labeled with white stars. In apoptotic cells, staining of PDC-E2 is present only in normal rat cholangiocyte (NRC) (J) and human salivary gland (HSG) (L). Representative images are shown. Original magnification was 100X.

The apoptotic fragment of the caspase substrate, poly-ADP ribose polymerase (PARP), is detected in all apoptotic lysates, regardless of oxidative state. Equivalent autoantibody recognition of PDC-E2 in reduced lysates of control and apoptotic cells excludes degradation as a potential mechanism of decreased recognition of PDC-E2 in HeLa. Conversely, the addition of glutathione disulfide (GSSG), a sulfhydryl-oxidizing agent, to lysates of both apoptotic NRC and control HeLa (data not shown) prior to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) abrogated blotting of PDC-E2.

In titration experiments using antiserum from four different patients with PBC, the difference in autoantibody recognition of PDC-E2 in GSSG- versus DTT-treated lysates of NRC ranged from 100- to 1000-fold (data not shown). The electrophoretic mobility and detection levels of [S^{35} Met]-labeled PDC-E2 immunoprecipitated from control cell lysate were not significantly different whether GSSG or DTT was added prior to SDS-PAGE (data not shown). Thus, by both immunostaining and immunoblotting, PBC patient autoantibody detection of the antigenic epitope of PDC-E2 following apoptosis was limited to cholangiocytes and salivary gland epithelial cells. Addition of sulfhydryl oxidizing agents to lysates eliminated PBC autoantibody recognition.

These studies indicated that the conformational form of PDC-E2 present in apoptotic cells varies by cell type. The sulfhydryl redox state of the cell types following apoptosis determined which form was present. The reduced

form present in apoptotic cholangiocytes and salivary gland epithelial cells was recognized by PBC patient autoantibodies, while the oxidized form present in other apoptotic cell types was not recognized. Considering that increased numbers of apoptotic cholangiocytes and salivary gland cells are observed in patients with PBC, selective autoantibody recognition of the form of PDC-E2 present in these apoptotic cells strongly suggests that apoptotic cholangiocytes and salivary gland cells are the source of PDC-E2 responsible for anti-PDC-E2 autoantibody production (Fig. 3). The lack of recognition by autoantibodies in the other apoptotic cell types indicates that they cannot be sources of PDC-E2 responsible for anti-PDC-E2 antibody production in all cells.

Genetic Associations

Substantial evidence suggests that an external trigger may initiate the disease in an otherwise susceptible individual. Evidence for the genetic susceptibility to the disease includes the disease's loose associations with various human leukocyte antigen (HLA) markers and an increased prevalence of familial PBC. There has also been a report of an inherited sulfoxidation disorder in PBC patients (37). Several genes and HLA patterns have been reported to be associated with PBC. The significance of these associations remains unclear. Several groups have found that having HLA DR8 increases the risk of PBC two- to seven-fold (38). This gene, however, is neither necessary nor sufficient to cause PBC. In the Japanese population, another

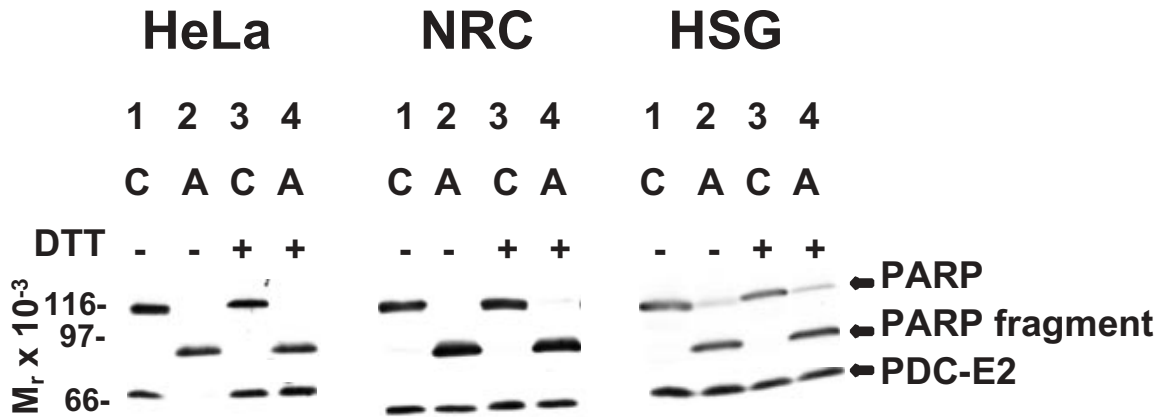


Fig. 2. Immunoblots of lysates from HeLa cells (left panel), normal rat cholangiocytes (NRC) (middle panel) and human salivary gland cells (HSG) (right panel) were incubated with systemic lupus erythematosus (SLE) patient antiserum (top row) specific for poly-ADP ribose polymerase (PARP) (116 kDa), and PBC patient antiserum (bottom row) specific for pyruvate dehydrogenase complex-E2 subunit (PDC-E2) (human: 72 kDa; rat: 66 kDa). 80 mg of protein was loaded in each lane with or without 5 mM dithiothreitol (DTT). The PARP cleavage fragment (89 kDa) is present in all apoptotic lysates. Note the lack of PDC-E2 blotting only in the non-reduced, apoptotic HeLa lysate (lane 2) which excludes degradation as a potential mechanism for its absence in HeLa cells. C = control; A = apoptotic.

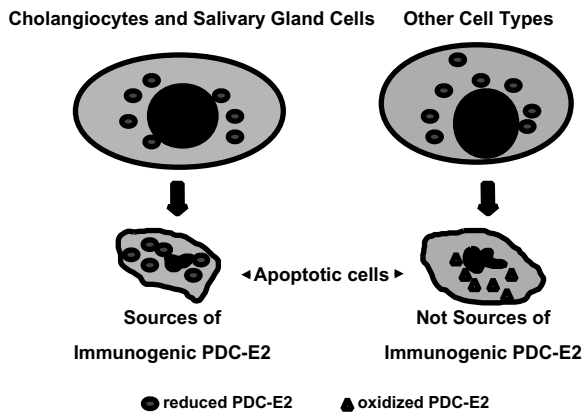


Fig. 3. Postulated immunogenicity of different non-apoptotic and apoptotic cell types. PBC patient autoantibodies recognize PDC-E2 in all non-apoptotic cells. Recognition in apoptotic cells is specific to cholangiocytes and salivary gland cells.

genotype, DP81*0501, was found to be strongly associated with PBC, although this finding has not been confirmed in Caucasian populations (39). Other associations such as tumor necrosis factor (TNF) alpha and cytotoxic lymphocyte antigen 4 polymorphisms have been less consistent observations (40, 41).

Several years ago, we systematically reviewed the family histories of PBC patients followed in our practice. Of the 564 patients identified, only 405 had sufficient family history available, including data on the parents, siblings and children of the patient, to be included in our review. We identified 26 patients with PBC who

had at least one family member with the disease. Using this information, we estimated the prevalence of the disease to be 6420 per 100,000. Seventeen families had more than one family member with documented PBC. Several families had more than two affected family members. The most common relationships were either mother and daughters or sisters.

In order to avoid duplication, we performed a separate calculation excluding those family members who were already patients in our practice. With this exclusion, the prevalence of the disease was 4282 per 100,000 (42). These figures greatly exceed the Mayo Clinic's recent prevalence estimate of 38 cases of PBC per 100,000 individuals in the general population (43). Since our study, several groups have confirmed the increased familial prevalence of this disease (Table 2) (44–46). Though each of the studies is fraught with errors that might either overestimate or underestimate the disease's prevalence, most investigators recognize an increased familial prevalence of this disease.

Association with Autoimmune Diseases

Several autoimmune diseases have been reported to occur with greater frequency in patients with PBC (2). Schaffner and co-workers at Mount Sinai in the late 1970s reported some of the original data establishing an association between thyroid disease and PBC (47). Though the exact percentage of patients with thyroid disease is not known, clearly there is an increased preva-

TABLE 2
Percentage of Patients with PBC Who Have a Relative with PBC

Author (ref.)	Affected Relative (%)
Jones et al. (44)	0.7 (1st degree only)
Brind et al. (45)	1.3
Bach and Schaffner (41)	4.3
Tsuji et al. (43)	5.1

lence of thyroid disease in PBC. Careful screening of PBC patients finds that as many as 72% of those with PBC have Sjögren's disease. Scleroderma has also been associated with PBC and as many as 20% of patients with PBC have joint problems, with a small percentage having rheumatoid arthritis. An association between PBC and celiac disease has also been appreciated for years (48), but controversy exists regarding the prevalence of celiac disease in PBC patients.

Recently five European groups (49–53) have reported celiac disease prevalence rates in patients with PBC ranging from 0–20% (Table 3). This led to our ongoing investigation to determine the prevalence of celiac disease in North American patients with PBC. Titers of various autoantibodies associated with celiac disease are being measured in PBC patients, in collaboration with the Mayo Clinic, Rochester, MN. We anticipate testing more than 300 PBC patients. Preliminary data is available on the first 78 patients (Table 4). Endomysial antibodies were found in 3% and tissue transglutaminase antibodies-IgA in 16% of the patients tested. The literature suggests that these markers are both very sensitive and specific for celiac disease (54). Further exploration of these findings is necessary. Unanswered questions about PBC and celiac disease include: (a) the true prevalence of the disease in North American patients with PBC; (b) the sensitivity and specificity of the various autoantibodies in patients with PBC; and (c) the role that celiac disease may play in causing complications of PBC such as hepatic osteodystrophy.

Conclusion

In summary, the outlook for patients with PBC has improved, with patients living longer, more comfortable lives. While the pathogenesis of PBC remains unclear, recent discoveries have increased our understanding of this disease and may lead to novel treatments to aug-

TABLE 3
Prevalence of Celiac Disease in Patients with PBC

Author (ref.)	Country	n	Prevalence (%)
Kingham and Parker (45)	United Kingdom	67	6
Dickey et al. (49)	Ireland	57	11
Bardella et al. (50)	Italy	65	0
Fidler et al. (51)	United Kingdom	87	2
Niveloni et al. (52)	Argentina	10	20

TABLE 4
Prevalence of Celiac Disease-Associated Autoantibodies in Patients with PBC

Autoantibody Specificity	Isotype	Positive (n)	Percentage (total n = 78)
Gliadin	IgA	9	12
Gliadin	IgG	5	6
Endomysium	Ig	2	3
Tissue transglutaminase	IgA	16	20
Tissue transglutaminase	IgG	32	41

ment ursodeoxycholate therapy. With the establishment of the Center for the Study of Primary Biliary Cirrhosis, it is clear that research at Mount Sinai will continue to contribute significantly to this process.

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