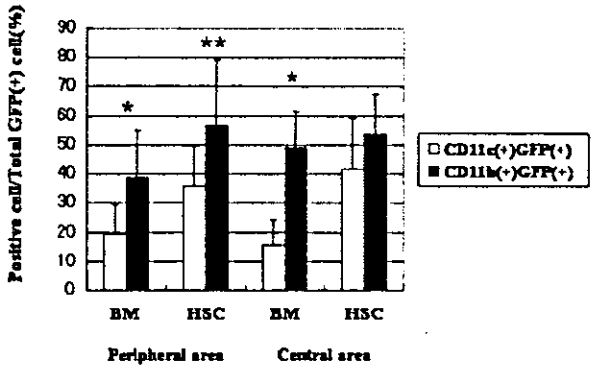

FIGURE 8. Quantitative analysis of the immunohistochemistry for CD11c and CD11b in the cornea of mice receiving transplants of BM cells or HSCs. The percentage of positive cells per section was calculated as the number of cells that coexpressed GFP and CD11c or GFP and CD11b, divided by the total number of GFP^+ cells × 100. The number of $CD11c^+$ cells in both the peripheral and central areas of the cornea was greater in mice receiving transplants of HSCs than in those receiving BM cells. Approximately 50% of GFP^+ cells were immunostained with CD11b in both BM cell and HSC recipients. There were statistically significant differences between $CD11c^+$ and $CD11b^+$ (Mann-Whitney test: $^*P < 0.01$, $^{**}P < 0.05$).

a significant number of resident dendritic cells in the corneal tissue.

Our observations in corneas receiving GFP-labeled BM cell transplants are of particular interest. Ours is the first report on the time course of the migration of GFP-labeled BM cells into the cornea. Within 2 months after BM cell transplantation, the density of GFP-labeled cells gradually increased; thereafter, cell density was comparatively stable, and finally, at 6 months, it reached a plateau. These findings led us to the interesting hypothesis that BM-derived cells continuously migrate into corneal tissue and contribute to corneal integrity. At present, we do not know the longevity of GFP-labeled BM cells in the mouse cornea. Using other experimental protocols, further cell biological study is needed to clarify this point.

There have been no reports on the distribution of hematopoietic stem/progenitor cells (not bone marrow cells) in the mouse cornea. Although the type of transplantation necessary to obtain these data is very difficult, our group has mastered the technique by using a unique protocol that facilitates our long-term observation of the eyes of transplant-recipient mice.

Our study demonstrates that most of the GFP^+ cells were distributed in the corneal stroma: Approximately 25% were found in the periphery and 7% in the center. In contrast, a small number, approximately 1%, were found in the corneal epithelium. The distribution rates of GFP^+ cells were similar in mice receiving with BM cells and HSCs. These results suggest that cells migrating into the corneal tissue may be definite populations of BM cells, such as HSCs or undifferentiated progenitor cells.

Based on our immunohistochemical results, we divided GFP^+ cells in the corneal tissue into four groups: GFP^+CD11c^+, GFP^+CD11b^+, GFP^+CD11c^-, and GFP^+CD11b^- cells. GFP^+CD11c^+ cells (approximately 40% in the HSC transplantation experiment) are thought to express the dendritic cell phenotype[20–22] and GFP^+CD11b^- cells (approximately 55% in HSCs) either the dendritic cell or macrophage phenotype.[23] Using a protocol similar to ours, Espinosa-Heidmann et al.[24] found that BM-derived progenitor cells contributed to experimental choroidal neovascularization. When they used the F4/80 antibody (monocyte marker), they observed $GFP^+F4/80^+$ cells in the limbus, ciliary body, and normal choroid and sclera, suggesting a high turnover and recruitment rate of infiltrated macrophages. Based on their findings and our observations, we postulate that some of the GFP^+ cells in the mouse cornea are BM-derived APCs.

Some of the GFP^+ cells were negative for cell-surface markers for APCs (CD11c and CD11b), and their origin is unclear. Corneal stroma is composed of both corneal keratocytes and a variety of extracellular matrices comprising collagen subtypes. In our experience, the morphology of GFP^+ cells in the corneal stroma and of corneal keratocytes is very similar. If BM-derived stem cells terminally transdifferentiate into corneal keratocytes, they can be expected eventually to lose surface CD45 expression. We posit that our immunologic experiment did not detect immature $Sca\text{-}1^+$ cells in the mouse cornea (data not shown), suggesting that transplanted hematopoietic stem/progenitor cells first homed to BM and engrafted in the recipient mice, and then provided mature BM-derived cells in the cornea. Based on our present results we cannot unequivocally claim that BM-derived GFP^+ cells can transdifferentiate into corneal cell phenotypes or neurons. Therefore, morphologic and immunohistochemical studies are under way to examine extracellular matrices and cell-surface markers that are uniquely synthesized by corneal keratocytes.

Several technical and conceptual issues deserve consideration in the interpretation of our results. It is important to note that even in eGFP mice significantly fewer than 100% of the cells express GFP. As this may be due to cell-cycle dependent expression of GFP, we suggest that our results underestimate the potential contribution of BM-derived cells in the mouse cornea. We are currently investigating whether the findings we made with our animal model are applicable to humans. Therefore, we are studying the distribution of BM-derived cells in human corneas.

In conclusion, ours is the first study that presents direct evidence for the migration into the cornea of GFP-labeled BM-derived cells. We provide immunohistochemical evidence that some of the migrating cells were BM-derived cells such as

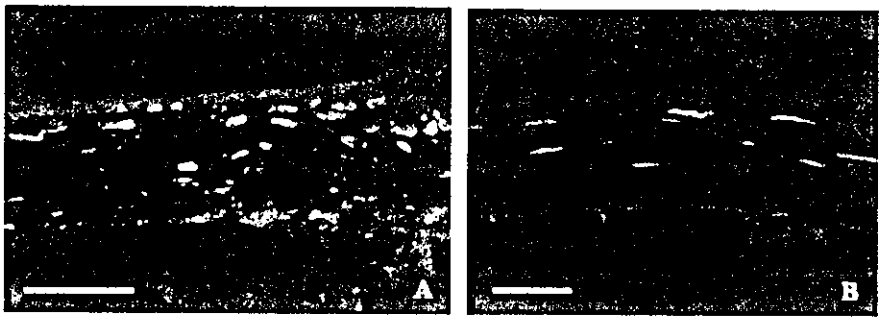

FIGURE 9. Representative immunohistochemical staining for CD45 (*red*) in the cornea of HSC-recipient mice. (A) Peripheral, (B) central retina. Most GFP^+ cells were immunostained with CD45 (*yellow*). Scale bars: (A) 100 μm; (B) 50 μm.

dendritic cells and macrophages. Cell biology studies will determine the lineage(s) of the other cells.

Acknowledgments

The authors thank Hisayo Sogabe for assisting in the immunohistochemical studies and quantitative evaluations.

References

1. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*. 1992;255:1707–1710.
2. Gage FH. Mammalian neural stem cells. *Science*. 2000;287:1433–1438.
3. Tropepe V, Coles BL, Chiasson BJ, et al. Retinal stem cells in the adult mammalian eye. *Science*. 2000;287:2032–2036.
4. Schermer A, Galvin S, Sun TT. Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. *J Cell Biol*. 1986;103:49–62.
5. Cotsarelis G, Cheng SZ, Dong G, Sun TT, Lavker RM. Existence of slow cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. *Cell*. 1989;57:201–209.
6. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*. 1997;276:71–74.
7. Weissman IL. Translating stem and progenitor cell biology to the clinic: barriers and opportunities. *Science*. 2000;287:1442–1446.
8. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284:143–147.
9. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multilineage engraftment by a single bone marrow-derived stem cell. *Cell*. 2001;105:369–377.
10. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*. 2002;418:41–49.
11. Hamrah P, Zhang Q, Liu Y, Dana MR. Novel characterization of MHC class II-negative population of resident corneal Langerhans cell-type dendritic cells. *Invest Ophthalmol Vis Sci*. 2002;43:639–646.
12. Brissette-Storkus CS, Reynolds SM, Lepisto AJ, Hendricks RL. Identification of a novel macrophage population in the normal mouse corneal stroma. *Invest Ophthalmol Vis Sci*. 2002;43:2264–2271.
13. Hamrah P, Liu Y, Zhang Q, Dana MR. The corneal stroma is endowed with a significant number of resident dendritic cells. *Invest Ophthalmol Vis Sci*. 2003;44:581–589.
14. Ishikawa F, Livingston AG, Wingard JR, Nishikawa S, Ogawa M. An assay for long-term engrafting human hematopoietic cells based on newborn NOD/SCID/beta2-microglobulin(null) mice. *Exp Hematol*. 2002;30:488–494.
15. Ishikawa F, Livingston AG, Minamiguchi H, Wingard JR, Ogawa M. Human cord blood long-term engrafting cells are CD34+ CD38−. *Leukemia*. 2003;17:960–964.
16. Hisatomi T, Sakamoto T, Sonoda KH, et al. Clearance of apoptotic photoreceptors: elimination of apoptotic debris into the subretinal space and macrophage-mediated phagocytosis via phosphatidylserine receptor and integrin alphavbeta3. *Am J Pathol*. 2003;162:1869–1879.
17. Okabe M, Ikawa M, Kominami K, Nakanishi T, Nishimune Y. "Green mice" as a source of ubiquitous green cells. *FEBS Lett*. 1997;407:313–319.
18. Nakamura T, Nishida K, Dota A, et al. Elevated expression of transglutaminase 1 and keratinization-related proteins in conjunctiva in severe ocular surface disease. *Invest Ophthalmol Vis Sci*. 2001;42:549–556.
19. Nakamura T, Nishida K, Dota A, Kinoshita S. Changes in conjunctival clusterin expression in severe ocular surface disease. *Invest Ophthalmol Vis Sci*. 2002;43:1702–1707.
20. Leenen, PJ, Radosevic, K, Voerman, JS, et al. Heterogeneity of mouse spleen dendritic cells: in vivo phagocytic activity, expression of macrophage markers, and subpopulation turnover. *J Immunol*. 1998;160:2166–2173.
21. Vremec, D, Zorbas, M, Scoolay, R, et al. The surface phenotype of dendritic cells purified form the mouse thymus and spleen: investigations of the CD8 expression by a subpopulation of dendritic cells. *J Exp Med*. 1992;176:47–58.
22. Metlay JP, Witmer-Pack MD, Agger R, et al. The distinct leukocyte integrins of mouse spleen dendritic cells as identified with new hamster monoclonal antibodies. *J Exp Med*. 1990;171:1753–1771.
23. Ross GD, Vetvicka V. CR3 (CD11b, CD18): a phagocyte and NK cell membrane receptor with multiple ligand specificities and functions. *Clin Exp Immunol*. 1993;92:181–184.
24. Espinosa-Heidmann DG, Caicedo A, Hernandez EP, Csaky KG, Cousins SW. Bone marrow-derived progenitor cells contribute to experimental choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2003;44:4914–4919.

CHAPTER 28

Primary Biliary Cirrhosis Bench to Bedside

Shinji Shimoda, Akiyoshi Nishio, Hiromi Ishibashi and M. Eric Gershwin

Abstract

Primary biliary cirrhosis (PBC) is an autoimmune liver disease that predominantly affects women and characterized by chronic progressive destruction of small intrahepatic bile ducts with portal inflammation and subsequent fibrosis. The serologic hallmark of PBC is the presence of antimitochondrial antibodies (AMA), which are found in 95% of patients with PBC. The AMA are directed against the 2-oxo-acid dehydrogenase complexes located on the inner membrane of mitochondria. Although the role of AMA in the pathogenesis of PBC is unknown, the presence of antibodies has allowed detailed immunological definition of the antigenic epitopes, the autoantibodies and the T-cell response. Theories have been proposed regarding the mechanism of immune-mediated bile duct damage in PBC, including the possible role of T-cell-mediated cytotoxicity and molecular mimicry. PBC is usually diagnosed based on the triad of elevated alkaline phosphatase, AMA, and characteristic histological changes on liver biopsy. Biochemical liver abnormalities tests are consistent with the presence of cholestasis and reveal an elevation of both serum alkaline phosphatase and γ-glutamyl transpeptidase, with or without elevation of aminotransferase levels. Ursodeoxycholic acid (UDCA), a dihydroxy bile acid, appears to be the only effective therapy in preventing or delaying the need for liver transplantation. However, a number of patients receiving UDCA still develop progressive disease and require transplantation; at present, liver transplantation is the only effective therapy for end-stage PBC.

Introduction

Primary biliary cirrhosis (PBC) is an organ specific autoimmune disease whose autoimmune targets are the small intrahepatic bile ducts. PBC primarily affects middle aged women and is associated with intrahepatic chronic progressive bile stasis without obstruction of the extrahepatic bile system. The signature of PBC is antimitochondrial antibodies (AMA). Most of the advances in PBC have occurred secondary to dissection of the anti-mitochondrial response at the level of the CD4, CD8 and B cell response. PBC is diagnosed based on clinical findings, the presence of AMA, elevation of alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (γ-GTP), and finally liver biopsy. The survival of PBC was once believed to be 5-10 years after the appearance of clinical finding, itching or jaundice. Recently many cases of asymptomatic PBC have been found, and prognosis of some patients with asymptomatic PBC is almost the same as healthy subjects. In fact, it is now suggested that some asymptomatic PBC will not progress to cirrhosis. In this review we will present a description of the fundamental pathology of PBC and then follow this presentation with a state-of-the art description of the autoimmune response.

The Pathophysiology of the Biliary Epithelia, edited by Gianfranco Alpini, Domenico Alvaro, Marco Marzioni, Gene LeSage, and Nicholas LaRusso.

Table 28.A. Clinical and pathological profiles of PBC

- Predominantly middle-aged women (M: F ratio is 1:9)
- Recurrent pruritus, lethargy, and progressive jaundice
- Elevation of serum alkaline phosphatase, g-glutamyl transpeptidase and IgM
- Antimitochondrial antibodies (AMA)
- Associated with other autoimmune diseases
  -Sjögren's syndrome, scleroderma, thyroid disease, etc.
- Classified histologically into four stages
  1) Inflammatory destruction of intrahepatic small bile ducts
  2) Proliferation of bile ductules and/or piecemeal necrosis
  3) Fibrosis and/or bridging necrosis
  4) Cirrhosis

  Noncaseating granulomas are sometimes but not always seen in the early stage
- Other possibilities need to be excluded in cases with the absence of AMA, male sex, or young age

Clinical Features

PBC presents insidiously with fatigue and pruritus; fatigue is found up to 80% of patients (Table 28.A). However, fatigue is relatively nonspecific and varies considerably between patients, as is the case of fatigue in patients with other chronic liver diseases. The cause of fatigue is unknown, but some recent work has indicated the possibility of altered neurotransmission. The lethargy of PBC must be differentiated from other causes of tiredness such as depression, hypothyroidism, adrenal disease or side effects of medications. Pruritus is the second commonest symptom in PBC, occurring in 50 to 60% of patients, and is characteristically worse on the palms and soles and not associated with cutaneous changes. When pruritus occurs, it does not appear to be related to the disease stage and may remit as the disease progresses; in some patients, it can develop during the late stages of the disease. Although several hypotheses are proposed, recent data support the importance of increased opioids tone in patients with PBC.

Jaundice usually follows months or years later; however, in 10% of patients, jaundice may be the presenting symptom. Darkening of the skin, hirsutism, anorexia, diarrhea, and weight loss may also be present. Less commonly, patients may present with a complication of portal hypertension, such as variceal bleeding or ascites, as evidence of more advanced liver disease. Occasionally, patients may present with problems related to cholestasis, such as severe osteoporosis or fat-soluble vitamin deficiency. Some patients present with malabsorption, but this is usually more common in patients with advanced disease. PBC may be associated with cutaneous changes such as generalized hyperpigmentation and facial xanthelasmata. The latter changes result from raised total cholesterol concentration.

Physical findings are variable and depend on the extent of the disease (Table 28.B). Hepatosplenomegaly, spider angiomas, palmar erythema, hyperpigmentation, hirsutism, and xanthoma may be present. An increasing susceptibility to urinary tract infection has been observed in women with PBC, but the cause of this associated problem is unknown. There appears to be an increased association between PBC and cancer of the breast. Increased risk of hepatocellular carcinoma among male patients with PBC has been also reported, but the magnitude of this implication is controversial.

In patients with PBC, elevated serum alkaline phosphatase and γ-glutamyl transpeptidase levels are the most characteristic abnormalities of liver chemistries. Hyperbilirubinemia is not frequently observed. However, serum bilirubin levels can rise to remarkably high levels as the disease progresses, and serum bilirubin is an excellent predictor of survival. Serum albumin

Table 28.B. Initial symptoms and signs at presentation of PBC

- Pruritus
- Fatigue
- Jaundice
- Appetite loss
- Weight loss
- Variceal bleeding
- Ascites
- Hepatosplenomegaly
- Hyperpigmentation
- Xanthelasma and/or xanthoma
- Palmar erythema

levels and prothrombin time are normal early in the course of the disease. A low serum albumin level and prolonged prothrombin time that is not corrected by vitamin K therapy are poor prognostic signs that indicate an advanced disease. Serum IgM levels are frequently increased, whereas IgG and IgA levels are usually within normal limits. The finding of a significant AMA titer (>1: 40) is strongly suggestive of PBC, even in the absence of symptoms and the presence of normal levels of serum alkaline phosphatase. Although demonstration of AMA by immunofluorescence has diagnostic specificity, newer techniques, such as enzyme-linked immunosorbent assay and immunoblotting, are more sensitive and specific than immunofluorescence.

Natural History

The natural history of patients with PBC is heterogeneous. Patients with prolonged survival with minimal progression of the disease and few or absent symptoms are well described. However, the prognosis largely depends on when the diagnosis of PBC has been established. Routine blood screening and increased awareness of PBC has led to the early detection of the disease, and approximately 60 to 70% of patients diagnosed as PBC are asymptomatic; the prognosis for asymptomatic patients with PBC is better than for symptomatic patients. Median predicted survival of patients from the time of diagnosis is twice as long for patients who present without symptoms compared to symptomatic patients. Approximately one third of asymptomatic patients become symptomatic within 5 years; 50% survival is seen at 12 years, as it is seen at only 8 years in symptomatic patients.[1,2] Overall survival of asymptomatic patients is shorter than that predicted for an age- and gender-matched control population, difference that becomes apparent only after 11 years of follow-up.[1]

There are no markers that can predict outcome in patients with PBC who are asymptomatic with normal serum bilirubin. There is a suggestion that the pattern of serum bile acids may be helpful, but such analyses are not readily available. There is correlation, albeit weak, between histology and outcome; patients found to be cirrhotic at diagnosis have a lesser chance of survival than those who are not cirrhotic. It is not unusual to find an asymptomatic patient with cirrhosis, and even baseline histology does not predict who will and who will not develop symptoms.[2] Serum bilirubin level is a reliable indication of final outcome.[3] In symptomatic patients, advanced age, decreased serum albumin levels, and cirrhosis each also correlated with shortened survival.

The increased use of liver transplantation for the treatment of PBC has highlighted the need for accurate assessment of prognosis. Several prognostic models based in Cox's regression analysis on follow-up of patients have been proposed for the estimation of survival probability. The Mayo survival model has gained the greatest popularity among them. The variables used

Table 28.C. Phenotypic changes to biliary epithelium of small bile ducts in PBC

	Normal	PBC	Other LD
Autoantigens			
PDC-E2	+	+++	+
PDC-E3BP	+	+++	+
Anion-Exchanger-2	+	-/+	+
Immune adhesion molecules			
MHC class I	+	+	+
MHC-class II (HLA-DR)	-	+*	+
CD 1d	-	+*	-
ICAM-1 (CD54)	-	-/+	-/+
VCAM-1	-	-/+	-
LFA-3 (CD58)	-	-/+	-/+
B7-1 (CD80)	-	-/+ ?	-
B7-2 (CD86)	-	-/+	-/+
Apoptosis-related molecules			
Fas (CD95)	-	+*	-/+
granzyme B	-	-/+	-/+
Perforin	-	-/+*	-/+
bcl-2	++	-**	++
Inflammatory cytokines			
IL-6	-	++	+
TNF-α	-/+	++	+
IL-6 receptor	-	-/+	-
TNF receptor	-/+	++	+
Others			
MUC1 apomucin	-	+*	+*
MUC6 apomucin	-/+	+*	+*
Lewis Y antigen	-	++*	++*
Heat-shock protein 27, 90	-	-/+	-/+

*: Increased expression in damaged bile ducts

**: Decreased expression in damaged bile ducts

in this model (age, serum bilirubin and albumin concentration, prothrombin time, and presence of edema) have most of the diagnostic information irrespective of prior treatment with UDCA. We note that the Mayo risk score is more precise but also more complicated to predict outcome than serum bilirubin levels. Post-transplantation outcome correlates well with the level of serum bilirubin level; optimal post-transplantation survival requires the bilirubin level to be less than 180 mmol/L at the time of transplantation. Although treatment with UDCA reduces the serum bilirubin level, it does not alter the validity of bilirubin level or the Mayo risk score in assessing prognosis.[4]

Pathology

Bile duct inflammation and destruction, with selective involvement of different size ducts, are the fundamental lesions in PBC.[5] Interlobular bile ducts, whose diameters are 30-100 μm, are affected displaying necrobiotic and proliferous changes of biliary epithelial cells,[6] with an intense peri-ductal lymphocytic infiltration. In the early stages of PBC, epitheloid granulomas are frequently noted but become less prominent with progressive disease.[7] T cells and other

inflammatory cells are frequently infiltrated in the biliary epithelial layer;[8,9] these features have suggested that PBC is similar to graft versus host disease. Indeed the striking similarity of the ultrastructural lesions in both PBC and graft versus host disease provides a morphological argument to suggest that certain common pathogenic mechanisms might be involved in the destruction of bile ducts.[8] However, despite such similarities, there are few if any common serologic links and the inflammatory lesions of PBC are strikingly mitochondrial antigen specific.

Current evidence suggests that T cell receptor (TCR) recognition of antigen bound to the major histcompatibility complex (Ag-MHC) is insufficient to lead to T cell proliferation or effector function. There is also a requirement for so-called 'costimulatory' or 'accessory' signals in addition to TCR ligation by Ag-MHC.[10,11] Cell-to-cell adhesions via ICAM-1 and LFA-1 or Mac-1, VCAM-1 and VLA-4, and LFA-3 and CD2 are well known as contributors to antigen nonspecific binding. In contrast, costimulatory factors such as the B7 families and its ligands CD28 and CTLA 4 are also significantly involved as second signals that are important for the activation of antigen-specific T cells.[10-14] B7-1 is normally expressed at low levels in professional antigen presenting cells, including dendritic cells. B7-2 is constitutionally expressed on the surfaces of dendritic cells and macrophages, and is rapidly up-regulated in B cells by the stimulation via the immunoglobulin receptor or in concert with cytokines. In fact, B7-2 is the major CD28 ligand which is activated in the clonal expansion of antigen-specific T cells.

Since the lymphocytes presumably must adhere to the bile ducts to initiate a cell-to-cell mediated destruction, Broome et al[15] have studied the expression of the LFA-1 together with its ligand, ICAM-1, and the expression of HLA-DR in PBC livers. Most lymphocytes expressed LFA-1. ICAM-1 expression was found on hepatocytes but was not seen on septal bile ducts and found only marginally on interlobular bile ducts. However, most bile ducts express HLA-DR. Proliferating bile ductules demonstrate a concomitant expression of ICAM-1 and HLA-DR. Thus, since most bile ducts involved in the disease process of PBC lack expression of ICAM-1, other mechanisms must be involved if a cell-to-cell mediated destruction accounts for the biliary destruction in PBC. The expression/induction of ICAM-1 on bile ducts may be important in the pathogenesis of bile duct damage in PBC and is further evidence to support an immune pathogenesis in PBC. The induction of ICAM-1 on hepatocytes may be an important factor in the liver-cell damage and fibrosis that occur during the development of cirrhosis.[16]

Activation of T cells to cytokine production and proliferation requires at least two distinct signals. The antigen-specific "first signal" is provided when the TCR interacts with antigen presented in the context of MHC expressed on APCs. The "second signal" or costimulatory signal is provided by a set of receptor-ligand interactions distinct from the TCR interactions.[13] It is generally accepted that interaction between members of the B7 family on antigen-presenting cells and the CD28 receptor on T cells causes transduction of a costimulatory signal, which is important for primary T cell activation.[17] Table 28.C summarizes what is known about adhesion molecules in PBC. Clearly further work is needed.

PBC is characterized therefore by chronic nonsuppurative cholangitis and progressive destruction. Biliary epithelial cells show proliferative change and papillary sheets of nuclei are seen. At the same time, eosinophilic changes, swellings, and destructive changes of biliary epithelial cells are found. Around destroyed bile ducts, various mononuclear cells, including lymphocytes and plasma cells are found; some lymphocytes invade into bile duct epithelial cells. Infiltration of eosinophils and CD68 positive monocytes are also seen. These bile duct changes are generally called chronic nonsuppurative destructive cholangitis (CNSDC). The progressive destruction of intrahepatic bile ducts is irreversible and there is no regeneration of bile ducts.[18] In the advanced stages, intrahepatic bile ducts vanish.

Histologically, PBC can be classified into four stages of increasing severity.[19] In stage 1, there is inflammatory destruction of the intrahepatic bile ducts involving interlobular bile ducts and bile ductules, which are up to 100 μm in diameter. These lesions are often seen

Table 28.D. Characteristics of antimitochondrial autoantibodies and mitochondrial antigens in PBC

Antigen	M.W. (kD)[1]	Frequency (%) in PBC[2]	Lipoyl Domain	B-Cell Epitopes	Major Ig Isotype	Inhibition of Function by AMA
PDC-E2	74	95	+	Outer and inner lipoyl domain	IgG3, IgM	+
BCOADC-E2	52	53-55	+	Lipoyl domain	NR	+
OGDC-E2	48	39-88	+	Lipoyl domain	IgG2, IgM	+
PDC-E1α	41	41-66	-	TPP binding and phosphorylation sites	NR	+
E3BP	55	95	+	NR	NR	NR

1. Determined by SDS-PAGE and immunoblotting against PBC sera; 2. Determined by immunoblotting or ELISA against recombinant proteins; NR: not reported

focally. The portal tracts are usually expanded by infiltration of lymphocytes and a few neutrophils or eosinophils, but the hepatic parenchyma is not prominently involved. Inflammation is also accompanied by granuloma formation that consists of histiocytes, lymphocytes, and occasionally giant cells. These granulomas are most often seen in stage 1 disease. In stage 2, the lesion is characterized by periportal inflammation and ductular proliferation. In this stage, inflammation extends from the portal tract into the hepatic parenchyma, so-called interface hepatitis or piecemeal necrosis. A prominent feature is the destruction of bile ducts with proliferation of bile ductules. In stage 3, the disease is characterized by scarring and fibrosis. Lymphocytic involvement of the portal and periportal areas as well as the hepatic parenchyma can be seen, but the main feature is the presence of subsequent fibrosis without regenerative nodules. Cholestasis is becoming increasingly evident. Stage 4 is represented by established cirrhosis that includes fibrous septa and regenerative nodules. The lesions develop at different rates in the different parts of the liver, and overlap among stages is frequently found. The histological features of early PBC may be seen in those with established cirrhosis. Therefore, it is often difficult to follow the course of the disease or the efficacy of a treatment by the histological findings from a single liver biopsy specimen. Other diseases, such as liver allograft rejection and chronic hepatitis, may also show bile duct lesions similar to those of PBC and should be differentiated from PBC.

Many infiltrating T cells around bile ducts are CD4 positive TCR α and β positive, and the ratio of CD4+/CD8+ is 2 to 2.5. In the early stages of PBC, there are many $CD8^+$ T cells. NK cells compose less than 5% of total infiltrated cells in the portal area. Plasma cells which produce anti-PDC antibody are very abundant in PBC liver, with a frequency much higher than blood.[20] Th1 type cytokines are found in PBC liver. For example,[21-24] Harada et al reported that mononuclear cells expressing IFN-γ and IL-4 mRNA were aggregated in inflamed portal tracts in PBC livers, and that IFN-γ mRNA expression was more commonly detected than IL-4 expression in PBC livers and that the levels of IFN-γ mRNA expression was highly correlated with the degree of portal inflammatory activity.[25] IL-6[26] is present in the bile ducts, suggesting the contribution of ADCC by Th2 type cytokines. In the livers of patients with PBC, eosinophils are abundant around the damaged bile ducts, around the portal vein branches and at the marginal regions, and they are scattered in the portal tracts. Peripheral blood eosinophils are often increased in patients with higher grades of eosinophilic infiltration of the portal tracts. Eosinophilic infiltration of the portal tracts may play a role in immunological injuries of the interlobular bile ducts in PBC.[27] Cytokine production profiles from portal area (biliary epithelial cells and endothelial cells) suggest it is important for generation of bile duct lesion in

PBC. Also it has been suggested that adherent molecules[28,29] and dendritic cells that produce NO all contribute to cytotoxic effects.[30,31] There is also increased fibronectin expression on the biliary basement membrane. Integrin alpha4-fibronectin interaction facilitates adhesion and the penetration of infiltrating alpha4-expressing lymphocytes into the biliary epithelial layer in PBC.[28] The selective impairment of dendritic cell function, increased production of NO by dendritic cells and restoration of blastogenesis using NO inhibitors in PBC all imply a role for NO and dysfunction of dendritic cells in the pathogenesis of PBC.[31] Nonetheless vigorous serial studies have not yet been performed.

Autoantibodies

AMA are highly specific for PBC,[32] but there is no clear relationship of AMA titer with prognosis or the progression of disease. The predominant AMA reactivity is directed to five autoantigens of the 2-OADC complexes; the E1α subunit of PDC, the E2 subunits of PDC, OGDC and BCOADC, and E3 binding protein (E3BP)[33-38] (Table 28.D). Serum autoantibodies from more than 90% of patients with PBC react with PDC-E2 by immunoblotting, whereas the frequency of reactivity against the E2 subunits of OGDC and BCOADC is lower, around 50-70%. Antibodies to PDC-E1α are present in lower titers. Approximately 10% of patients react only to OGDC-E2 and/or BCOADC-E2. Antimitochondrial reactivity is usually observed against some, or even all of the 2-OADC, but serologic cross-reactivity is only found between PDC-E2 and E3BP.

The E2 enzymes have a common structure, which consists of the N-terminal domain containing the lipoyl group(s); the peripheral subunit-binding domain, responsible, at least in part, for binding the E1 and E3 components together; and the C-terminal inner core domain, which houses the active site responsible for the acetyltransferase activity. Several studies using oligopeptides or recombinant proteins have shown that the predominant epitope of PDC-E2 is located within the lipoyl domain of PDC-E2.[39] AMA also react with the outer lipoyl domain, but at a 100-fold lower titer, and only a minority of PBC sera reacts weakly to the E1/E3-binding domain. The mapping of B-cell epitopes using truncated constructs reveals that the reactive AMA to BCOADC-E2, OGDC-E2 and E3BP each also recognize a conformational epitope including the lipoyl domain.[40-42] These domains contain the signature motifs of amino acids with lipoic acid covalently bound to lysine residue.

The highly conserved structure in the E2 subunits of 2-OADC and their lipoyl domains suggest that lipoic acid may be a part of the immunodominant epitope. Several studies have been conducted to address the contribution of lipoic acid to the reactivity of the autoantibodies, and the results are somewhat contradictory.[43-45] However, the data clearly show that AMA are capable of binding to both lipoylated and unlipoylated PDC-E2.

AMA also reacts with the outer domain, but at a 100-fold lower titer, and only one in 26 PBC sera react weakly to the E1/E3 binding region.[39] The mapping of B cell epitopes using truncated constructs and the combination of peptides reveals that the reactive AMA to PDC-E2, BCOADC-E2, and OGDC-E2 each recognize a conformational epitope including the inner lipoyl domain.[39,41,46,47] These domains contain amino acids motif ETDKA, ETDK(T), (GlnS)DKA with lipoic acid covalently bound to the ε group of lysine (K).

An additional target of some AMA is the E1α subunit of PDC (PDC-E1α) which lacks the lipoyl domain (Table 28.A).[48] Its autoepitope of interest is located at the phosphorylation and TPP-binding site.[49] Interestingly, AMA, especially of the IgA isotype, and the autoantigens, PDC-E2, OGDC-E2, and BCOADC-E2 have all been readily detected in the bile of patients with PBC.[50]

Approximately 30% to 50% patients with PBC have antinuclear antibodies. Interestingly some ANA subtypes are nearly 100% specific for PBC including peripheral labeling of the nuclear envelope and multiple nuclear dots when examined by immunofluorescence microscopy.[51] However the predominant ANA recognizes a nuclear envelope antigen coined gp210.[52] Gp210 is a 210 KD integral membrane glycoprotein of the nuclear pore membrane with the

majority of its molecular mass in the perinuclear space, a single transmembrane segment and a carboxyl-terminal tail of 58 amino acids that faces the nuclear pore complex.[53,54] The results of several studies show that from about 10% to 40% of patients with PBC have gp210 autoantibodies.[55-58] Less frequently, patients with PBC have autoantibodies against other nuclear envelope antigens, including (Lamin B receptor) LBR,[57-59] a polytopioc integral protein of the inner nuclear membrane,[60] and possibly nucleoporin p62,[61,62] a nonmembrane protein of the nuclear pore complex.[63] The predominant antigen recognized by autoantibodies that produce the multiple nuclear dot pattern is Sp100, a protein of nuclear bodies.[64] Various studies have reported that anti-Sp100 autoantibodies are present in approximately 20% to 40% of patients with PBC.[65-67] The promyelocytic leukemia protein of nuclear bodies (PML) is also recognized by autoantibodies in some patients with PBC.[67,68]

Antigens in Biliary Epithelium

In PBC, immune responses are directed against similar intracellular mitochondrial antigens that are sheltered from the immune system by double membrane barriers. One obvious question is how the immune response is confined to the biliary tissue of the liver despite ubiquitous distribution of mitochondrial antigens in the body. Several studies have shown that autoantigens relevant to PBC may be present on the surface of biliary epithelial cells of livers from PBC patients using antibodies specific for PDC-E2.[69,70] Monoclonal antibodies (mAbs) to OGDC-E2 and BCOADC-E2 also showed a disease-specific pattern of reactivity.[71] Increased production of these mitochondrial proteins in bile duct cells seems unlikely in patients with PBC.[72] Nonetheless, the abundance of such disease-specific determinants raises the possibility that novel molecules exist in the biliary epithelial cells of PBC, which cross-react with PDC-E2, OGDC-E2 and BCOADC-E2.

Emerging data suggest that this specific staining is due to a large fragment of PDC-E2, if not the entire molecule, as the disease-specific mAbs recognize the same region of PDC-E2, but not the same epitope.[73] It has been reported that the bile from patients with PBC contains AMA of IgG and IgA classes and mitochondrial autoantigens and that there is a positive correlation between the two.[50] The existence of AMA and mitochondrial autoantigens in bile creates the hypothesis in which the disease-specific staining is primarily caused by uptake of mitochondrial autoantigens by bile duct cells. In patients with PBC, simultaneous presence of mitochondrial autoantigens and AMA, especially IgA AMA, may form antigen-antibody complexes and be trapped or accumulated in bile duct cells during their transport to the bile duct lumen via the polyimmunoglobulin receptors found only bile duct cells. Such immune complex formation in bile duct cells may cause conformational change of mitochondrial antigens, which bring about limited expression of antigen determinant, resulting in the recognition of antigens by only a subset of mAbs.

There is evidence that BECs undergo apoptosis in PBC using in situ nick-end labeling methods for the detection of DNA fragmentation of apoptosis, although the mechanisms responsible for inducing apoptosis are presently unclear.[74,75] Related studies have shown increased expression of perforin and granzymes in PBC. In addition, Fas (CD95) is upregulated on the biliary epithelial cell membrane, it is possible that both of these pathways are involved.[74,76]

Genetics

Published studies have shown marked variation in prevalence rates, with high levels found among Northern European populations and low levels found in Japan. There is no consensus regarding whether PBC is more common in urban or rural populations. Estimates of disease prevalence range from 19 to 150 cases per million population, and annual incidence rates vary between 4 and 30 cases per million.[77] The point prevalence of PBC has risen from 19 per million in 1976 to 128 per million in 1987[78] and the reported incidence has also increased. Such apparent increases might only represent an improved diagnosis due to greater awareness and the increased use of autoantibody screening in primary care. However, it is impossible to

Table 28.E. Extrahepatic autoimmune diseases associated with PBC

- Sjögren's syndrome
- Rheumatoid arthritis
- Autoimmune thyroiditis
- Scleroderma (CREST syndrome)
- Mixed connective tissue disease (MCTD)
- Polymyositis
- Renal tubular acidosis
- Systemic lupus erythematosus (SLE)
- Myasthenia gravis
- Ulcerative colitis
- Exocrine pancreatic insufficiency

exclude a genuine increase in the frequency of the disease. Geographical clusters of disease have been also reported, but no causative environmental agents have been identified.[79] There is increasing evidence of a family predisposition for PBC: the prevalence of disease among first degree relatives is markedly increased compared with that of the general population and the relative risk of siblings for PBC was 10.5 in a population based study.[80] When different generations are affected, the second generation usually presents earlier and tends to have more rapid progression. Furthermore, family members of PBC patients have positive AMA as well as other autoantibodies more frequently than controls and exhibit abnormal immunoglobulin levels.[81] These data provide clues regarding the etiology of the disease, and support a genetic component.

Studies of genetic background have largely focused on the major histocompatibility complex (MHC). MHC class I molecules are not associated with susceptibility to PBC. In contrast, some association of susceptibility to PBC with MHC class II molecules have been shown, especially for DR8 (DRB1*0801) in patients from Europe and North America, although the results are not uniform.[82-84] In many cases, the association between MHC class II and PBC is weaker than what has been reported for other autoimmune diseases.

The development of PBC is likely determined by the interaction of multiple genes, whose contribution can only be determined by examining large numbers of PBC patients or performing sibling studies, i.e., inheritance by descent. It also needs to be kept in mind that environmental factors, such as infectious agents, environmental chemicals, and hormonal stimulation may be implicated in the precipitation of disease. Finally, we note the increased frequency of other autoimmune diseases in patients with PBC (Table 28.E).

T Cell Response in PBC

The presence of lymphoid infiltration in the portal tracts, the specific destruction of small bile ducts and the aberrant expression of MHC class II antigen on biliary epithelium in PBC have suggested that an intense autoimmune response is directed against biliary epithelial cells. It has been hypothesized that the destruction of biliary tract in PBC is mediated by autoreactive liver-infiltrating T cells through either cytotoxicity or lymphokine production. The evidence was initially derived from immunohistochemical studies of liver tissues,[85] in which a predominance of T cell infiltration, mainly $CD3^+$, $CD4^+$ T cells bearing T cell receptor $\alpha\beta$, was shown in the liver and in particular around the portal tracts. Later several studies have been reported on analysis of T-cell lines that proliferate in the presence of putative mitochondrial antigens. However, despite accumulating data that T cell-mediated immune responses are involved in PBC, studies to determine the T cell epitopes of the mitochondrial antigens in PBC are still limited. The data available on the T-cell epitope of PDC-E2 in PBC show that there is some

overlapping in the PDC-E2-specific T and B cell epitopes. This may be a result of not only more efficient uptake and processing of the autoantigen by autoimmune B cells but also could be due to the fact that the epitopes may be protected from degradation during antigen processing. A study by Van de Water et al demonstrated for the first time that T cell clones reacting with PDC-E2 and/or BCOADC-E2 were present in the liver biopsy specimens of patients with PBC.[86] These clones were CD4+, TCRαβ+ and produced IL-2 specifically in response to PDC-E2 or BCOADC-E2.[86] The precise T cell autoepitopes of PDC-E2 have been characterized by Shimoda et al.[87,88] During the course of these studies, a number of PDC-E2 specific T cell clones from not only the peripheral blood, but also from the explanted livers and regional lymph nodes (RLH) of patients with PBC were established. Using a battery of overlapping peptides covering the full length PDC-E2 molecule, the minimal autoepitopes recognized by these T cell clones was defined and found to locate within the same region of PDC-E2 peptide 163-176 (GDLLAEIETDKATI), which is contained within the inner lipoyl domain of human PDC-E2.[88] Additional peptide specificity studies revealed a degree of cross-reactivity for the T cell clones with specificity for the PDC-E2 peptide 163-176. Thus, such PDC-E2 163-176 was also shown to proliferate with PDC-E2 peptide 36-49 and OGDC peptide 100-113. A fine analysis of the peptides that react with such T cell clones led to the identification of a *common T cell epitope motif* ExExDK.[87,88] *These data provide evidence for a major role for* the PDC-E2 peptide 163-176 and/or peptides bearing a similar motif in the pathogenesis of PBC. *Interestingly, autoreactive T cells and autoantibodies from PBC patients both recognize* the same dominant epitope. The importance of such T cell clones was highlighted by the *finding that there is a disease-specific 100-150-fold increase in the precursor frequency of such* PDC-E2 163-176-specific T cells in the hilar lymph nodes and liver when compared with concordant PBMC samples from the patients with PBC. In addition, in the early or moderate stage of PBC, the frequency of peripheral T cells responding to peptide 163-176 is significantly higher than in end-stage PBC, although it is not currently known whether this represents the disappearance of cells during the course of the disease or a progressive homing of cells to the liver or the RLH.[87]

Several TCR Vβ usage studies have been performed on liver-derived T cells. Some studies showed that the T cell clones established from PBC patients had limited diversity and sequence analysis of CDR3 and revealed the presence of conserved residues, no random N additions, and a common motif within the CDR3.[89,90] However, another study showed that TCR-Vβ usage by PDC-reactive T cell clones infiltrating the liver was remarkably heterogeneous.[91] The reasons for such discrepant results are not clear. By the nature of techniques utilized, all the T cell clones established thus far express CD3+, CD4+, CD45RO, and TCR αβ+ denoting that each of these T cell clones is a memory helper T cells. The role, characteristics and functions of CD8+ cytotoxic T cell in PBC to date remain largely unknown. Clearly additional studies are required to define a role if any for CD8+ cytotoxic T cells in PBC.

While a large body of data has accumulated on the precise identification of the autoantigens that serve as targets for humoral immune responses and significant data exists on the nature of the autoantigen-specific $CD4^+$ helper T cell responses in primary biliary cirrhosis (PBC), information on the nature of autoantigen-specific cytotoxic T lymphocyte (CTL) responses is relatively unknown (121,122). Characterization of the $CD8^+$ CTL response in PBC is important as such cells are likely involved in the lysis of biliary epithelial cells (BECs), the major target involved in the pathogenesis of this disease. Identification of the autoantigen peptide, that is the target of MHC Class I restricted CTLs in PBC, will provide an important initial step not only in determining the mechanism by which tolerance to the self peptide is abrogated, but also in facilitating the potential for therapeutic strategies.

Several sequence patterns or motifs for peptides that bind to particular MHC molecules or groups of MHC molecules have been defined by analysis of peptides presented by MHC class I molecules.[92,93] Amongst the best-studied motif is that of the MHC class I HLA-A2 molecule which is prevalent in 50% of the Caucasian population in the Western Hemisphere.[94,95] In

fact, motif prediction analysis of HLA-A2 has led to the identification of the dominant epitopes of the human myelin basic proteins[96] and glutamic acid decarboxylase,[97] the key autoantigens in multiple sclerosis and insulin-dependent diabetes mellitus, respectively.

Using PBMC from PBC, we identified an HLA-A2 restricted CTL epitope of the E2 component of pyruvate dehydrogenase (PDC-E2), the immunodominant mitochondrial autoantigen. This peptide, aa 159-167 of PDC-E2, induces specific MHC-class I restricted $CD8^+$ CTL lines from 10/12 $HLA\text{-}A2^+$ PBC patients, but not controls, following in vitro stimulation with antigen pulsed dendritic cells (DCs). PDC-E2 specific CTLs could also be generated by pulsing DCs with full length recombinant PDC-E2 protein. Furthermore, using soluble PDC-E2 complexed with either PDC-E2 specific human mAb or affinity purified autoantibodies against PDC-E2, the generation of PDC-E2 specific CTLs, occurred at 100-fold and 10 fold less concentration respectively, compared to soluble antigen alone. Collectively, these data demonstrate that autoantibody, helper and CTL epitopes all contain a shared peptide sequence. The finding that autoantigen-immune complexes can not only cross present but also that presentation of the autoantigen is of a higher relative efficiency, for the first time defines a unique role for autoantibodies in the pathogenesis of an autoimmune disease.

PDC-E2 specific autoreactive CTLs appear primed in vivo only in PBC patients. Our data prompted us to hypothesize that a previously described nonconventional mechanism of antigen presentation, "cross-priming" or "cross-presentation", is involved in the maintenance and / or amplification of CTL response against PDC-E2, with PDC-E2-specific autoantibody playing a key role. Professional APCs have been shown capable of intracellular processing of exogenous antigen using proteosomes to generate peptides presented by MHC class I,[98,99] the exogenous antigen being presented by the same APCs to both $CD4^+$ and $CD8^+$ T cells.[100] This is important for the induction of immunity to pathogens that avoid professional APCs.[101] The precise nature of the APCs that are able to take up, process and present exogenous antigens in association with MHC class I molecules remains to be defined. However, in vitro studies suggest that DCs,[102-104] macrophages,[105] or B cells[106] might be involved. Cross-priming is generally inefficient, but the efficiency is much higher with macropinocytosis[107] and phagocytosis of the particle.[108,109] Phagocytosis of apoptotic cells also results in efficient MHC class I-restricted antigen presentation in macrophages and / or DCs.[104,110,111] DCs in murine models have been shown to mediate internalization of antigen-immunoglobulin complexes (immune complexes, ICs), and promote efficient MHC class I as well as class II-restricted antigen presentation.[112] In terms of MHC class II presentation, Fcγ receptors, which bind ICs, represent a privileged antigen internalization route for efficient MHC class II-restricted antigen presentation in DCs.[113] In murine systems, it has been suggested that DCs are capable of taking up ICs, and presenting the appropriate processed antigenic peptide to $CD4^+$ T cells, which in turn activate DCs, and convert them into DCs capable of priming $CD8^+$ T cells in vivo.[112]

The estimated frequency of PDC-E2 specific $CD8^+$ T cells from peripheral blood was significantly higher in early stages of PBC than in the more advanced disease stages. This is in agreement with a previous report showing that the frequency of peripheral blood $CD4^+$ T cells that respond to the helper T cell epitope 163-176 was significant higher in early stage of PBC as compared with advanced stage.[114] In previous studies directly comparing the frequency of hepatitis C virus (HCV) specific $CD8^+$ T cells in peripheral blood and liver, higher frequencies of HCV-specific $CD8^+$ T cells were found in liver biopsies than in the blood of patients with chronic HCV infection.[115,116] In another study using end stage cirrhotic liver, the HCV-specific T cell could not be found in the liver,[117] suggesting that the level of virus-specific intrahepatic CTLs declines as the viral hepatitis progress. Supposing that declination of the disease specific CTL with the disease progression occurs in PBC, the PDC-E2-specific $CD8^+$ T cells in the liver may be even more abundant during the earlier stages of PBC and be playing an important role in the disease development. In addition, the autoreactive CTL response in PBC may also involve other epitopes restricted by HLA-A*0201, as well as epitopes restricted by other HLA molecules.

Table 28.F. Possible pathogenetic determinants in PBC

1.	INCREASED EXPRESSION OF PDC-E2 ON BEC	
	a.	Isoform/cross-reactive antigen?
	b.	IgA-PDC-E2 immune complex
2.	ABERRANT MOLECULAR TRAFFICKING	
	a.	MHC Class I (and Class II) association of autoantigenic molecules
	b.	Surface expression of autoantigen with MHC
3.	STIMULATION OF CYTOTOXIC CD8 CELLS	
	a.	Genetic background?
	b.	Cytokine release - interferon g and other stimulatory molecules
	c.	Necrosis of biliary epithelial cells
4.	ASSOCIATION OF ANTIGEN WITH CLASS II	
	a.	Genetic background: DRW8 possible but not evident in all studies.
	b.	Null alleles for C4A0
5.	STIMULATION OF CD4 T CELLS	
	a.	Production of autoantibody
6.	REACTION WITH SURFACE-EXPRESSED ANTIGEN	
	a.	Necrosis
7.	MOLECULAR MIMICRY	
8.	SELECTIVE INDUCTION OF APOPTOSIS	
9.	XENOBIOTIC EXPOSURE	

Etiology

Although the etiology of PBC is unknown, there are several clues and hypotheses (Table 28.F). First, it is never found in childhood. Second, it is more common in Westernized countries and the incidence may be increasing.[118] Third, despite the absence of correlation with the MHC, the risk factor for developing PBC in a first degree relative is 100-800 fold more common and the onset of disease in relatives is often within a few years of each other's diagnosis.[119] Fourth, there is a long incubation time between the appearance of AMA and clinical disease. Fifth, AMA, detected by using recombinant autoantigens as targets, are pathognomonic of PBC. Sixth, there is no objective data supporting a role for microbial agents in the etiology of PBC.[120]

Xenobiotics are foreign compounds that may either alter or complex to defined self proteins, inducing a change in the molecular structure of the native protein sufficient to induce an immune response. Such immune responses may then result in the recognition of not only the modified or altered protein, but also the unmodified native protein.[121,122] The chronic presence of the self protein serves to perpetuate the immune response initiated by the xenobiotic-induced adduct and leads to autoimmunity.[123,124] Many xenobiotics are metabolized in the liver, thereby increasing the potential for liver-specific alteration of proteins.[125] In fact, a liver-specific autoimmune disease can be observed in some patients exposed to chlorofluorohydrocarbon anesthetics.[126,127] Previous work has reported that immunization with halothane, whose trifluoroacetyl (TFA) metabolite covalently links to lysine on cytochrome p450 2E1,[128] induces the formation of antibodies that cross-react with not only the haptenated (TFA) immunogen, but also to lipoated PDC-E2, the major autoantigen of PBC.[129,130] This finding has important implications in the pathogenic mechanisms associated with PBC, an autoimmune disease marked by the presence of antimitochondrial antibodies (AMA).[131,132] The target of AMA are the E2 components of the 2-oxo acid dehydrogenase pathway, particu-

larly PDC-E2,[131] and the primary B cell epitope of PDC-E2 recognized by AMA includes a lipoated lysine residue.[114,133]

Our group hypothesized that the lipoic acid residue of PDC-E2 serves as a xenobiotic target which, following the modification of the lipoyl lysine residue, becomes immunogenic and initiates or perpetuates an AMA response. We further hypothesized that the AMA response is induced by a modified self protein and that the antibody specificities present in such sera include those that recognize the xenobiotic modification. To address the hypothesis that PBC is induced by xenobiotic exposure, we took advantage of ab initio quantum chemistry and synthesized the inner lipoyl domain of PDC-E2, replacing the lipoic acid moiety with synthetic structures designed to mimic a xenobiotically-modified lipoyl hapten, and we quantitated the reactivity of these structures with sera from PBC patients. Interestingly, AMA from all seropositive patients with PBC, but no controls, reacted against 3 of the 18 organic modified autoepitopes significantly better than to the native domain. By structural analysis, the features that correlated with autoantibody binding included synthetic domain peptides with a halide or methyl halide in the meta- or para-position containing no strong hydrogen bond accepting groups on the phenyl ring of the lysine substituents, and synthetic domain peptides with a relatively low rotation barrier about the linkage bond. Many chemicals including pharmaceuticals and household detergents have the potential to form such halogenated derivatives as metabolites. This data reflects the first time that an organic compound has been shown to serve as a mimeotope for an autoantigen and further provides evidence for a potential mechanism by which environmental organic compounds may cause primary biliary cirrhosis.

The liver is an important organ for metabolism/degradation for xenobiotics and an altered immune response.[134] A large number of chemicals, including halogenated compounds, are detoxified through the liver and secreted in the bile. Hence, exposure to an agent that would uniquely modify the mitochondrial antigens within biliary epithelium, could lead to a breakdown of tolerance and induction of a self-reactive response that is target-specific. Moreover, there is evidence based on in vivo studies in guinea pigs exposed to halothane, that Kupffer cells carry trifluoroacetylated protein adducts;[135] these protein adducts are not found in other organs, including hilar lymph nodes. This provides evidence that the generation of autoreactivity to the protein adducts is likely a local liver response. We should also note the possibility that xenobiotics have other immunotoxic potential, including a selective stimulation or inhibition of components of the immune system.[123] Such effects, of course, would be independent of any modification of autoantigens.

We postulate that people genetically predisposed to PBC have inherited such predisposition based on either the cytochrome p450 pathway or another metabolic process responsible for degrading halogenated compounds. A large number of common pharmaceuticals such as diuretic agents are halogenated structures. In fact, halogens are common substituents in pharmaceuticals that modulate binding, activity and metabolism. In addition, there are large numbers of detergents, commonly used at home and commercially, that are rich in halogenated derivatives. Estrogens have already been shown to modulate the expression of many liver metabolic pathways and may explain the preponderance of women with PBC. Finally, the presence of primarily small bile duct destruction may be reflective of the local mucosal immune response, which is more prominent on epithelial surfaces.[136] Indeed, PBC is often referred to as an epithelitis with involvement not only of bile ducts, but also of salivary glands.

The destructive phase of PBC appears to be mediated locally by an intense mucosal immune response. High titer IgA PDC-E2 specific autoantibodies have been found in the bile, saliva and even the urine of patients with PBC.[137,138] Moreover, there is unique staining of the cell surface, of both bile duct and salivary gland epithelial cells, with monoclonal antibodies to PDC-E2 that colocalizes with IgA transcytosing the cell, suggesting that these may be IgA-PDC-E2 complexes.[138,139] Furthermore, the precursor frequency of PDC-E2-specific CD4 T cells is 100-150 folds higher in liver than in the peripheral blood of patients with PBC.[114] In addition, we have recently observed the presence of PDC-E2 peptide specific MHC Class I

restricted CD8 T cells only in patients with PBC (manuscript in preparation). Chronic intense PDC-E2 specific responses, either individually or in concert, contribute to the overall pathology. It is ironic that the liver, known to contribute to the induction of tolerance, is precisely the organ that is the target of a central breakdown in tolerance. Towards that end, the influence of these xenobiotics on cellular immune responses in PBC is currently underway. Defining the precise sequence and the molecular basis by which xenobiotics initiate the cascade of autoimmune responses is the next challenge for understanding the etiology and pathogenesis of PBC.

REFERENCES

1. Mahl TC, Shockcor W, Boyer JL. Primary biliary cirrhosis: survival of a large cohort of symptomatic and asymptomatic patients followed for 24 years. Journal of Hepatology 1994; 20:707-713.
2. Springer J, Cauch-Dudek K, O'Rourke K et al. Asymptomatic primary biliary cirrhosis: a study of its natural history and prognosis. Am J Gastroenterol 1999; 94:47-53.
3. Shapiro JM, Smith H, Schaffner F. Serum bilirubin: a prognostic factor in primary biliary cirrhosis. Gut 1979; 20:137-140.
4. Kilmurry MR, Heathcote EJ, Cauch-Dudek K et al. Is the Mayo model for predicting survival useful after the introduction of ursodeoxycholic acid treatment for primary biliary cirrhosis?. Hepatology 1996; 23:1148-1153.
5. Nakanuma Y, Tsuneyama K, Gershwin ME et al. Pathology and immunopathology of primary biliary cirrhosis with emphasis on bile duct lesions: recent progress. Seminars in Liver Disease 1995; 15:313-328.
6. Nakanuma Y, Ohta G, Takeshita H et al. Florid duct lesions and extensive bile duct loss of the intrahepatic biliary tree in chronic liver diseases other than primary biliary cirrhosis. Acta Pathologica Japonica 1983; 33:1095-1104.
7. Nakanuma Y, Ohta G. Quantitation of hepatic granulomas and epithelioid cells in primary biliary cirrhosis. Hepatology 1983; 3:423-427.
8. Bernuau D, Feldmann G, Degott C et al. Ultrastructural lesions of bile ducts in primary biliary cirrhosis. A comparison with the lesions observed in graft versus host disease Hum Pathol 1981; 12:782-793.
9. Nakanuma Y, Ohta G, Kono N et al. Electron microscopic observation of destruction of biliary epithelium in primary biliary cirrhosis. Liver 1983; 3:238-248.
10. Thompson CB. Distinct roles for the costimulatory ligands B7-1 and B7-2 in T helper cell differentiation?. Cell 1995; 81:979-982.
11. June CH, Bluestone JA, Nadler LM et al. The B7 and CD28 receptor families. Immunol Today 1994; 15:321-331.
12. Freeman GJ, Borriello F, Hodes RJ et al. Uncovering of functional alternative CTLA-4 counter-receptor in B7- deficient mice. Science 1993; 262:907-909.
13. Hathcock KS, Laszlo G, Pucillo C et al. Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. J Exp Med 1994; 180:631-640.
14. Lenschow DJ, Su GH, Zuckerman LA et al. Expression and functional significance of an additional ligand for CTLA- 4. Proc Natl Acad Sci USA 1993; 90:11054-11058.
15. Broome U, Hultcrantz R, Scheynius A. Lack of concomitant expression of ICAM-1 and HLA-DR on bile duct cells from patients with primary sclerosing cholangitis and primary biliary cirrhosis. Scandinavian Journal of Gastroenterology 1993; 28:126-130.
16. Adams DH, Hubscher SG, Shaw J et al. Increased expression of intercellular adhesion molecule 1 on bile ducts in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 1991; 14:426-431.
17. Allison JP. CD28-B7 interactions in T-cell activation. Curr Opin Immunol 1994; 6:414-419.
18. Portmann B, Popper H, Neuberger J et al. Sequential and diagnostic features in primary biliary cirrhosis based on serial histologic study in 209 patients. Gastroenterology 1985; 88:1777-1790.
19. Scheuer P. Primary biliary cirrhosis. Proceedings of the Royal Society of Medicine 1967; 60:1257-1260.
20. Björkland A, Loof L, Mendel-Hartvig I et al. Primary biliary cirrhosis. High proportions of B cells in blood and liver tissue produce anti-mitochondrial antibodies of several Ig classes Journal of Immunology 1994; 153:2750-2757.
21. Berg PA, Klein R, Rocken M. Cytokines in primary biliary cirrhosis. Semin Liver Dis 1997; 17:115-123.

22. Harada K, Van de Water J, Leung PS 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 1997; 25:27-32.
23. Dienes HP, Lohse AW, Gerken G et al. Bile duct epithelia as target cells in primary biliary cirrhosis and primary sclerosing cholangitis. Virchows Arch 1997; 431:119-124.
24. Tjandra K, Sharkey KA, Swain MG. Progressive development of a Th1-type hepatic cytokine profile in rats with experimental cholangitis. Hepatology 2000; 31:280-290.
25. Harada K, Van de Water J, Leung PS et al. In situ nucleic acid hybridization of cytokines in primary biliary cirrhosis: predominance of the Th1 subset. Hepatology 1997; 25:791-796.
26. Yasoshima M, Kono N, Sugahara H et al. Increased expression of interleukin-6 and tumor necrosis factor-a in pathologic biliary epithelial cells: in situ and culture study. Laboratory Investigation 1998; 78:89-100.
27. Terasaki S, Nakanuma Y, Yamazaki M et al. Eosinophilic infiltration of the liver in primary biliary cirrhosis: a morphological study. Hepatology 1993; 17:206-212.
28. Yasoshima M, Tsuneyama K, Harada K et al. Immunohistochemical analysis of cell-matrix adhesion molecules and their ligands in the portal tracts of primary biliary cirrhosis. J Pathol 2000; 190:93-99.
29. Yasoshima M, Nakanuma Y, Tsuneyama K et al. Immunohistochemical analysis of adhesion molecules in the micro-environment of portal tracts in relation to aberrant expression of PDC-E2 and HLA-DR on the bile ducts in primary biliary cirrhosis. J Pathol 1995; 175:319-325.
30. Tanimoto K, Akbar SM, Michitaka K et al. Immunohistochemical localization of antigen presenting cells in liver from patients with primary biliary cirrhosis; highly restricted distribution of CD83-positive activated dendritic cells. Pathol Res Pract 1999; 195:157-162.
31. Yamamoto K, Akbar SM, Masumoto T et al. Increased nitric oxide (NO) production by antigen-presenting dendritic cells is responsible for low allogeneic mixed leucocyte reaction (MLR) in primary biliary cirrhosis (PBC). Clin Exp Immunol 1998; 114:94-101.
32. Berg PA, Klein R, Lindenborn-Fotinos J et al. ATPase-associated antigen (M2): marker antigen for serological diagnosis of primary biliary cirrhosis. Lancet 1982; 2:1423-1426.
33. Fregeau DR, Davis PA, Danner DJ et al. Antimitochondrial antibodies of primary biliary cirrhosis recognize dihydrolipoamide acyltransferase and inhibit enzyme function of the branched chain alpha-ketoacid dehydrogenase complex. J Immunol 1989; 142:3815-3820.
34. Fregeau DR, Prindiville T, Coppel RL et al. Inhibition of alpha-ketoglutarate dehydrogenase activity by a distinct population of autoantibodies recognizing dihydrolipoamide succinyltransferase in primary biliary cirrhosis. Hepatology 1990; 11:975-981.
35. Fregeau DR, Roche TE, Davis PA et al. Primary biliary cirrhosis. Inhibition of pyruvate dehydrogenase complex activity by autoantibodies specific for E1 alpha, a nonlipoic acid containing mitochondrial enzyme. J Immunol 1990; 144:1671-1676.
36. Surh CD, Roche TE, Danner DJ et al. Antimitochondrial autoantibodies in primary biliary cirrhosis recognize cross-reactive epitope(s) on protein X and dihydrolipoamide acetyltransferase of pyruvate dehydrogenase complex. Hepatology 1989; 10:127-133.
37. Surh CD, Danner DJ, Ahmed A et al. Reactivity of primary biliary cirrhosis sera with a human fetal liver cDNA clone of branched-chain alpha-keto acid dehydrogenase dihydrolipoamide acyltransferase, the 52 kD mitochondrial autoantigen. Hepatology 1989; 9:63-68.
38. Van de Water J, Fregeau D, Davis P et al. Autoantibodies of primary biliary cirrhosis recognize dihydrolipoamide acetyltransferase and inhibit enzyme function. J Immunol 1988; 141:2321-2324.
39. Surh CD, Coppel R, Gershwin ME. Structural requirement for autoreactivity on human pyruvate dehydrogenase-E2, the major autoantigen of primary biliary cirrhosis. Implication for a conformational autoepitope. J Immunol 1990; 144:3367-3374.
40. Dubel L, Tanaka A, Leung PS et al. Autoepitope mapping and reactivity of autoantibodies to the dihydrolipoamide dehydrogenase-binding protein (E3BP) and the glycine cleavage proteins in primary biliary cirrhosis. Hepatology 1999; 29:1013-1018.
41. Leung PS, Chuang DT, Wynn RM et al. Autoantibodies to BCOADC-E2 in patients with primary biliary cirrhosis recognize a conformational epitope. Hepatology 1995; 22:505-513.
42. Moteki S, Leung PS, Dickson ER et al. Epitope mapping and reactivity of autoantibodies to the E2 component of 2-oxoglutarate dehydrogenase complex in primary biliary cirrhosis using recombinant 2-oxoglutarate dehydrogenase complex. Hepatology 1996; 23:436-444.
43. Quinn J, Diamond AG, Palmer JM et al. Lipoylated and unlipoylated domains of human PDC-E2 as autoantigens in primary biliary cirrhosis: significance of lipoate attachment. Hepatology 1993; 18:1384-1391.
44. Leung PS, Iwayama T, Coppel RL et al. Site-directed mutagenesis of lysine within the immunodominant autoepitope of PDC-E2. Hepatology 1990; 12:1321-1328.

45. Fussey SP, Ali ST, Guest JR et al. Reactivity of primary biliary cirrhosis sera with *Escherichia coli* dihydrolipoamide acetyltransferase (E2p): characterization of the main immunogenic region. Proc Natl Acad Sci USA 1990; 87:3987-3991.
46. Cha S, Leung PS, Coppel RL et al. Heterogeneity of combinatorial human autoantibodies against PDC-E2 and biliary epithelial cells in patients with primary biliary cirrhosis. Hepatology 1994; 20:574-583.
47. Moteki S, Leung PS, Coppel RL et al. Use of a designer triple expression hybrid clone for three different lipoyl domain for the detection of antimitochondrial autoantibodies. Hepatology 1996; 24:97-103.
48. Fussey SP, Bassendine MF, Fittes D et al. The E1 alpha and beta subunits of the pyruvate dehydrogenase complex are M2'd' and M2'e' autoantigens in primary biliary cirrhosis. Clin Sci 1989; 77:365-368.
49. Iwayama T, Leung PS, Coppel RL et al. Specific reactivity of recombinant human PDC-E1 alpha in primary biliary cirrhosis. Journal of Autoimmunity 1991; 4:769-778.
50. Nishio A, Van de Water J, Leung PS et al. Comparative studies of antimitochondrial autoantibodies in sera and bile in primary biliary cirrhosis. Hepatology 1997; 25:1085-1089.
51. Worman HJ, Terjung B, Courvalin J-C. Nuclear protein antigens in primary biliary cirrhosis. In: M.P. Manns, G. Paumgartner and U. Leuschner, eds. In Immunology and the Liver: Proceedings of Falk Symposium 114. Dordrecht.: Kluwer Academic Publishers, 2000:257-267.
52. Courvalin JC, Lassoued K, Bartnik E et al. The 210-kD nuclear envelope polypeptide recognized by human autoantibodies in primary biliary cirrhosis is the major glycoprotein of the nuclear pore. Journal of Clinical Investigation 1990; 86:279-285.
53. Greber UF, Senior A, Gerace L. A major glycoprotein of the nuclear pore complex is a membrane-spanning polypeptide with a large lumenal domain and a small cytoplasmic tail. Embo J 1990; 9:1495-1502.
54. Wozniak RW, Bartnik E, Blobel G. Primary structure analysis of an integral membrane glycoprotein of the nuclear pore. J Cell Biol 1989; 108:2083-2092.
55. Lozano F, Pares A, Borche L et al. Autoantibodies against nuclear envelope-associated proteins in primary biliary cirrhosis. Hepatology 1988; 8:930-938.
56. Lassoued K, Brenard R, Degos F et al. Antinuclear antibodies directed to a 200-kilodalton polypeptide of the nuclear envelope in primary biliary cirrhosis. A clinical and immunological study of a series of 150 patients with primary biliary cirrhosis Gastroenterology 1990; 99:181-186.
57. Nickowitz RE, Wozniak RW, Schaffner F et al. Autoantibodies against integral membrane proteins of the nuclear envelope in patients with primary biliary cirrhosis. Gastroenterology 1994; 106:193-199.
58. Bandin O, Couvalin J, Poupon R et al. Specificity and sensitivity of gp210 autoantibodies detected using an enzyme-linked immunosorbent assay and a synthetic polypeptide in the diagnosis of primary biliary cirrhosis. Hepatology 1996; 23:1020-1024.
59. Courvalin JC, Lassoued K, Worman HJ et al. Identification and characterization of autoantibodies against the nuclear envelope lamin B receptor from patients with primary biliary cirrhosis. Journal of Experimental Medicine 1990; 172:961-967.
60. Worman HJ, Evans CD, Blobel G. The lamin B receptor of the nuclear envelope inner membrane: a polytopic protein with eight potential transmembrane domains. J Cell Biol 1990; 111:1535-1542.
61. Wesierska-Gadek J, Honenauer H, Hitchman E et al. Autoantibodies against nucleoporin p62 constitute a novel marker of primary biliary cirrhosis. Gastroenterology 1996; 110:840-847.
62. Miyachi K, Shibata M, Onozuka Y et al. Primary biliary cirrhosis sera recognize not only gp210 but also proteins of the p62 complex bearing N-acetylglucosamine residues from rat liver nuclear envelope. Anti-p62 complex antibody in PBC Mol Biol Rep 1996; 23:227-234.
63. Davis LI, Blobel G. Identification and characterization of a nuclear pore complex protein. Cell 1986; 45:699-709.
64. Szostecki C, Guldner HH, Netter HJ et al. Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. Journal of Immunology 1990; 145:4338-4347.
65. Evans J, Reuben A, Craft J. PBC 95k, a 95-kilodalton nuclear autoantigen in primary biliary cirrhosis. Arthritis & Rheumatism 1991; 34:731-736.
66. Orth T, Gerken G, Meyer Zum Buschenfelde KH et al. Antineutrophil nuclear antibodies (ANNA) in primary biliary cirrhosis: their prevalence and antigen specificity. Z Gastroenterol 1997; 35:113-121.
67. Zuchner D, Sternsdorf T, Szostecki C et al. Prevalence, kinetics, and therapeutic modulation of autoantibodies against Sp100 and promyelocytic leukemia protein in a large cohort of patients with primary biliary cirrhosis. Hepatology 1997; 26:1123-1130.

68. Sternsdorf T, Guldner HH, Szostecki C et al. Two nuclear dot-associated proteins, PML and Sp100, are often co autoimmunogenic in patients with primary biliary cirrhosis. Scand J Immunol 1995; 42:257-268.
69. 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 1991; 14:442-447.
70. Van de Water J, Turchany J, Leung PS et al. Molecular mimicry in primary biliary cirrhosis. Evidence for biliary epithelial expression of a molecule cross-reactive with pyruvate dehydrogenase complex-E2. J Clin Invest 1993; 91:2653-2664.
71. Migliaccio C, Nishio A, Van de Water J et al. Monoclonal antibodies to mitochondrial E2 components define autoepitopes in primary biliary cirrhosis. Journal of Immunology 1998; 161:5157-5163.
72. Harada K, Sudo Y, Kono N et al. In situ nucleic acid detection of PDC-E2, BCOADC-E2, OGDC-E2, PDC-E1alpha, BCOADC-E1alpha, OGDC-E1, and the E3 binding protein (protein X) in primary biliary cirrhosis. *Hepatology 1999; 30:36-45.*
73. Migliaccio C, Van de Water J, Ansari AA et al. Heterogeneous response of antimitochondrial autoantibodies and bile duct apical staining monoclonal antibodies to pyruvate dehydrogenase complex E2: the molecule versus the mimic. Hepatology 2001; 33:792-801.
74. Harada K, Ozaki S, Gershwin ME et al. Enhanced apoptosis relates to bile duct loss in primary biliary cirrhosis. Hepatology 1997; 26:1399-1405.
75. Koga H, Sakisaka S, Ohishi M et al. Nuclear DNA fragmentation and expression of Bcl-2 in primary biliary cirrhosis. Hepatology 1997; 25:1077-1084.
76. Kuroki T, Seki S, Kawakita N et al. Expression of antigens related to apoptosis and cell proliferation in chronic nonsuppurative destructive cholangitis in primary biliary cirrhosis. Virchows Arch 1996; 429:119-129.
77. Watson RG, Angus PW, Dewar M et al. Low prevalence of primary biliary cirrhosis in Victoria, Australia. Melbourne Liver Group Gut 1995; 36:927-930.
78. Myszor M, James OF. The epidemiology of primary biliary cirrhosis in north-east England: an increasingly common disease?. Quarterly Journal of Medicine 1990; 75:377-385.
79. Triger DR. Primary biliary cirrhosis: an epidemiological study. British Medical Journal 1980; 281:772-775.
80. Jones DE, Watt FE, Metcalf JV et al. Familial primary biliary cirrhosis reassessed: a geographically-based population study. J Hepatol 1999; 30:402-407.
81. Galbraith RM, Smith M, Mackenzie RM et al. High prevalence of seroimmunologic abnormalities in relatives of patients with active chronic hepatitis or primary biliary cirrhosis. New England Journal of Medicine 1974; 290:63-69.
82. Begovich AB, Klitz W, Moonsamy PV et al. Genes within the HLA class II region confer both predisposition and resistance to primary biliary cirrhosis. Tissue Antigens 1994; 43:71-77.
83. Gregory WL, Mehal W, Dunn AN et al. Primary biliary cirrhosis: contribution of HLA class II allele DR8. Q J Med 1993; 86:393-399.
84. Zhang L, Weetman AP, Bassendine M et al. Major histocompatibility complex class-II alleles in primary biliary cirrhosis. Scand J Immunol 1994; 39:104-106.
85. Krams SM, Van de Water J, Coppel RL et al. Analysis of hepatic T lymphocyte and immunoglobulin deposits in patients with primary biliary cirrhosis. Hepatology 1990; 12:306-313.
86. Van de Water J, Ansari AA, Surh CD et al. Evidence for the targeting by 2-oxo-dehydrogenase enzymes in the T cell response of primary biliary cirrhosis. J Immunol 1991; 146:89-94.
87. Shimoda S, Van de Water J, Ansari A et al. Identification and precursor frequency analysis of a common T cell epitope motif in mitochondrial autoantigens in primary biliary cirrhosis. Journal of Clinical Investigation 1998; 102:1831-1840.
88. Shimoda S, Nakamura M, Ishibashi H et al. HLA DRB4 0101-restricted immunodominant T cell autoepitope of pyruvate dehydrogenase complex in primary biliary cirrhosis: evidence of molecular mimicry in human autoimmune diseases. J Exp Med 1995; 181:1835-1845.
89. Tsai S, Lai M, Chen D. Analysis of rearranged T cell receptor (TCR) V beta transcripts in livers of primary biliary cirrhosis: preferential V beta usage suggests antigen-driven selection. Clin Exp Immunol 1996; 103:99-104.
90. 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 1990; 20:889-896.
91. Van de Water J, Ansari A, Prindiville T et al. Heterogeneity of autoreactive T cell clones specific for the E2 component of the pyruvate dehydrogenase complex in primary biliary cirrhosis. J Exp Med 1995; 181:723-733.

92. Sidney J, Southwood S, del Guercio MF et al. Specificity and degeneracy in peptide binding to HLA-B7-like class I molecules. J Immunol 1996; 157:3480-3490.
93. Sidney J, Grey HM, Southwood S et al. Definition of an HLA-A3-like supermotif demonstrates the overlapping peptide-binding repertoires of common HLA molecules. Hum Immunol 1996; 45:79-93.
94. Nayersina R, Fowler P, Guilhot S et al. HLA A2 restricted cytotoxic T lymphocyte responses to multiple hepatitis B surface antigen epitopes during hepatitis B virus infection. J Immunol 1993; 150:4659-4671.
95. Cerny A, McHutchison JG, Pasquinelli C et al. Cytotoxic T lymphocyte response to hepatitis C virus-derived peptides containing the HLA A2.1 binding motif. J Clin Invest 1995; 95:521-530.
96. Tsuchida T, Parker KC, Turner RV et al. Autoreactive CD8+ T-cell responses to human myelin protein-derived peptides. Proc Natl Acad Sci USA 1994; 91:10859-10863.
97. Panina-Bordignon P, Lang R, van Endert PM et al. Cytotoxic T cells specific for glutamic acid decarboxylase in autoimmune diabetes. J Exp Med 1995; 181:1923-1927.
98. Rock KL, Gamble S, Rothstein L. Presentation of exogenous antigen with class I major histocompatibility complex molecules. Science 1990; 249:918-921.
99. Rock KL, Rothstein L, Gamble S et al. Characterization of antigen-presenting cells that present exogenous antigens in association with class I MHC molecules. J Immunol 1993; 150:438-446.
100. Shen Z, Reznikoff G, Dranoff G et al. Cloned dendritic cells can present exogenous antigens on both MHC class I and class II molecules. J Immunol 1997; 158:2723-2730.
101. Sigal LJ, Crotty S, Andino R et al. Cytotoxic T-cell immunity to virus-infected nonhaematopoietic cells requires presentation of exogenous antigen. Nature 1999; 398:77-80.
102. Kurts C, Kosaka H, Carbone FR et al. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8(+) T cells. J Exp Med 1997; 186:239-245.
103. Kurts C, Carbone FR, Barnden M et al. CD4+ T cell help impairs CD8+ T cell deletion induced by cross- presentation of self-antigens and favors autoimmunity. J Exp Med 1997; 186:2057-2062.
104. Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. Nature 1998; 392:86-89.
105. Kovacsovics-Bankowski M, Clark K, Benacerraf B et al. Efficient major histocompatibility complex class I presentation of exogenous antigen upon phagocytosis by macrophages. Proc Natl Acad Sci USA 1993; 90:4942-4946.
106. Ke Y, Kapp JA. Exogenous antigens gain access to the major histocompatibility complex class I processing pathway in B cells by receptor-mediated uptake. J Exp Med 1996; 184:1179-1184.
107. Norbury CC, Chambers BJ, Prescott AR et al. Constitutive macropinocytosis allows TAP-dependent major histocompatibility complex class I presentation of exogenous soluble antigen by bone marrow-derived dendritic cells. Eur J Immunol 1997; 27:280-288.
108. Kovacsovics-Bankowski M, Rock KL. A phagosome-to-cytosol pathway for exogenous antigens presented on MHC class I molecules. Science 1995; 267:243-246.
109. Reis e Sousa C, Germain RN. Major histocompatibility complex class I presentation of peptides derived from soluble exogenous antigen by a subset of cells engaged in phagocytosis. J Exp Med 1995; 182:841-851.
110. Albert ML, Pearce SF, Francisco LM et al. Immature dendritic cells phagocytose apoptotic cells via alphavbeta5 and CD36, and cross-present antigens to cytotoxic T lymphocytes. J Exp Med 1998; 188:1359-1368.
111. Bellone M, Iezzi G, Rovere P et al. Processing of engulfed apoptotic bodies yields T cell epitopes. J Immunol 1997; 159:5391-5399.
112. Regnault A, Lankar D, Lacabanne V et al. Fcgamma receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. J Exp Med 1999; 189:371-380.
113. Lanzavecchia A. Mechanisms of antigen uptake for presentation. Curr Opin Immunol 1996; 8:348-354.
114. Shimoda S, Van de Water J, Ansari A et al. Identification and precursor frequency analysis of a common T cell epitope motif in mitochondrial autoantigens in primary biliary cirrhosis. J Clin Invest 1998; 102:1831-1840.
115. He XS, Rehermann B, Lopez-Labrador FX et al. Quantitative analysis of hepatitis C virus-specific CD8(+) T cells in peripheral blood and liver using peptide-MHC tetramers. Proc Natl Acad Sci USA 1999; 96:5692-5697.
116. Grabowska AM, Lechner F, Klenerman P et al. Direct ex vivo comparison of the breadth and specificity of the T cells in the liver and peripheral blood of patients with chronic HCV infection. Eur J Immunol 2001; 31:2388-2394.