Title

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Journal

Hepatology Communications, 1(4)

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Publication Date

2017-06-01

DOI

10.1002/hep4.1044

Peer reviewed

Toward Solving the Etiological Mystery of Primary Biliary Cholangitis

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Primary biliary cholangitis (PBC) is considered a model autoimmune disease due to its signature anti-mitochondrial antibody (AMA) autoantibody, female predominance, and relatively specific portal infiltration and cholestasis. The identification and cloning of the major mitochondrial autoantigens recognized by AMA have served as an immunologic platform to identify the earliest events involved in loss of tolerance. Despite the relatively high concordance rate in identical twins, genome-wide association studies have not proven clinically useful and have led to suggestions of epigenetic events. To understand the natural history and etiology of PBC, several murine models have been developed, including spontaneous models, models induced by chemical xenobiotic immunization, and by "designer" mice with altered interferon metabolism. Herein, we describe five such models, including 1) NOD.c3c4 mice, 2) dominant negative form of transforming growth factor receptor type II mice, 3) interleukin-2R $\alpha^{-/-}$ mice, 4) adenylate-uridylate-rich element Del$^{-/-}$ mice, and 5) 2-octynoic acid-conjugated bovine serum albumin immunized mice. Individually there is no perfect murine model, but collectively the models point to loss of tolerance to PDC-E2, the major mitochondrial autoantigen, as the earliest event that occurs before clinical disease is manifest. Although there is no direct association of AMA titer and PBC disease progression, it is noteworthy that the triad of PBC monocytes, biliary apotopes, and AMA leads to an intense proinflammatory cytokine burst. Further, the recurrence of PBC after liver transplantation indicates that, due to major histocompatibility complex restriction, disease activity must include not only adaptive immunity but also innate immune mechanisms. We postulate that successful treatment of PBC may require a personalized approach with therapies designed for different stages of disease. (*Hepatology Communications* 2017;1:275-287)

Introduction

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease characterized by immune-mediated destruction of small and medium-sized intrahepatic bile ducts.[1,2] PBC predominantly affects women in their fifth and sixth decades of life, with a female to male ratio of 10:1. The serologic diagnostic hallmark of PBC is detection of anti-mitochondrial antibodies (AMAs) targeting the 2-oxo acid dehydrogenase complex (2-OADC) enzymes located in the inner lipoyl domain of the mitochondria.[3,4] Typical histologic findings of PBC include dense infiltration of mononuclear cells in the vicinity of small or medium-sized intrahepatic bile ducts, known as chronic nonsuppurative destructive cholangitis.[5,6] Numerous previous studies have suggested that immunologic activity against small biliary epithelial cells (BECs) leads to clinical disease.[7-10] In PBC, as with other autoimmune diseases, both genetic and environmental factors contribute to the development of pathology[11-14]; however, the precise etiology of this disease remains unclear.[15,16]

Abbreviations: 2-OA, 2-octynoic acid; 2-OADC, 2-oxo acid dehydrogenase; AMA, antimitochondrial antibodies; APC, antigen-presenting cell; ARE, adenylate-uridylate-rich element; BECs, biliary epithelial cells; dnTGFβRII, dominant negative form of transforming growth factor β receptor type II; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; LA, lipoic acid; NOD, nonobese diabetic; PBC, primary biliary cholangitis; PDC-E2, E2 subunit of pyruvate dehydrogenase; TGF, transforming growth factor; Th1, T helper 1; UDCA, ursodeoxycholic acid.

Received March 22, 2017; accepted April 16, 2017.

Supported in part by National Institutes of Health grants DK39588, DK090019, and DK067003.

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DOI 10.1002/hep4.1044

Potential conflict of interest: Nothing to report.

While the introduction of ursodeoxycholic acid (UDCA) for the treatment of PBC greatly improved the outcome,[17] nearly 30% of patients treated with UDCA show an incomplete response and disease progression.[18-20] Recently, obeticholic acid, a selective ligand of the farnesoid X receptor, was approved for patients who are refractory to UDCA.[21] However, the efficacy of obeticholic acid is still suboptimal, and additional therapeutic approaches are urgently needed.[7,22-26]

Our laboratory identified mitochondrial autoantigens recognized by AMAs as 2-OADC in 1987.[27] Since then, we have intensively investigated the etiology of PBC, a prototype organ-specific autoimmune disease (Fig. 1). In this review, we assess our results regarding the etiology of PBC.

Definition of AMA Epitopes

AMAs are the most disease-specific autoantibodies in human immunopathology and are detected in 90%-95% of patients with PBC.[28,29] A high titer of autoantibody in the sera of patients with PBC was observed by Mackay more than 60 years ago,[30] and AMA was found to be an effective serologic tool for the diagnosis of PBC.[31] However, the immunodominant epitopes of AMA were not determined until the identification of pyruvate dehydrogenase complex E2 subunit (PDC-E2) as the mitochondrial autoantigen of PBC by complementary DNA cloning.[27] 2-OADC, a family of mitochondrial enzymes located in the inner membrane of mitochondria, are targets of AMA and include PDC-E2, branched chain 2-oxo-acid dehydrogenase complex E2, 2-oxo-glutarate dehydrogenase complex E2, and dihydrolipoamide dehydrogenase binding protein.[32] All these E2 enzymes have a common structure consisting of an N-terminal domain with a single or multiple attachment sites to lysine (^{173}K in mammalian PDC-E2) of lipoic acid (LA). The dominant epitope sites recognized by AMA are in contiguity with the LA attachment site(s) as the lipoyl domains of these target antigens.[33-35] The amino acid residues critical to maintaining the structural integrity of PDC-E2 lipoyl domain have been revealed by site-directed mutagenesis.[36] Furthermore, while AMA is typically not found in other liver diseases or autoimmune diseases, a positive AMA in otherwise healthy individuals indicates a substantial risk of future PBC development.[37-39] These findings suggest that AMAs are not simply serologic markers for diagnosis but are important in the immunopathology of PBC.

Overlapping of T-Cell Epitopes

The histologic signature of PBC includes a dense infiltration of mononuclear cells in the portal tracts near small or medium-sized bile ducts. In the typical pathology of PBC, infiltrated lymphocytes are found adjacent to BECs in damaged bile ducts.[15] Immunohistochemical examination of these lymphocytes reveals a predominance of CD4+ and CD8+ T cells with B and natural killer cells.[40,41] BECs and hepatocytes in the liver of patients with PBC also express large amounts of human leukocyte antigen (HLA) class I and II molecules.[42,43] Therefore, both CD4+ and CD8+ autoreactive T cells play a crucial role in the pathogenesis of PBC.

In the case of CD4+ T cells, Shimoda et al.[44] established HLA DRB4 0101-restricted PDC-E2-specific T-cell clones from the peripheral blood of patients with PBC and mapped immunodominant T-cell epitopes as PDC-E2 peptide 163-176

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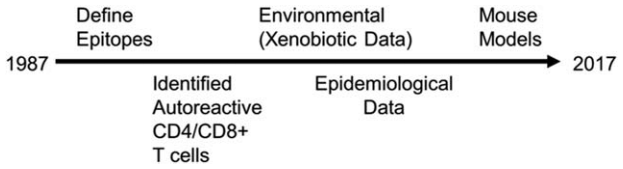

FIG. 1. Toward solving the etiological mystery.

(GDLLAEIETDKATI), which overlapped with the B-cell epitope of human PDC-E2 (Table 1). Importantly, the frequency of PDC-E2-specific CD4+ T cells was 100-fold to 150-fold higher in the liver and hilar lymph nodes than in the peripheral blood.[45] Our laboratory also characterized a major histocompatibility complex class I (HLA-A2)-restricted epitope for CD8+ T cells as PDC-E2 peptide 159-167 (KLSEGDLLA), which again mapped to the same region of the inner lipoyl domain as the autoantigen PDC-E2 (Table 1).[46] The frequency of CD8+ cytotoxic T lymphocytes specific for this epitope was 10-fold higher in the liver than in the blood.[40] Interestingly, cytotoxic T lymphocyte cell lines specific for PDC-E2 can be efficiently generated from peripheral blood mononuclear cells of patients with PBC by using soluble PDC-E2 complexed with anti-PDC-E2 autoantibodies, which results from cross-presentation of the PDC-E2 epitope by antigen-presenting cells (APCs).[46] The finding that autoantigen-immune complexes can cross-present the autoantigen with high efficiency reveals a unique role for anti-PDC-E2 antibodies in the pathogenesis of PBC.

Why Biliary Epithelial Cells? The "ABC" of PBC

We determined that AMA and autoreactive helper and cytotoxic T cells contain a shared peptide sequence of the human PDC-E2 (Table 1). However, PDC-E2 is a ubiquitous protein located in nearly all nucleated cells in the human body. Why do autoreactive T cells specific for PDC-E2 elicit cytotoxicity against only biliary epithelial cells? We should note that PBC recurs even after liver transplantation, indicating that the immunopathologic susceptibility of BECs in PBC is not major histocompatibility complex-specific, but a general feature shared with autologous BECs. To answer this question, we demonstrated that only human intrahepatic BECs could maintain PDC-E2 immunologically intact within apoptotic blebs (apotopes) during apoptosis but not control epithelial cells. This supports data in which AMA-containing sera reacted with PDC-E2 on apoptotic BECs without permeabilization.[47] We then examined the ability of BECs to induce cytokine secretion from mature monocyte-derived macrophages, with and without AMAs and observed intense inflammatory cytokine production in the presence of a unique triad consisting of BEC apotopes, macrophages from patients with PBC, and AMAs.[8] Macrophages from healthy controls did not produce inflammatory cytokines even when cocultured with apoptotic bodies from human intrahepatic BECs and AMAs (Fig. 2). Thus, we propose that A (AMA, apotope, and APC), B (blebs from apoptotic BECs), and C (complex formation and cytokine secretion) constitute the crucial triad in the inflammatory cascade of PBC.

Genetic and Environmental Influences: Xenobiotic Modification of PDC-E2

As with many other autoimmune diseases, genetic factors are known to play a decisive role in conferring susceptibility to PBC. The prevalence of PBC is higher in families with an affected member.[48] High concordance rates among monozygotic twins have also been observed.[49,50] To identify the genetic background and immunologic pathways responsible for PBC development, multiple genome-wide association studies have been conducted in several populations.[51-56] Although differences in several genes were reported, their clinical implications and relevance remain elusive. In fact, in PBC and all other autoimmune diseases, the results of genome-wide association studies have been disappointing, and recent efforts have been directed to both deep sequencing and consideration of epigenetic events.[57-64]

TABLE 1. OVERLAPPING EPITOPES OF HUMAN PDC-E2 RECOGNIZED BY B CELLS, CD4+ T CELLS, AND CD8+ T CELLS

	155 160 165 170 175 180 185
B cell	**KVGEKLSEGDLLAEIETDK*ATIGFEVQEEGY**
CD4+ T cell	KVGEKLSE**GDLLAEIETDK*ATI**GFEVQEEGY
CD8+ T cell	KVGE**KLSEGDLLA**EIETDK*ATIGFEVQEEGY

Bold characters define an epitope on the PDC-E2 recognized by each cell type.
^{173}K*, attachment site of lipoic acid.

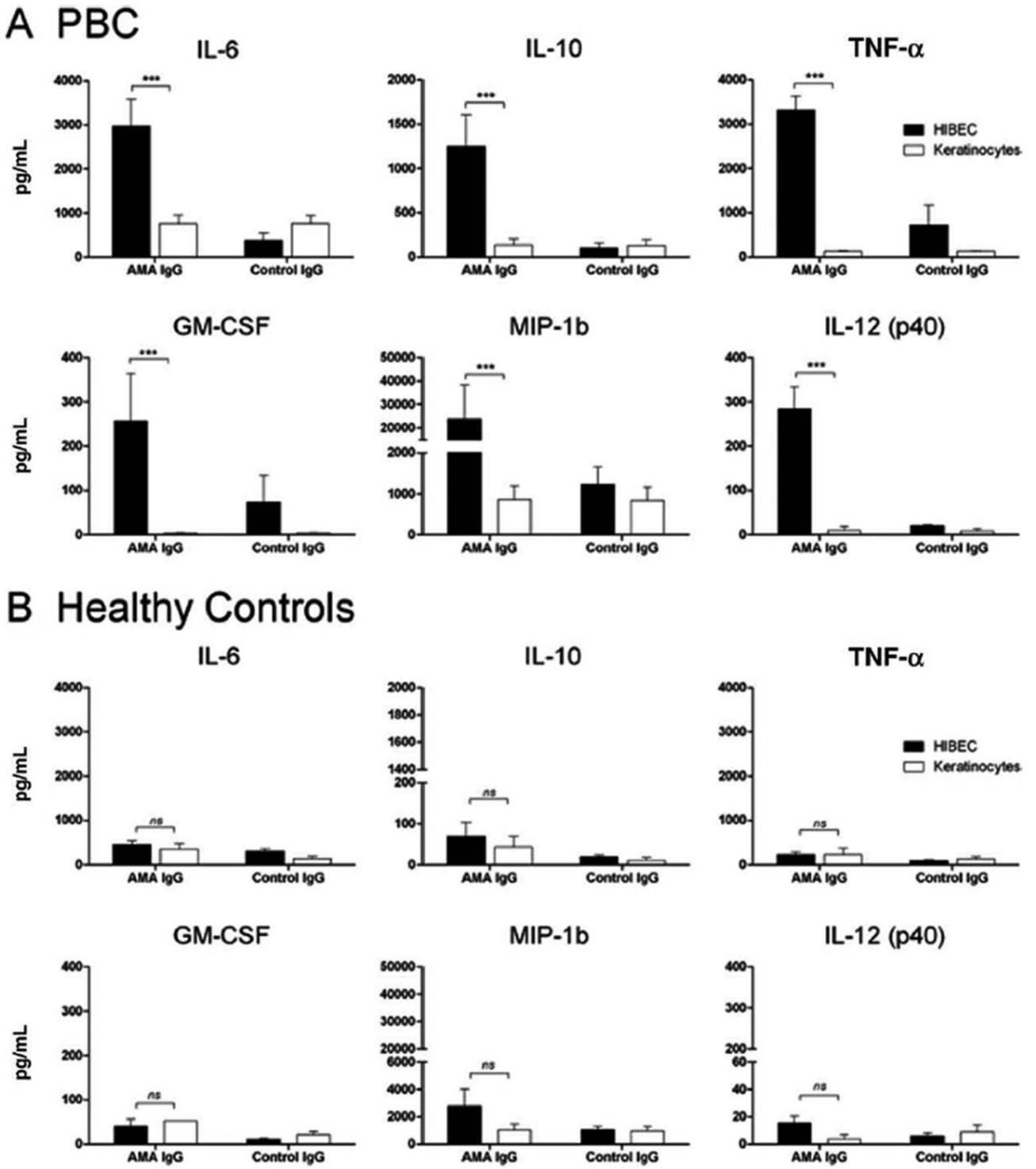

FIG. 2. Macrophages from patients with PBC and from healthy controls. (A) Macrophages from patients with PBC cocultured with apoptotic bodies from HIBECs, and secreted proinflammatory cytokines in the presence of AMAs. (B) Macrophages from healthy controls did not produce inflammatory cytokines, even when cocultured with apoptotic bodies from HIBECs in the presence of AMAs. Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; HIBECs, human intrahepatic BECs; IL-6, interleukin 6; IL-10, interleukin -10; IL12p40; interleukin 12 p40 subunit; MIP-1b, MIP, macrophage inflammatory protein 1b; TNF-α, tumor necrosis factor alpha. Error bar represents ± standard error of the mean. Level of significance is denoted as $^{***}P < 0.001$; two tailed Mann-Whitney test with 95% CI. Reproduced with permission, Lleo et al., Hepatology 2010;52:987-998.[8]

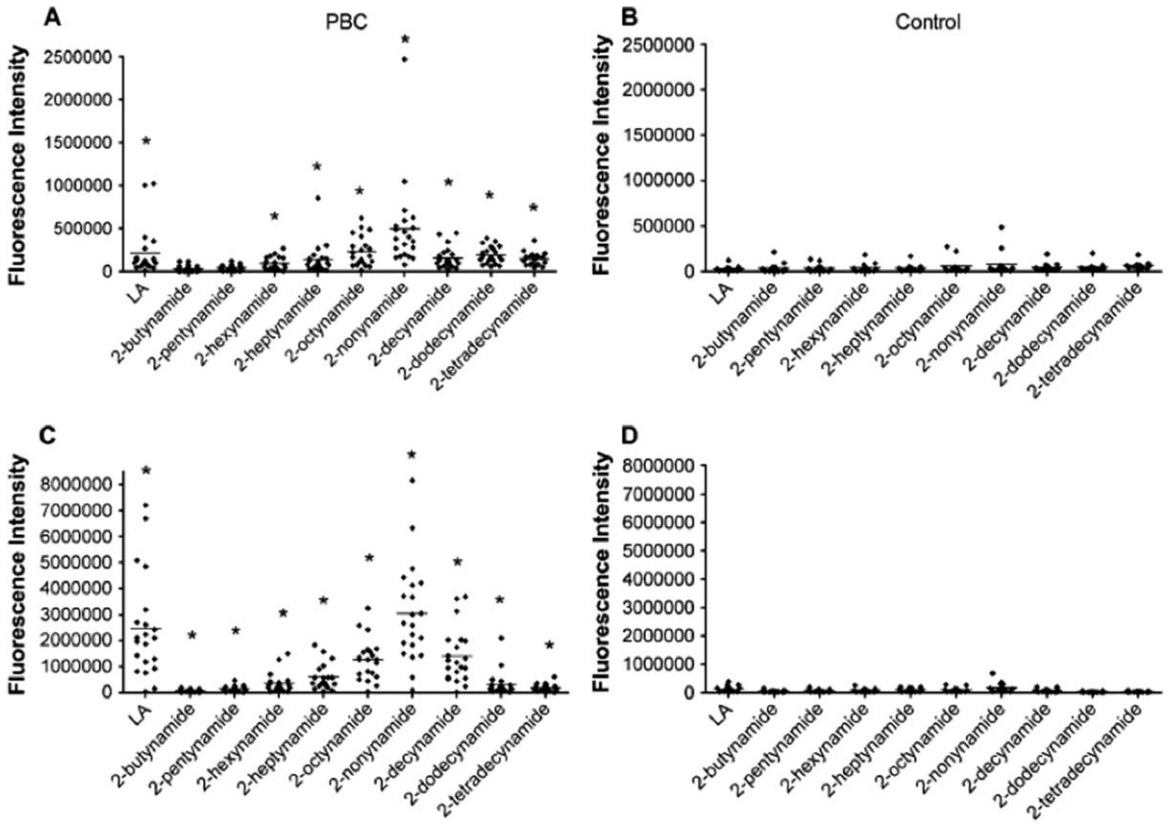

FIG. 3. Reactivity of 2-alkynamide-modified PDC-E2 peptide with (A,C) PBC sera and (B,D) healthy control sera were analyzed by microarray for IgG (A,B) and IgM (C,D) reactivity against PDC-E2 peptide modified with 2-alkynoic acids. Reproduced with permission, Rieger et al., J Autoimmun 2006;27:7-16.[69] Abbreviation: Ig, immunoglobulin. Level of significance is denoted as $^{***}P < 0.05$ (unpaired t-test); horizontal bar indicates mean of pixel intensity.

Despite the importance of genetics, environmental influences should not be underestimated in their ability to trigger or exacerbate PBC. We previously reported a large-scale epidemiological study that evaluated risk factors and comorbidities in PBC, including an interview-based study of 1,032 patients and controls matched for number, sex, age, race, and geographic location. In families with a first-degree relative with PBC, history of urinary tract infections, past cigarette smoking, use of reproductive hormone replacement, and to a minor degree, frequent use of nail polish were associated with increased risk in PBC.[65] Other studies suggested significant clustering of patients with PBC, particularly surrounding toxic sites.[66,67] These epidemiological data, along with the crucial role of AMA in the immunopathology of PBC, prompted us to search for environmental mimotopes in the form of xenobiotics.

We performed a detailed, quantitative, structure-activity relationship analysis on xenobiotics. We found 107 potential xenobiotic mimics that coupled to the lysine residue of the immunodominant 15-amino acid peptide of the PDC-E2 inner lipoyl domain. PBC sera were more reactive with a number of xenobiotic-modified PDC-E2 peptides than with the native lipoylated peptide. Among them, 2-octynamide, the conjugate derived from 2-octynoic acid (2-OA) present in cosmetics, lipsticks, and some chewing gums, was unique in both its quantitative structure-activity relationship analysis and reactivity.[68] We evaluated the chemical structure that leads to enhanced AMA recognition and found that 2-nonyamide provided an optimal chemical structure of the xenobiotic-modified epitope recognized by AMA-positive PBC sera (Fig. 3).[69] Indeed, significant molecular mimicry between lipoamide and 2-nonynamide was demonstrated (Fig. 4). These findings illustrate that xenobiotic modification of PDC-E2 with chemicals abundant in daily life plays a role in generating immunogenic neoantigens and breaking tolerance in PBC.

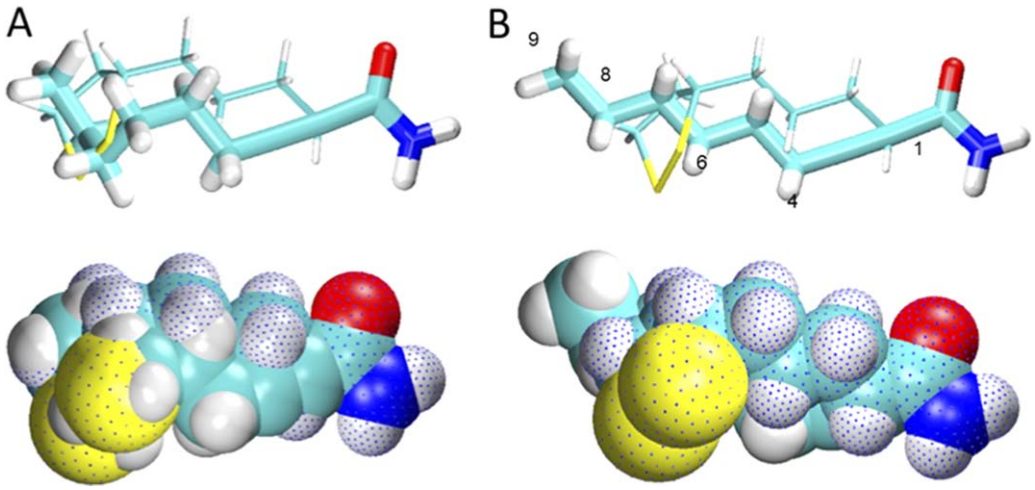

FIG. 4. Molecular mimicry between lipoamide and 2-nonynamide. Superimposed models of lipoamide (dotted) and 2-nonynamide in space-filled and bond representations with 2-nonynamide in either (A) "corkscrew" or (B) straight chain conformation. Reproduced with permission, Rieger et al., J Autoimmun 2006; 27:7-16.[69]

Efforts to Establish Mouse Models of PBC

In addition to *in vitro* studies, mouse models are important for understanding the etiology and natural history of PBC. Patients newly diagnosed with PBC have often past their initial phase. Animal models that reflect many important aspects of the disease are therefore needed to explore the initiating events and interactions between genetic and environmental factors. The animal model should have the same physiological mechanisms observed in human PBC, such as female predominance; chronic cholestasis; AMA production; histologic features, including lymphocyte infiltration into the liver; and bile duct involvement.

In particular, recognition of a strong gender (female) is essential to understand PBC. Sex hormones, X-linked genes, and sex-specific microbiota may contribute to the immune difference between male and female individuals.[70-73] However, the physiological mechanisms accounting for the strong female predominance in PBC remain unclear.[74] PBC risk factors may function synergistically in accelerating the loss of tolerance. One theory proposes that haploinsufficiency for specific X-linked genes leads to female susceptibility to PBC and that enhanced monosomy X in the peripheral lymphocytes of affected women induce PBC.[75,76] Data from another study suggest a potential association of PBC and the loss of the Y chromosome.[77] Epigenetic analysis revealed a significant difference in the X chromosome DNA methylation profile of CD4+ T, CD8+ T, and CD14+ cells in patients with PBC, in particular with an aberrant demethylation on the CXCR3 promoter.[78] However, how these genetic and environmental factors interact with the immune system to elicit autoimmunity in PBC remains enigmatic.

To date, we have established several murine models that develop autoimmune cholangitis resembling PBC in a spontaneous or xenobiotically induced manner (Table 2). These mice share some of the important clinical phenotypes of PBC.[79] There are three mice models that spontaneously develop autoimmune cholangitis: nonobese diabetic (NOD).c3c4 mice, dominant negative form of transforming growth factor (TGF)-β receptor type II (dnTGFβRII) mice, and interleukin (IL)-2R $\alpha^{-/-}$ mice. The NOD.c3c4 mice have multiple B6- and B10-derived insulin-dependent diabetes-resistant alleles on chromosomes 3 and 4, respectively. These mice are protected from autoimmune diabetes but spontaneously develop lymphocytic peribiliary infiltrates and AMA positivity.[80,81] Notably, AMAs were detected in female mice, indicating female predominance as in human PBC. However, pathologic examination of the liver revealed biliary polycystic diseases in both the intrahepatic and extrahepatic biliary ducts and little evidence of chronic nonsuppurative destructive cholangitis. The dnTGFβRII mice also mimicked phenotypes of human PBC.[82]

TABLE 2. CHARACTERISTICS OF PBC MOUSE MODELS

	Spontaneous Model				Induced Model
	NOD.c3c4	DnTGFβRII	IL-2R$\alpha^{-/-}$	ARE Del$^{-/-}$	2-OA-BSA Immunized
Female dominance	Yes	No	No	Yes	No
Cholestasis	-	+	-	+	+
AMA seropositivity	50-60%	100%	100%	100%	100%
Portal inflammation	+++	+++	+++	Yes	+
Granulomas	+	−	−	+	+
Other features	Biliary polycystic lesions	Moderate colitis	Severe anemia, inflammatory bowel diseases, and short life span		Peritonitis

Abbreviations: BSA, bovine serum albumin; −, not detected; +, present; +++, strongly present.

These mice are transgenic for the expression of a dominantly negative form of TGF-β receptor type II directed by the CD4 promoter. DnTGFβRII mice spontaneously produce AMAs directed to the same mitochondrial autoantigens as human PBC. Lymphocytic liver infiltration with periportal inflammation is analogous to the histologic profile of human PBC. The complexity of the IL-12/IL-23 cytokine milieu in autoimmunity in dnTGFβRII mice was examined by generating a series of cytokine knockouts: interferon (IFN)$\gamma^{-/-}$, IL-12p35$^{-/-}$, IL-12/IL-23p40$^{-/-}$, IL-23p19$^{-/-}$, and IL-17A$^{-/-}$ dnTGFβRII mice. Collectively, our data indicated that the IL-12/T helper type 1 (Th1) pathway is essential for biliary disease pathogenesis, and IFN-γ production is significant for triggering Th1 cell responses in this model.[83-86]

The third spontaneous mouse model is the IL-2R $\alpha^{-/-}$ mice, which lack the IL-2R cytokine crucial for differentiation of regulatory T cells and their eventual reduction.[87] These mice develop portal inflammation, biliary ductular damage, and a Th1 cytokine bias, resembling human PBC. In addition, AMAs are targeted to the inner lipoyl domain of PDC-E2. However, female predominance was not observed.

We have also examined possible environmental triggers of autoimmune cholangitis in mice, particularly chemical xenobiotics. We immunized C57BL/6 mice with 2-OA, which was suggested as a candidate xenobiotic present in the environment in our previous study,[68] coupled to bovine serum albumin. We found that anti-PDC-E2 antibodies were present in the serum as early as 4 weeks after immunization (Fig. 5), indicating loss of tolerance to PDC-E2 with xenobiotic immunization. In addition, these mice demonstrated portal infiltration of CD4+ and CD8+ T cells, granulomas, and elevated tumor necrosis factor-α and IFN-γ expression levels.[88] Using several unique gene-deleted mice immunized with 2-OA-bovine serum albumin,[89] we also found that both IL-12/Th1 and IL-23/Th17 were involved in autoimmune cholangitis. The IL-12/Th1 signaling pathway elicited the pathology, while deletion of IFN-γ prevented autoimmune cholangitis (Fig. 6).

Adenylate-Uridylate-Rich Element Del$^{-/-}$ Mice as a Novel PBC Model

We are currently focusing on IFN-γ using a "designer" mouse with dysregulation of IFN-γ, in which the adenylate-uridylate-rich element (ARE) of the IFN-γ 3′-untranslated region is deleted and IFN-γ is constitutively produced.[90] Through various assays, we found that IFN-γ is crucial to the pathogenesis of autoimmune cholangitis in this model.[89,91] We should note that activation of naïve CD4 T cells from healthy women produces higher levels of IFN-γ and lower levels of IL-17 than in healthy men.[92] Increased IFN-γ levels have also been observed in patients with autoimmune diseases.[70,93]

ARE Del$^{-/-}$ mice spontaneously developed many manifestations similar to human PBC, including nonsuppurative destructive cholangitis, AMA production, and elevated serum total bile acid levels.[94] These features were also found predominantly in female mice. In male ARE Del$^{-/-}$ mice, portal inflammation was rarely observed and serum titers of AMA were elevated but not significantly compared to wild-type mice (Fig. 7). Total bile acid levels were comparable. In addition, gene expression analysis revealed that up-regulated genes in female ARE Del$^{-/-}$ mice specifically overlapped with the gene expression signature of BECs in human PBC. Therefore, female ARE Del$^{-/-}$ mice closely mimic human PBC.[95]

Female predominance occurs in ARE Del$^{-/-}$ mice likely because female hormones and genetics cause immune cells in female mice to favor production of

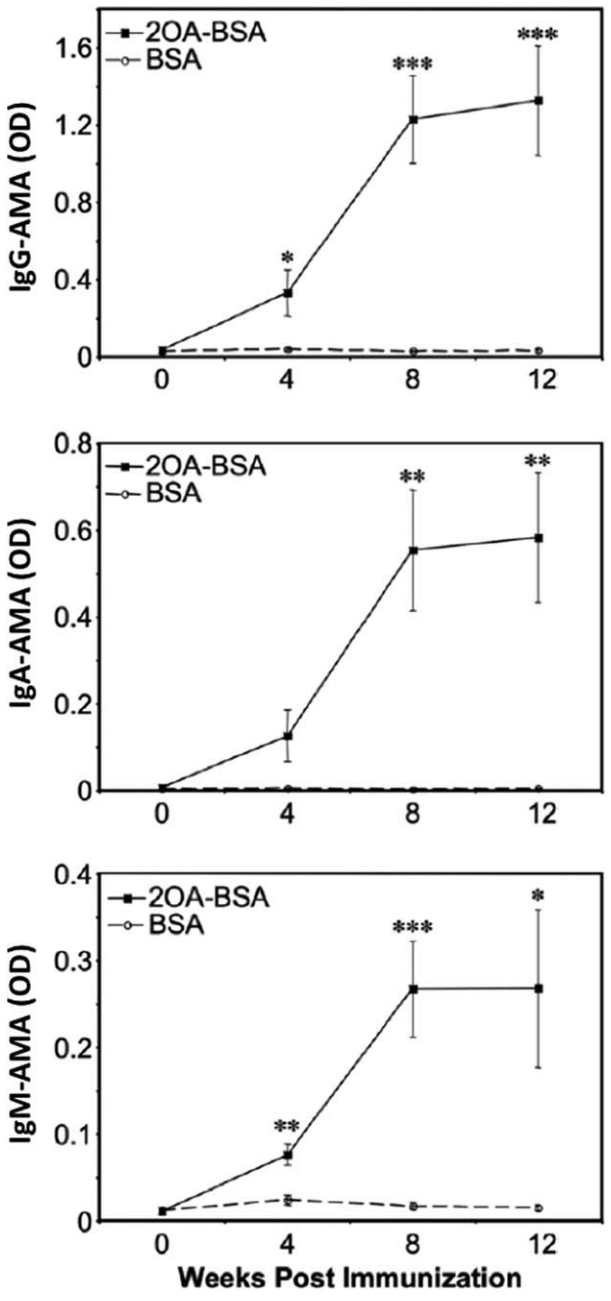

FIG. 5. Detection and quantification of anti-PDC-E2 antibody in sera of 2-OA-BSA-immunized mice by using enzyme-linked immunosorbent assay at 2-week intervals after immunization. A significant increase in OD was observed after immunization with xenobiotic modification compared to control. Reproduced with permission, Wakabayashi et al., Hepatology 48:531-540.[88] Abbreviation: BSA, bovine serum albumin; Ig, immunoglobulin; OD, optical density. Level of significance denoted as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Error bar represents ± standard deviation.

additional IFN-γ-producing cells. In contrast, male mice may be protected by androgens, which favor up-regulation of regulatory cells and down-regulation of IFN-γ-producing cells. Female hormones activate T lymphocytes to express higher levels of IFN-γ in female mice in this mouse model. Although numerous murine spontaneous and induced models have been reported as PBC mouse models,[79,85] no single model exhibits female dominance as observed in ARE Del$^{-/-}$ mice.

ARE Del$^{-/-}$ mice also provide clues regarding the immunopathology of PBC. IFN-γ may play a pathogenic role in BECs during the initiation stage of PBC, and changes in expression levels of IFN-γ are critical to the development of PBC in susceptible individuals. Furthermore, we demonstrated that transfer of CD4 T cells from ARE Del$^{-/-}$ mice to B5/Rag1$^{-/-}$ mice (an immune-deficient strain producing no mature T or B cells) induced moderate portal and parenchymal inflammation, indicating that CD4+ T cells contribute to the induction of cholangitis.

Using ARE Del$^{-/-}$ mice, we learned the following: First, this is a female-predominant model for PBC that allows for intensive investigation of the female predominance of the disease. We comprehensively analyzed sexual dimorphic physiological systems, including hormones, immune differences, and the microbiome. We can manipulate estrogen and estrogen receptors on cells to determine their effect on the pathology. We also examined the female microbiome and found that it was similar to the male microbiome; we can raise these mice in a germ-free environment, followed by the introduction of a selected set of bacteria. Second, this model will help determine which gene pathways help or hinder the progress of PBC in both sexes. Third, this model allows us to study the interaction of multiple cellular messengers and their relationships with cellular immune transformation to the autoimmune state. Finally, this model has implications for clinical research. The heterogeneity and the natural history of PBC can be examined from an early asymptomatic stage to a late stage with full liver involvement, and new biomarkers at different stages may be identified. This mouse is an effective tool for assessing the effect of drugs currently used and those under development and for the design and screening of novel more-effective compounds for curing PBC.

Where We Are and Future Directions

Since the cloning and identification of PDC-E2 as the major mitochondrial autoantigen of PBC in 1987, our understanding of the immunologic mechanisms,

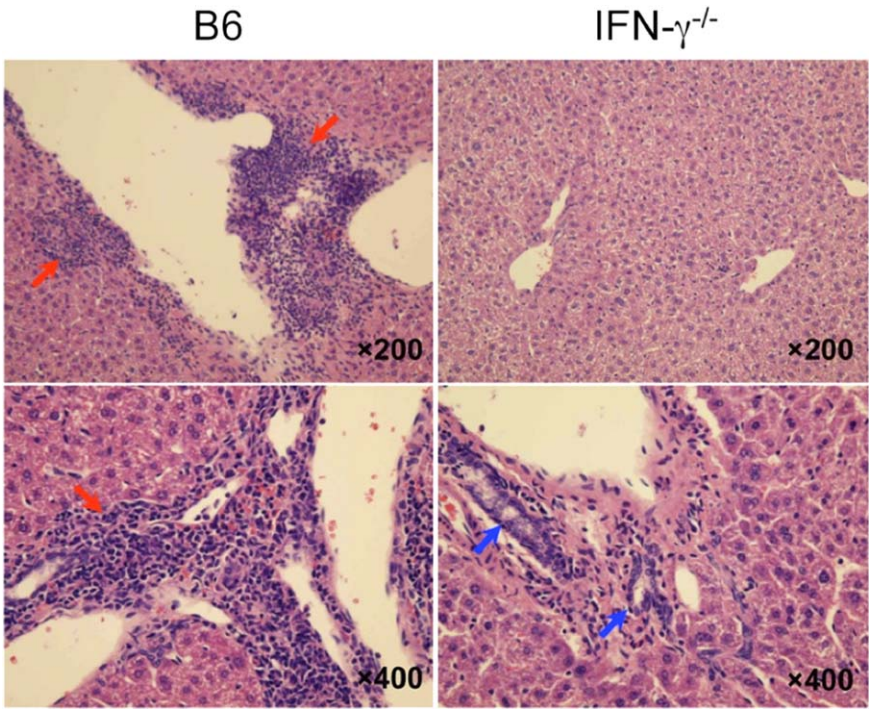

FIG. 6. IFN-γ knockout completely abolished autoimmune cholangitis. Portal inflammatory changes and interlobular bile duct damage (red arrows) were observed in wild-type mice (B6 mice) and normal bile ducts in IFN-$\gamma^{-/-}$ mice (blue arrows). Reproduced with permission Kawata et al., PLoS One 2013; 8:e74225.[89]

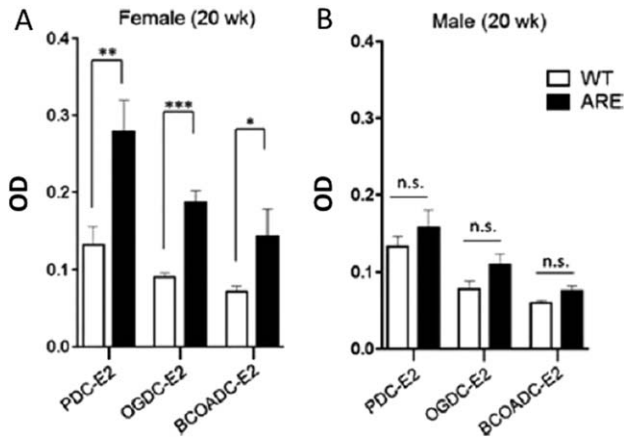

FIG. 7. AMA levels in ARE Del$^{-/-}$ (A) female and (B) male mice at 20 weeks. Abbreviations: BCOADC-E2, E2 component of branched chain 2-oxo-acid dehydrogenase complex; n.s., not significant; OGDC-E2, E2 component of 2-oxo-acid dehydrogenase complex; WT, wild-type. Error bar represents ± standard deviation. All data are representative of at least three independent experiments. Level of significance is denoted as $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (unpaired student t-test). Reproduced with permission, Bae et al., Hepatology 2016;64:1189-1201.[94]

pathologic process, genetics, etiology, and natural history of PBC has significantly increased.[4,7,8,15,16,65,79,91,96-100] The vast amount of information gained from these studies points to the thesis that the loss of tolerance to the PDC-E2 lipoyl domain is a linchpin in the development of the biliary pathology of PBC.

To help understand these events, Fig. 8 illustrates illustrates a hypothetical pathway from tolerance breakdown to biliary pathology in PBC. BECs express PDC-E2 on apotopes in an immunologically intact form during apoptosis. Notably, the capability to do this is observed only in BECs and not in other epithelial cells, possibly explaining the tissue specificity of PBC. Intact lipoylated PDC-E2, presumably after modification with xenobiotics, such as 2-octynamide or 2-nonyamide, from the environment are endocytosed by APCs and presented to CD4+ or CD8+ T cells. Immune complexes with PDC-E2 and anti-PDC-E2 autoantibody cross-present autoantigens in a more efficient manner. Finally, orchestrated immunologic responses against BECs with activated CD4+ and CD8+ T cells, AMA, and immunoglobulin A transcytosis result in subsequent pathology. We

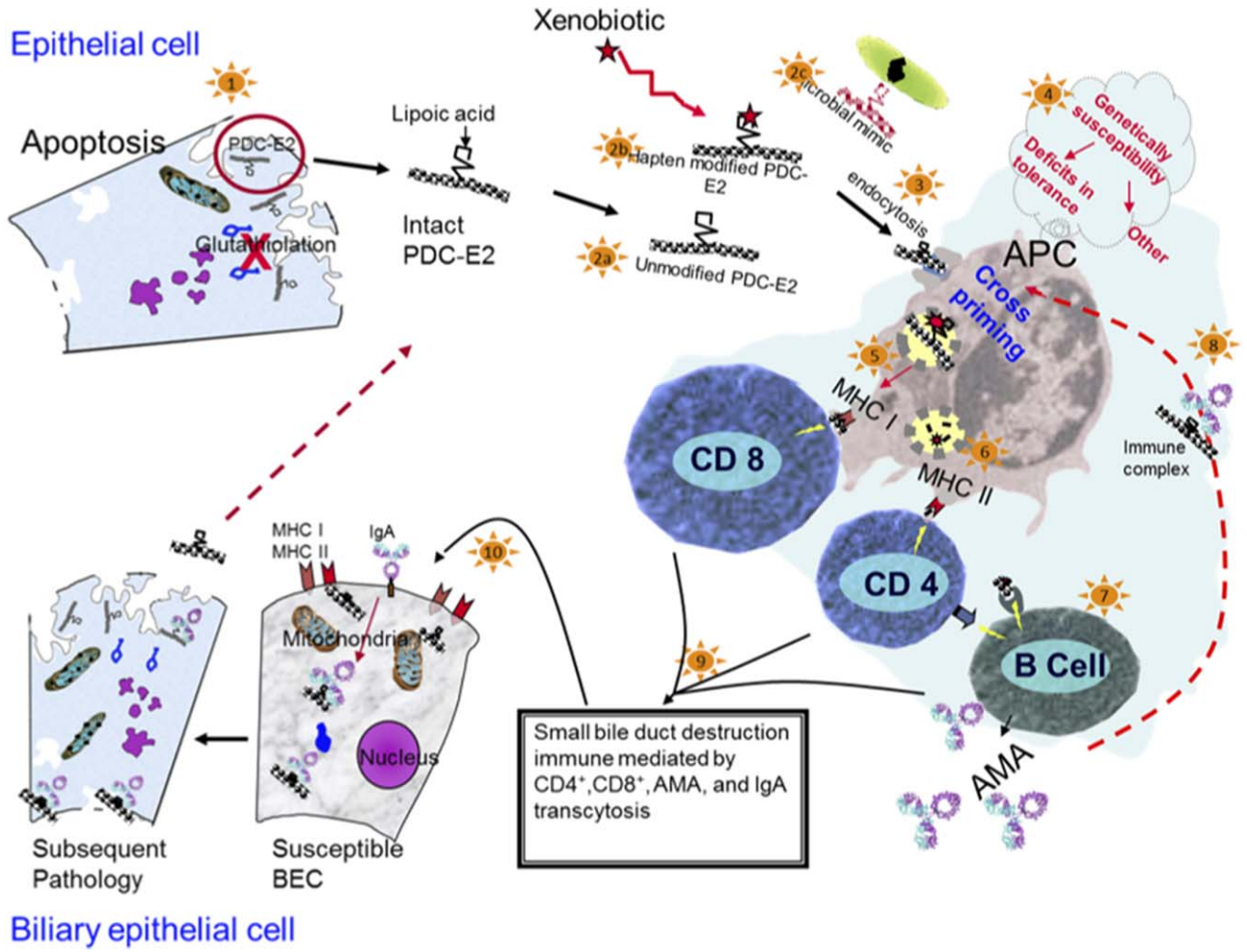

FIG. 8. Tolerance breakdown against PDC-E2 expressed on apotopes through xenobiotic modification, leading to activation of autoreactive T cells and cholangitis in PBC. Abbreviations: Ig, immunoglobulin; MHC, major histocompatibility complex.

currently have ARE Del$^{-/-}$ model mice that mimic human PBC, including female dominance, and will use this model to elucidate the immunopathology of PBC. Expanding our knowledge regarding this pathology from a very early stage of disease will provide a foundation for "curing" PBC.

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