Therapeutic and immunological interventions in primary biliary cholangitis: from mouse models to humans

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Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease that predominantly affects women in their fifth and sixth decades. The diagnostic hallmarks of PBC are detection of anti-mitochondrial antibodies (AMAs) and chronic non-suppurative destructive cholangitis (CNSDC) of small- and medium-sized intrahepatic bile ducts in liver histological examination [1, 2]. A significant amount of data suggests that immunological activity against small biliary epithelial cells (BECs), found histologically as portal inflammation, leads to clinical disease. In PBC, as with other autoimmune diseases, both genetic and environmental factors contribute to the development of pathology [3–8]. The first-line therapy of PBC is ursodeoxycholic acid (UDCA), although obeticholic acid (OCA) has been approved recently for patients with an incomplete response to UDCA [9–11]. Unfortunately, unlike other autoimmune diseases, no successful clinical trials of biologics have been conducted, and the mechanisms of action of UDCA and OCA are not fully understood [12–16].

The clinical phenotype and the natural history of PBC vary between patients and can have other overlapping autoimmune diseases [12, 17–19]. For example, some patients may have mild elevation of liver enzyme levels and remain asymptomatic for life. By contrast, other patients can develop signs of liver failure and rapidly decompensate despite therapy, requiring liver transplantation. A presumption is that these differences are due to genetic and environmental factors, both contributing to the development of PBC to various degrees in each patient [4, 7, 20]. Although multiple genome-wide association studies (GWASs) have been reported differences in several genes [21–24], their clinical implications and relevance remain elusive [25]. In fact, in PBC, in autoimmune diseases, including PBC, the results of GWASs have been disappointing, and efforts have been made recently for both deep sequencing and study of epigenetic events [26–30]. To understand the importance of developing a useful mouse model, other aspects of PBC such as spectra of disease and gender dominance should be considered [18, 31].

Toward solving the etiological mystery

Most autoimmune diseases, including PBC, have a strong sex bias towards women [32, 33]. Male and female immune responses differ and

are affected by sex hormones, X-linked genes, and sex-specific microbiota [34–38]. Despite extensive studies with clinical samples and animal models of PBC, no demonstrated physiological mechanisms account for the strong female predominance in PBC [32, 39–48]. Epidemiological studies on PBC show that frequent exposure to environmental chemicals such as nail polish, chemicals in tobacco smoke, and hormone replacement therapies are significantly associated with an increased risk of PBC [7]. Other PBC risk factors include recurrent urinary tract infections and increased prevalence of reproductive complications [49, 50]. These risk factors may work synergistically in accelerating loss of tolerance. One theory proposes that haploinsufficiency for specific X-linked genes leads to female susceptibility to PBC and suggests that enhanced monosomy X in the peripheral lymphocytes of affected women plays a role in the induction of PBC [51, 52]. However, how these genetic and environmental factors interact with the immune system to elicit autoimmunity in PBC remains unclear [53].

Newly diagnosed PBC patients are often past their initial stages and present with elevated cholestatic enzymes, AMA positivity, and dense aggregation of lymphocytes in the liver. Investigation of these patients for very early events of PBC is therefore inappropriate, and animal models that reflect many of the important aspects of the disease are much needed. The animal model should have the same physiological mechanisms that are observed in human PBC, such as female predominance; chronic cholestasis; AMA production; histological features, including lymphocyte infiltration into the liver; and bile duct immune involvement.

Efforts to establish mouse models of PBC

Several groups including, our own, have established mouse models of PBC in a spontaneous or induced manner (Table I). These mice share some of the important clinical phenotypes of PBC, including portal lymphocytic infiltration, presence of AMA against mitochondrial antigens, and chronic cholestasis.

Spontaneous murine models

The NOD.c3c4 mice, which have multiple B6- and B10-derived insulin-dependent diabetes (*Idd*)-resistant alleles on chromosomes 3 and 4, respectively, of the non-obese diabetic (NOD) mice. NOD.c3c4 mice were demonstrated to be protected from autoimmune diabetes and spontaneously develop lymphocytic peribiliary infiltrates and AMA positivity [54, 55]. AMAs were detectable in 60% (3/5) and 57% (4/7) of mice at 9–10 weeks and 14–20 weeks of age, respectively, and were directed against the inner lipoyl domain of PDC-E2, the same mitochondrial autoantigens as recognized in human PBC [54]. Moreover, it is noteworthy that AMA detection was observed in female mice, indicating female predominance like in human PBC. However, pathological examination of the liver revealed biliary polycystic diseases in both intrabiliary and extrabiliary ducts, and little evidence of CNSDC. The expression levels of anti-PDC-E2 antibodies declined as the mice got older. Chronic biochemical cholestasis was not observed. Nevertheless, NOD.c3c4 mice clearly indicated that genetic manipulation in a susceptible strain could produce AMA with identical antigenicity as human PBC for the first time, supporting the importance of genetic contribution to PBC.

Table I. Characteristics of some PBC mouse models

Clinical and immunological features	Spontaneous model					Induced model 2-OA-BSA immunized
	NOD.c3c4	dnTGFbRII	IL-2Rα$^{-/-}$	Ae2$_{a,b}$$^{-/-}$ mice	ARE Del$^{-/-}$	
Female dominance	Yes	No	No	No	Yes	No
Cholestasis	−	+	−	Yes	+	+
AMA seropositivity	50–60%	100%	100%	30%	100%	100%
Portal inflammation	+++	+++	+++	++	++	+
Granulomas	+	−	−	−	+	+
Other features	Biliary polycystic lesions	Moderate colitis	Severe anemia, inflammatory bowel diseases, and short life span	Osteopetrosis in long bones	Mild lupus-like disease	Peritonitis

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The dominant-negative TGF-β receptor II (dnTGFβRII) mice, studied intensively by our group, also mimicks phenotypes of human PBC [56]. These mice are transgenic for the directed expression of a dominantly negative form of TGF-β receptor type II, under the direction of the CD4 promoter. Thus, TGF-β signaling is not completely abolished and dnTGFβRII mice can survive as do non-transgenic mice. DnTGFβRII mice spontaneously produce AMAs directed to the same mitochondrial autoantigens as in human PBC, namely PDC-E2, BCOADC-E2, and OGDC-E2. By weeks 22–24, 100%, 95%, and 68% of the mice sera reacted with PDC-E2, OGDC, and BCOADC, respectively. Lymphocytic liver infiltration with periportal inflammation is analogous to the histological profile of human PBC (Figure 1). In addition, the serum cytokine profile of affected mice mimics data in human PBC. DnTGFβRII mice, although lacking female predominance, demonstrate the role of TGF-β pathways in the immunopathogenesis of PBC and served as a model for investigating the efficacy of B-cell depletion therapy for PBC [57].

The third spontaneous mouse model is IL-2Rα$^{-/-}$ mice, lacking IL-2R that is crucial for differentiation of regulatory T cells (Tregs) and eventually reduction in Tregs [58]. We reported the case of a 5-year-old boy with IL-2α deficiency who developed liver dysfunction with serological expression of PBC, and documented paucity in the frequency and absolute numbers of circulating CD4+CD25^{high} natural Tregs in patients with PBC and their first-degree relatives [59]. These observations prompted us to examine whether IL-2Rα$^{-/-}$ mice would serve as PBC model mice. The results were striking because the mice developed portal inflammation, biliary ductular damage, and a Th1 cytokine bias in sera, resembling human PBC. In addition, AMAs were detectable in all IL-2Rα$^{-/-}$ mice and targeted to the inner lipoyl domain of PDC-E2. However, female predominance was not observed in the IL-2Rα$^{-/-}$ mice. Furthermore, absence of granulomas, short life span, and presence of clinical manifestations such as anemia and inflammatory bowel diseases made the IL-2Rα$^{-/-}$ mice less attractive as a PBC model mice.

The fourth spontaneous model is the Ae2a,b$^{-/-}$ mice. AE2 is an Cl$^-$/HCO$_3^-$ anion exchanger 2 which is involved in intracellular pH regulation and trans-epithelial acid-base transport. In 2008, Salas *et al.* reported that Ae2a,b$^{-/-}$ mice develop elevated levels of IL-12p70 and IFN-γ and increased numbers of CD8+ T cells. Interestingly, AMA and increased levels of IgM, IgG and alkaline phosphatase were also observed in Ae2a, b$^{-/-}$ mice. Histologically, 30% of Ae2a,b$^{-/-}$ mice showed infiltration of CD4$^+$ and CD8$^+$ T cells in the portal areas and around the damaged bile duct with slight fibrosis around areas of bile duct obstruction [60].

The mechanism of this model has been explained by a change in biliary epithelial cell homeostasis due to AE2 deficiency, which increases intracellular pH and promotes the proliferation, differentiation and activity of lymphocytes, leading to an increase in the targeting sensitivity of CD8$^+$ T cells, leading to biliary pathology. Although Ae2a,b$^{-/-}$ mice develop hepatobiliary and immunological features resembling human PBC, the large variations in liver pathology, lack of female predominance and difficulties in breeding made Ae2a,b$^{-/-}$ mice limit its applications for preclinical studies.

Induced murine models

The three above-mentioned mice spontaneously developed serological and immunopathological features that mimic human PBC after genetic manipulation (Table I), clearly indicating the importance of genetics in the development of the disease. Meanwhile, robust evidence supports the crucial contribution of environmental factors; in this regard, stimulation of genetically susceptible mice with environmental factors would be another attractive approach for breaking immunological tolerance against PDC-E2. To establish these "induced" mice model, we have studied chemical xenobiotics as potential environmental factors, particularly and hypothesized that xenobiotic modification of native PDC-E2
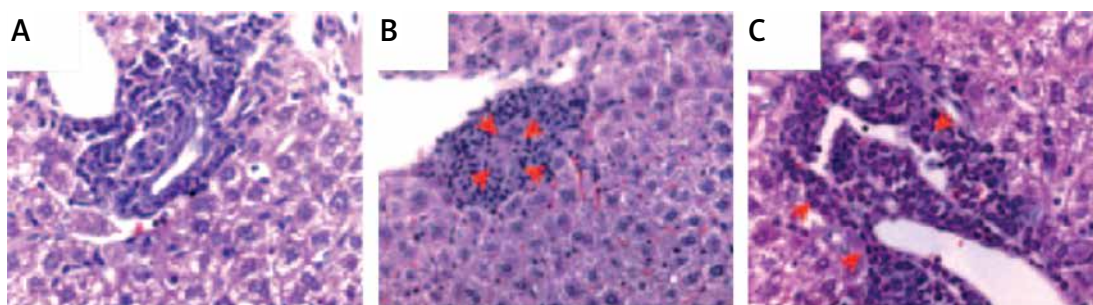

Figure 1. Histological features of the liver in dnTGFβRII mice at 24–28 weeks. Different degrees of lymphocytic infiltrations (red arrows) surrounding the small bile ducts, within the portal tracts (hematoxylin-eosin staining) [56]

could elicit autoimmune response to PDC-E2 and break tolerance.

Based on a detailed, quantitative structure-activity relationship analysis with 107 potential xenobiotic mimics coupled to the lysine residue of the immunodominant 15-amino acid peptide of the PDC-E2 inner lipoyl domain, we found that PBC sera were more reactive to a number of xenobiotics modified PDC-E2 peptide than to the native lipoylated peptide. Among them, 2-Octynoic acid (2-OA) was unique in both its quantitative structure-activity relationship analysis and reactivity [20]. 2-OA is a xenobiotic chemical that is widely used as a cosmetic ingredient and for seasoning foods. Thus, we immunized C57BL/6 mice with 2-OA coupled to bovine serum albumin (BSA; Figure 2) and demonstrated that anti-PDC-E2 antibodies were seropositive as early as 4 weeks after immunization (Figure 3), indicating tolerance breakdown against PDC-E2 with xenobiotic immunization. In addition, these mice have shown portal infiltration of CD4+ and CD8+ T cells and granulomas, and elevation of TNF-α and IFN-γ expression levels [61]. Therefore, 2-OA-BSA-immunized mice provides a suitable model for investigating immunopathogenesis in the early stage of PBC and for testing the therapeutic efficacy of new compounds.

To further investigate potential roles of cytokine pathways, we systemically constructed several unique gene-deleted mice, including mice with IL-12p40, IL-12p35, IFN-γ, IL-23p19, IL-17A, IL-17F, and IL-22 deletions. Then, we immunized these mice with 2-OA-BSA and followed their natural history [62]. The results are summarized as follows: (1) both IL-12/T helper type 1 (Th1) and IL-23/Th17 were involved in autoimmune cholangitis, (2) the IL-12/Th1 signaling pathway elicited pathology, and (3) IFN-γ deletion prevented autoimmune cholangitis (Figure 4). Furthermore, 2-OA-BSA mice were also studied for the potential efficacy of CTLA-4-based therapy on cholangitis by using CTLA4-Ig [63].

In summary, these model mice develop clinical and immunological characteristics as human PBC, including cholestasis, AMA positivity, and portal inflammation. However, female predominance, an important feature of PBC and other autoimmune diseases, was not manifested in most of the mice except for NOD.c3c4 mice. A mouse model with immunological characteristics of PBC and female predominance will more closely reflect the human disease. Therefore, we focused on IFN-γ based on extensive efforts of Dr. Howard Young *et al.* on a interesting IFN-γ designer mouse, described below, and also in part on numerous studies that reported that IFN-γ plays an essential role in the development and severity of female-dominant autoimmune diseases [34].

IFN-γ as a key molecule of PBC

There are several lines of evidence that IFN-γ is involved in the pathogenesis of autoimmune cholangitis: (i) Immunohistochemistry staining in PBC livers demonstrates that, IL-12/Th1 cytokines are dominant in the inflamed portal area with IFN-γ-positive monocular cells surrounding degenerated cholangiocytes in the early stage of disease, whereas the Th1/Th17 balance is skewed toward IL-23/Th17 in advanced stages of PBC [64]. (ii) As described previously, liver histology in a xenobiotic induced model demonstrated that both IL-12/Th1 and IL-23/Th17 are involved in cholangitis but indicated that the IL-12/Th1 signaling pathway elicits pathology. The disease was prevented by IFN-γ deletion (Figure 4) [62, 65]. (iii) Th17 signaling suppressed the accumulation of IFN-γ-producing cells in the liver during the early phase of cholangitis [62], supporting the hypothesis that IFN-γ is a key factor in the induction of biliary autoimmunity. Thus, we emphasize that the pathology of PBC is mediated through IFN-γ-related pathways. For example, one may postulate that
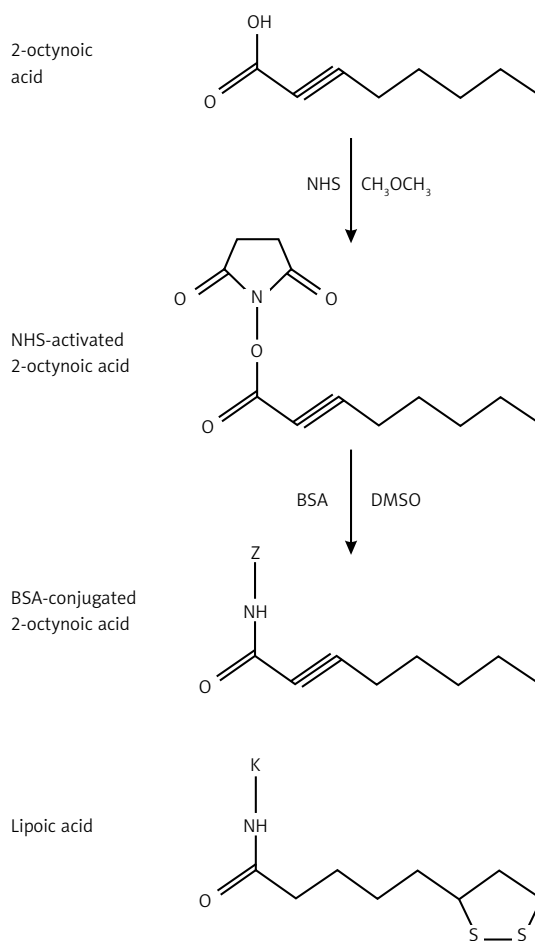

Figure 2. Structures of lipoic acid and 2OA-BSA. 2OA is conjugated with BSSA and used for immunization. "Z" represents any lysine residue of BSA. NHS, *N*-hydroxysuccinimide [61]

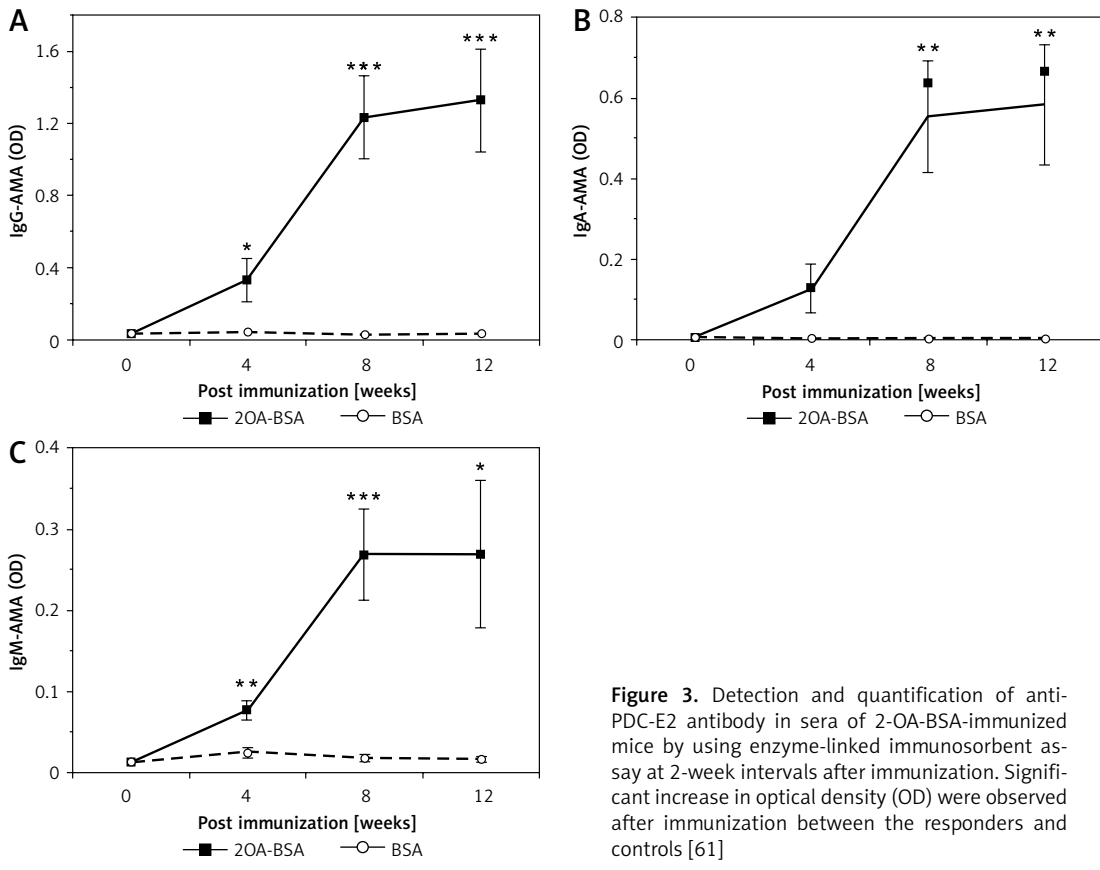Atsushi Tanaka, Patrick S.C. Leung, Howard A. Young, M. Eric Gershwin

Figure 3. 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. Significant increase in optical density (OD) were observed after immunization between the responders and controls [61]
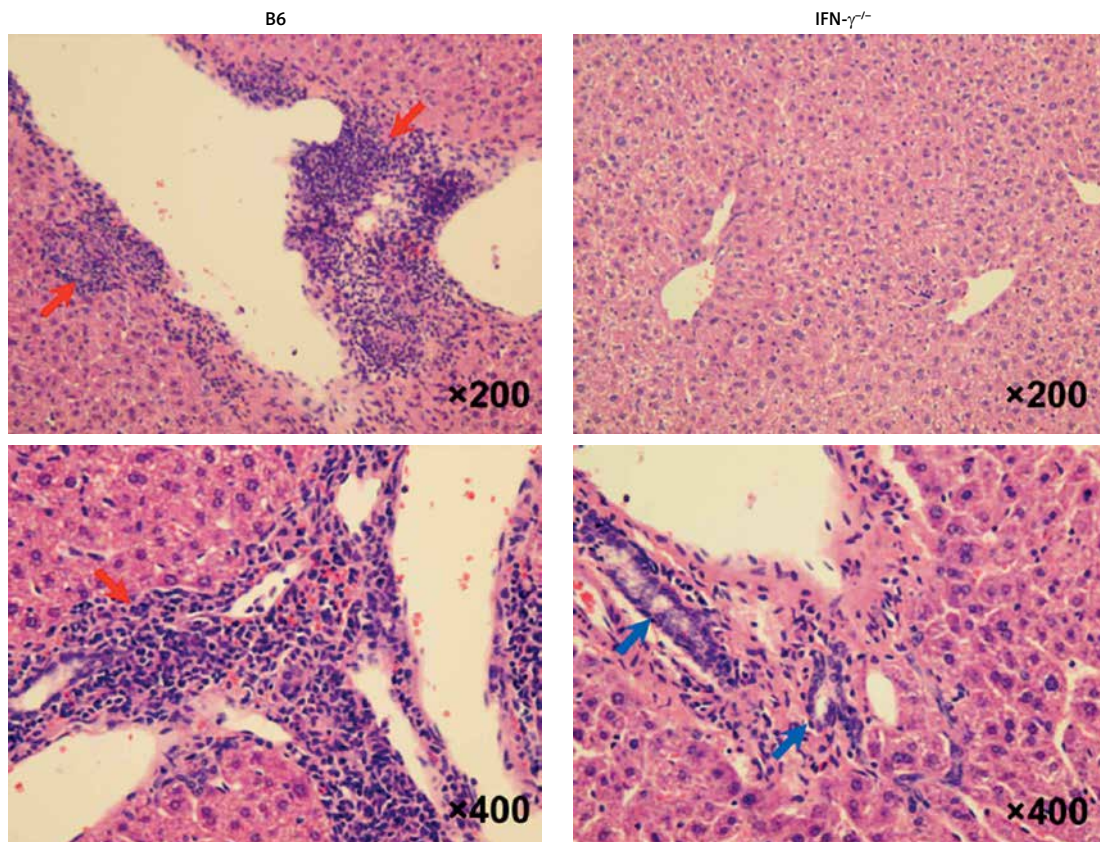

Figure 4. IFN-γ knockout completely abolished autoimmune cholangitis. Portal inflammatory changes with interlobular bile duct damage (red arrow) were observed in wild-type mice (B6 mice) and normal bile ducts in IFN-γ$^{-/-}$ mice (blue arrows) [62]

after a liver environmental insult, local up regulation of IFN-γ in a genetically susceptible host will lead to loss of tolerance. These events may occur serially, each leading to an up regulation of MHC in target tissue, further contributing to an auto-immune response. We should note that activation of naive CD4 T cells from healthy women produces higher levels of IFN-γ and lower levels of IL-17 than those from healthy men [66]. Increased IFN-γ levels have also been demonstrated in patients with autoimmune diseases [34, 67]. However, no optimal model has been developed to determine how IFN-γ and gender together could elicit female-biased cholangitis in patients with PBC. In particular, whether IFN-γ is the first trigger in breaking tolerance needs to be addressed.

An ARE Del$^{-/-}$ mice as a novel PBC model

To solve this problem, we took advantage of a "designer" mouse with dysregulation of IFN-γ, in which adenylate uridine-rich element (ARE) of the IFN-γ 3'-untranslated region was deleted [68]. In these mice, regulation of IFN-γ is disrupted and is constantly produced.

The ARE Del$^{-/-}$ mice have mild autoimmune manifestations similar to lupus early in life. Adult mice reflect many symptoms similar to human PBC, including liver histology, AMA production, and elevation of serum total bile acid (TBA) levels [69]. Furthermore, these features are predominantly found in female mice. In male ARE Del$^{-/-}$ mice, portal inflammation was scarcely found, and serum titers of AMA were elevated but not significantly in comparison with those in wild-type mice (Figure 5). The TBA levels were comparable. Furthermore, gene expression analysis revealed that upregulated genes in female ARE Del$^{-/-}$ mice specifically overlapped with the gene expression signature of BECs in human PBC. Therefore, female ARE Del$^{-/-}$ mice mimic human PBC closely, making ARE Del$^{-/-}$ mice an ideal PBC mouse model.

Female predominance occurs in ARE Del$^{-/-}$ mice probably because female hormones and genetics shape the immune system in female mice to generate more IFN-γ-producing cells. On the other hand, 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 the T lymphocytes to express more IFN-γ in female mice in this mouse model. Although many spontaneous and induced murine models have been reported as PBC mouse models [65], no single model has exhibited female dominance as observed in this ARE Del$^{-/-}$ mice.

IFN-γ may play a pathogenic role in BECs in the initiation stage of PBC, and alteration of expression levels of IFN-γ would be critical for the development of PBC in susceptible individuals. Furthermore, we demonstrated that transfer of CD4 T cells from ARE Del$^{-/-}$ mice to B6/Rag1$^{-/-}$ mice (an immune-deficient strain lacking mature T or B cells) induced moderate portal and parenchymal inflammations, implying that CD4+ T cells mainly contribute to the induction of cholangitis [69].

What then are the therapeutic targets in PBC?

Although the pathogenesis of PBC is still not fully understood, progression of the disease from early to late stage is divided into 3 steps: (i) loss of tolerance against PDC-E2, which leads to inflammation and bile duct damage; (ii) insults to BECs with toxic bile acids due to chronic cholestasis; and (iii) liver fibrosis. Thus, treatment targets are mainly considered as inhibition of autoimmunity or modification of lymphocytes targeted to the liver, detoxification of harmful bile acids, and reversal of the fibrotic process [31]. As anti-fibrotic drugs are expected to be effective for all liver diseases regardless of etiologies, we will focus on the modification of autoimmunity and detoxification of harmful bile acids (Table II).

PBC is an autoimmune-mediated liver disease, and homing of autoantigen specific T lymphocytes
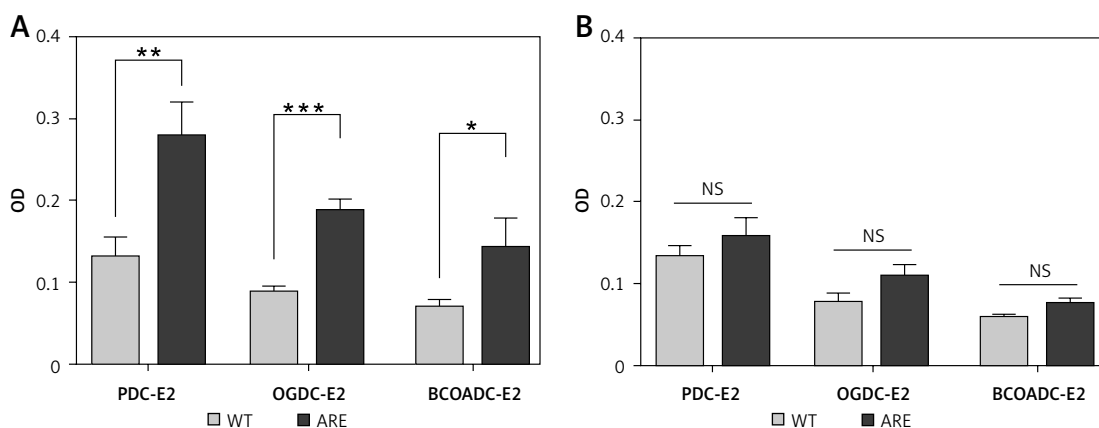

Figure 5. AMA levels in ARE-Del$^{-/-}$ female (**A**) and male (**B**) mice at 20 weeks [69]

WT – wild type.

to the liver plays a crucial role in its pathogenesis. However, clinical trials with immunosuppressive agents have essentially failed, including corticosteroids, methotrexate, azathioprine budenoside and select biologics. Among these trials, B-cell depletion with anti-CD20 had been expected to be effective in PBC [57]. Although non-specific inhibition of activation of T cells was thought to be a candidate for therapeutic trials, overall results from clinical trials with anti-CD40, chimeric CTLA4 (abatacept), and anti-IL12/IL23 (ustekinumab) were disappointing [70].

Biliary epithelial cells damaged by innate and/or acquired immunity impede smooth flow of bile within the bile ducts, which leads to chronic cholestasis. In normal conditions, the "bicarbonate umbrella", a layer of secreted bicarbonate onto biliary epithelial cells, protects them from toxic bile acids. Impairment of this umbrella contributes to development of cholestasis in PBC [71]. Hydrophobic bile acids such as lithocholic acid are highly toxic to biliary epithelial cells and hepatocytes. Therefore, reducing the toxicity of hydrophobic bile acids through decreasing bile acid synthesis and stimulating choleresis would be another possible regimen in cholestatic liver diseases such as PBC [72]. So far, many compounds have been developed for detoxification of hydrophobic bile acids, and mechanisms of these drugs are largely classified into three categories: reduction of intrahepatic *de novo* synthesis of bile acids, choleresis through modification of hepatic bile acid transporters, and blocking bile acid transporters at the ileum for disturbing the reabsorption of bile acids [73].

Modification of autoimmunity

Rituximab that leads to B-cell depletion and ant-fractalkine antibody were expected to be effective and clinical trials are in preparation. Rituximab is anti-CD20 antibody, clinically used for several indications associated with B lymphocytes such as non-Hodgkin lymphoma. B-cell depletion with anti-CD20 antibody ameliorates cholangitis in dnTGF-βRII mice, but preliminary data in clinical trials suggested conflicting results [74, 75]. Recently, several novel anti-CD20 antibodies have been developed and approved for B-cell lymphoma [76] and are expected to be investigated in patients with PBC as well.

Pathogenic T lymphocytes are activated and recruited into the vicinity of the bile ducts from the portal vein. During this homing process of T lymphocytes, chemokines play an important role. Previous studies demonstrated that CX3CL1 (a chemokine called fractalkine) production was augmented with TLR3 by biliary epithelial cells in PBC, and CX3CR1 (the receptor of fractalkine)-positive lymphocytes invade into biliary tract by engaging CX3CL1, thereby causing cholangitis [77]. Anti-fractalkine antibody (E6011), originally developed for rheumatoid arthritis [78] and Crohn's disease, is thus expected to be effective for PBC, and the phase 2 trial of E6011 for PBC with incomplete response to UDCA will be soon launched in 2017.

Obeticholic acid

Alternative treatment options for PBC patients refractory to UDCA have been long awaited. In 2016, the Food and Drug Administration (FDA) officially approved OCA, based on the results of the phase 3 clinical trial of OCA (POISE trial) [10]. OCA is a selective ligand of the farnesoid X receptor (FXR). Bile acid toxicity against biliary epithelial cells and hepatocytes is decreased by FXR signaling through impairment of bile acid synthesis and stimulation of choleresis. The endogenous FXR ligand is chenodeoxycholic acid (CDCA), a primary bile acid. Compared with CDCA, OCA is approximately 100 times more potent than CDCA in activating FXR [79].

In the POISE trial, 217 patients with PBC who showed an inadequate response (serum alkaline phosphatase (ALP) level of > 1.67 × ULN) or an abnormal total bilirubin level (< 2 times × ULN), or intolerance to UDCA were enrolled and received 5–10 mg of OCA, 10 mg of OCA, and placebo for 1 year. The primary end point was ALP level of < 1.67 × ULN with > 15% reduction from the baseline and normal bilirubin level. Of the patients,

Table II. Pathogenic and therapeutic targets of PBC

Mechanisms	Drug name	Drug class	Phase of development
Modification of autoimmunity	Abatacept	Chimeric CTLA4	Phase 4
	Ustekinumab	Anti-IL12 Ab	Phase 2
	Rituximab	Anti-CD20 Ab	Phase 2
	E6011	Anti-fractalkine Ab	Phase 2
Detoxification of toxic bile acids	Obeticholic acid	FXR agonist	Approved and phase 4
	Fenofibrate	Fibrates	Phases 2 and 3
	Bezafibrate	Fibrates	Phase 2
	GSK2330672	Inhibitors of ileal bile acid transporter	Phase 2
	NGM282	FGF19	Phase 2

46–47% of patients achieved the primary end point [10]. Consequently, OCA received accelerated FDA approval on May 27, 2016.

While OCA has become the second-line drug officially approved for PBC and is well accepted, it remains suboptimal for several reasons: First, the response rate was at most 50%, which means that half of the patients did not respond to OCA. Second, pruritus, a frequent symptom of PBC, appeared as an adverse effect in 56–68% of patients treated with OCA. Furthermore, whether the primary end points (ALP level < 1.67 × ULN with > 15% reduction from the baseline and normal bilirubin level) are associated with improvement of long-term outcomes has not been confirmed yet [80]. In this regard, follow-up studies of the POISE trial were required by the FDA, and a phase 3 study is currently ongoing (COBALT, NCT02308111). Finally, OCA treatment should be continued for patients refractory to UDCA; thus, OCA may be prescribed lifelong along with UDCA. Based on the high cost of OCA ($69,350 per year), this unlimited prescription of OCA may cause substantial economic burden on both patients and society, Samur *et al.* recently demonstrated that the price should be decreased to $18,450 per year to make OCA cost-effective [81].

Fibrates

Fibrates (fenofibrate and bezafibrate) were originally labeled for dyslipidemia and used for decreasing serum cholesterol and triglycerides. Fibrates are PPAR-alfa and PXR agonists, resulting in reduction of de novo bile acid synthesis and up regulation of bile acid transporters [82]. Bezafibrate was first reported as biologically effective for PBC patients refractory to UDCA in 1999 [83]; since then, it has been used as an off-label second-line drug in Japan. A large-scale retrospective cohort study in Japan demonstrated that patients with good response to bezafibrate (normalization of ALT) exhibited a comparable prognosis to those treated with UDCA alone and significantly better outcomes than those with poor response to bezafibrate [84]. Recently, a prospective randomized study in Japan revealed that long-term outcomes were not significantly different between the UDCA plus bezafibrate and UDCA only groups, but the sample size in this study might be too small to establish enough statistical power [85]. Another prospective, randomized phase 3 study of bezafibrate for patients with PBC with incomplete responses to UDCA is ongoing in France (NCT01654731). Fenofibrate was reported to decrease serum ALP levels in studies in Japan and China [86, 87], while adjunct use of fenofibrate to UDCA showed no association with decreased serum ALP levels

in a UK cohort [88]. Participants are being recruited for a prospective randomized study in China (NCT02965911). However, these two prospective clinical trials showed improved liver enzyme levels notably at 12 or 24 months as the primary end point. Even after these trials are terminated, whether long-term outcomes are improved with additional treatment with fibrates is still unknown, and follow-up studies of these trials are needed.

Ileal bile acid transporter inhibitors

Bile acids are essential to the human body, and 98% of bile acids excreted into the intestine are reabsorbed from the ileum bile acid transporter (IBAT) and returned to the liver via portal tract, known as enterohepatic circulation. In cholestatic liver disease, ileal bile acid absorption is increased [89]. Therefore, another strategy for reducing bile acids is blocking IBAT. One of these compounds, GSK2330672, did not significantly decrease serum ALP levels in patients with incomplete responses to UDCA but was demonstrated to significantly improve pruritus in comparison with placebo [90]. A prospective, randomized, placebo-controlled phase 2 study of GSK2330672 is launched in 2017 (NCT02966834).

Future direction with the ARE Del$^{-/-}$ mouse model

Although PBC is clearly a heterogeneous disease, the therapeutic approach is similar for all patients and not satisfactory at all even with OCA. This is partly due to the lack of animal models that could be used for investigating the immunopathology of PBC and the efficacy of newly developed compounds. In this regard, ARE Del$^{-/-}$ mice, which closely mimic human PBC, is currently the most attractive model. ARE Del$^{-/-}$ mice provides the following benefits. First, this is a female-predominant model for PBC, which allows for investigation on mechanisms of female predominance in the disease. We can conduct a comprehensive analysis on sexual dimorphic physiological systems, including hormones, immune differences, and microbiome. We can alter estrogen and estrogen receptors on cells to study their effect on disease process and pathology. We can also examine the female microbiome to resemble the male microbiome and raise these mice in a germ-free environment for further systematic introduction of selected bacteria. Second, this model will aid in determining which gene pathways may help or hinder the progress of PBC in both sexes. Third, this model also enables us to examine over time the interaction of multiple cellular messengers and their role in the development of autoimmune state. Finally, this model has

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vast implications for clinical research. It allows for examination of the heterogeneity and the natural history of PBC, from the early, asymptomatic stage to the late stage and the possibility to discover new biomarkers along different stages. This mouse also provides an effective tool for assessing the effect of drugs currently used or under development, and for designing novel, more-effective drugs for the clinical management of PBC.

Conflict of interest

The authors declare no conflict of interest.

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