

# Chapter 14

## Animal Models for Primary Biliary Cirrhosis

Yuki Moritoki and Yoshiyuki Ueno

**Abstract** Recent PBC mouse models underwent extensive gene modifications and immunomodulations to elucidate functions of their products and signaling pathway in autoimmune cholangitis. Expanded hepatic memory CD8<sup>+</sup> T cells and elevated T helper type 1 and proinflammatory cytokines are the commonly observed in these models. B cell depletion studies revealed opposite B cell functions in the time/disease course of dnTGF-βRII mice. NKT cells positively contributed to the development of autoimmune cholangitis in the early phase in both dnTGF-βRII mice and 2-octynoic acid-conjugated BSA (2OA-BSA)-immunized mice models. Further, the depletion of the IL-12p40 subunit, comprising cytokines IL-12 and IL-23, significantly ameliorated PBC-like liver disease. In contrast, IL-12p35 depletion delayed but similarly developed cholangiopathy, but led to liver fibrosis in dnTGF-βRII mice. Inhibitory signaling, derived from exogenous costimulatory immunoreceptor-conjugated immunoglobulin, i.e., cytotoxic T lymphocyte antigen 4 (CTLA-4)-Ig, was quite efficient to improve developed cholangitis in 2OA-BSA-immunized mice. These results highlighted the pivotal roles of T cells, especially CD8<sup>+</sup> T cells, the regulatory and proinflammatory opposite functions of B cells, the indispensable role of IL-12p40 to develop cholangiopathy, and the negative regulatory roles of IL-12p35 and CTLA-4-Ig to suppress liver fibrosis and bile duct damage, respectively. These studies emphasize that failure to maintain self-tolerance in CD8<sup>+</sup> T cells and the expansion of these cells induce and promote

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inflammatory responses in autoimmune cholangitis. Further characterization of these models will support to elucidate the pathogenesis of PBC. This chapter summarizes the recent progress of understandings about the pathogenesis and pathophysiology of autoimmune cholangitis in the context of the PBC mouse model.

**Keywords** CD8<sup>+</sup> T cells • CTLA-4 • Liver fibrosis • Mouse model • Primary biliary cirrhosis

## 14.1 Introduction

Interlobular bile ducts are the primary targets in primary biliary cirrhosis (PBC), of which the term was first introduced more than six decades ago [1]. A certain condition comprising genetics, epigenetics, and environmental factors is thought to affect susceptible individuals, resulting in the development of PBC [2–4]. Compatible for this concept, some of the congenic, transgenic, gene-deleted (knocked-out), and chemical xenobiotic-immunized mice have been reported as faithful animal models with accompanying close similarity of features seen in human PBC. In addition to the enthusiastic researches on human PBC, these animals have advanced our understandings of the pathogenesis of PBC.

Five spontaneous models and one faithfully induced mouse model have been reported for human PBC in a decade [5–11]. These models overcome the limitation to access human liver tissues and have enabled to elucidate the pathogenesis of PBC in detail (i.e., contribution of each immune cell population, cholangiocytes, humoral immune components, cytokines/chemokines, and their receptors). Recent findings of PBC mouse models are summarized and compared to the features of PBC (Table 14.1).

## 14.2 dnTGF- $\beta$ RII Mice

Transforming growth factor (TGF)- $\beta$  mediates pleiotropic functions on various cells and plays a role in the central negative regulation in autoimmunity. TGF- $\beta$  receptor II is essential for signal transduction of TGF- $\beta$  that regulates the activation of lymphocytes. The mouse expressing the dominant-negative form of TGF- $\beta$  receptor II (dnTGF- $\beta$ RII) on CD4<sup>+</sup> and CD8<sup>+</sup> cells demonstrates closely resembling features of human PBC [5, 57]. dnTGF- $\beta$ RII has a truncated intracellular domain of the normal receptor, resulting in the incapacity to transduce signal in both CD4<sup>+</sup> and CD8<sup>+</sup> cells after TGF- $\beta$  ligation. Although anti-mitochondrial antibodies (AMAs) are not sufficient to assure specific models of PBC [58, 59], these mice demonstrate 100 % of serum AMA positivity in a time-dependent manner, and those against PDC-E2, BDOADC-E2, and OGDC-E2 inhibit its enzymatic activity in vitro. In addition, antinuclear antibodies directed against two nuclear proteins, gp210 and

**Table 14.1** Comparison between human PBC and the mouse models

Index	PBC	dnTGF- $\beta$ RII	IL-2Ra <sup>-/-</sup>	NOD.c3c4	Ae2a,b <sup>-/-</sup>	Scurfy	20A-BSA <sub>ip</sub>
<i>Species</i>	Human	Mice	Mice	Mice	Mice	Mice	Mice
<i>Classification</i>	N/A	Spontaneous	Spontaneous	Spontaneous	Spontaneous	Spontaneous	Induced
<i>Background strain</i>	N/A	C57BL/6	C57BL/6	NOD congenic	FVB/N, 129/Sv, Balb/c, SJL	C57BL/6	C57BL/6, NOD congenic
<i>Clinical features</i>							
Female/male ratio	9:1	1:1	1:1	1:1	1:1	(X/X females: fatal)	1:1
Age of onset	40–60 years	?	?	?	?	?	?
<i>Genetic contribution</i>	60 % concordance, identical twin	1	1	1	1	1	1
<i>Environment factor</i>	Yes	Yes	?	?	?	?	?
<i>Serum biochemistry</i>							
Alkaline phosphatase	+++	?	?	?	++	?	?
<i>B cell immunity</i>							
Immunoglobulins	IgM +—+++ , IgG +	IgM +, IgA +++, IgG +	IgA +++, IgG +	IgM +, IgA ++	IgM ++, IgG ++	IgM +++, IgA ++, IgG +	?
AMA	90–95 %	100 %	100 %	50–60 %	40–80 %	100 %	100 %
Dominant AMA target protein	PDC-E2	PDC-E2	PDC-E2	PDC-E2	PDC-E2	PDC-E2	PDC-E2
Dominant epitope	Lipoyl domain	Lipoyl domain	Lipoyl domain	Lipoyl domain	Lipoyl domain	Lipoyl domain	Lipoyl domain
Reactivity to 20A-modified PDC-E2	+++	?	?	?	?	?	?
ANA	40–50 %	100 %	80 %	80–90 %	?	?	?
<i>Liver histology</i>							
Portal lymphoid infiltrates	+++	+++	+++	+++	+—+++	+—+++	+
CD4 cell	+	+	+	++	+	+	+
CD8 cell	++	++	++	+	++	++	++
B cell	+	+	+	—	+	+	+

(continued)

Table 14.1 (continued)

Index	PBC	dnTGF-βRII	IL-2Ra <sup>-/-</sup>	NOD.c3c4	Ae2a,b <sup>-/-</sup>	Scurfy	20A-BSA <sub>ip</sub>
Granuloma	+ + + +	-	-	+	?	-	+
Eosinophilia	+	-	-	+	+	(+)	-
Bile duct destruction	+ + + + +	+ + + +	+ + + +	+	+ + + +	+ + + +	+
AE2 on cholangiocytes	↓	?	?	?	-	?	?
Liver fibrosis	- - - + + +	- (+ + in IL-12p35 <sup>-/-</sup> )	-	- - - ±	- - ±	-	- - ± (+by poly I:C, ++ by α-GalCer.)
<i>Cytokines/chemokines</i>							
IFN-γ	↑	↑	↑	(↑)	↑	↑	↑
TNF-α	↑	↑	↑	?	NC	↑	↑
IL-12	↑	↑	?	?	↑	↑	?
IL-12p40	-	↑	↑	?	?	↑	?
IL-23	↑	?	?	?	?	↑	?
IL-5	↑	?	?	(↑)	?	?	NC
IL-6	↑	↑	↑	?	NC	↑	?
IL-8	↑	?	?	?	NC	?	?
IL-17A	↑	↑	↑	?	?	↑	?
CXCL10 (IP-10)	↑	↑	?	?	?	?	?
Disadvantages	N/A	Moderate colitis	Severe colitis, severe hemolytic anemia	Biliary dilatation	Late onset	Short life span	Peritonitis
References	[2, 3, 12-31]	[5, 32-45]	[6, 45-47]	[7, 45, 48-50]	[8, 45]	[9, 45]	[10, 11, 45, 51-56]

20A 2-Octynoic acid, Ae anion exchanger, α-GalCer α-galactosylceramide, AMA anti-mitochondrial antibody, ANA antinuclear antibody, NC no change

sp100, were detectable in all examined sera out of 21 dnTGF- $\beta$ RII mice at 24 weeks of age [41]. Liver histology demonstrates lymphoid cell infiltrates in portal tracts accompanied with bile duct injury [5]. dnTGF- $\beta$ RII mice show an increase in the number and frequency of the CD44<sup>+</sup> memory phenotype of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, a decreased CD4<sup>+</sup>/CD8<sup>+</sup> T cell ratio in liver lymphoid cell infiltrates, and an increment of B cells and natural killer T (NKT) cells in the liver [5]. In addition, serum levels of proinflammatory cytokines such as TNF- $\alpha$ , IFN- $\gamma$ , IL-12p40, and IL-6 are significantly higher in this strain than in control mice.

### 14.2.1 T Cell Contribution in dnTGF- $\beta$ RII Mice

To examine the contribution of T and B cells to the development of PBC-like disease, dnTGF- $\beta$ RII mice were crossed with recombinase-deficient Rag1<sup>-/-</sup> mice that lack a diversified B and T cell receptor repertoire, leading to the absence of B and T cells. Rag1<sup>-/-</sup>-dnTGF- $\beta$ RII mice do not develop liver pathology, suggesting that a specific condition of T cells with impaired TGF- $\beta$ -signaling in the presence or absence of B cells is involved in the pathogenesis of PBC-like disease of this mouse [5]. Thus, the pathology developed in this strain could be hypothesized due to either a structural or functional change of TGF- $\beta$ RII signaling in T cells or following a breakdown of self-tolerance.

To assess the contribution of T and B cells to the development of PBC-like disease, various series of adoptive transfer studies were performed: transferring splenic CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells derived from dnTGF- $\beta$ RII mice into Rag1<sup>-/-</sup> recipients [33, 34]. Whole splenocytes in dnTGF- $\beta$ RII mice were sufficient to develop features of liver disease similar to human PBC in Rag1<sup>-/-</sup> recipients, suggesting that the loss of self-tolerance in splenic T and B cells is sufficient to induce cholangitis, and specific abnormality in the biliary targets was dispensable for the onset of the disease, compatible to the “innocent victim” concept of cholangiocytes in human PBC [15, 60]. More importantly, adoptive transfer of CD8<sup>+</sup> but not CD4<sup>+</sup> T cells into Rag1<sup>-/-</sup> mice led to cholangiopathy quite similar to PBC livers, emphasizing a pivotal role of CD8<sup>+</sup> T cells in the pathogenesis of both human and murine PBCs [18, 33, 61]. In contrast, Rag-1<sup>-/-</sup> recipients of CD4<sup>+</sup> T cells of dnTGF- $\beta$ RII mice predominantly developed inflammatory bowel disease associated with higher levels of serum interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$ . These data clarified that CD8<sup>+</sup> T cells are the primary contributors for bile duct destruction in this model [33]. Of note, breakdown of T cell self-tolerance to liver autoantigens was indispensable to develop cholangiopathy in dnTGF- $\beta$ RII mice [42]. Ovalbumin (OVA)-specific CD8<sup>+</sup> T cell (OT-I) or OVA-specific CD4<sup>+</sup> T cell (OT-II) mice were utilized to develop OT-I/dnTGF- $\beta$ RII/Rag-1<sup>-/-</sup> and OT-II/dnTGF- $\beta$ RII/Rag-1<sup>-/-</sup> mice in which the entire T cell repertoire was substituted for OVA-specific CD8<sup>+</sup> and CD4<sup>+</sup> T cells and to examine the specificity of autoantigens in dnTGF- $\beta$ RII mice. Adoptive transfer of CD8<sup>+</sup> T cells from dnTGF- $\beta$ RII mice but not from OT-I/dnTGF- $\beta$ RII/Rag-1<sup>-/-</sup> mice or

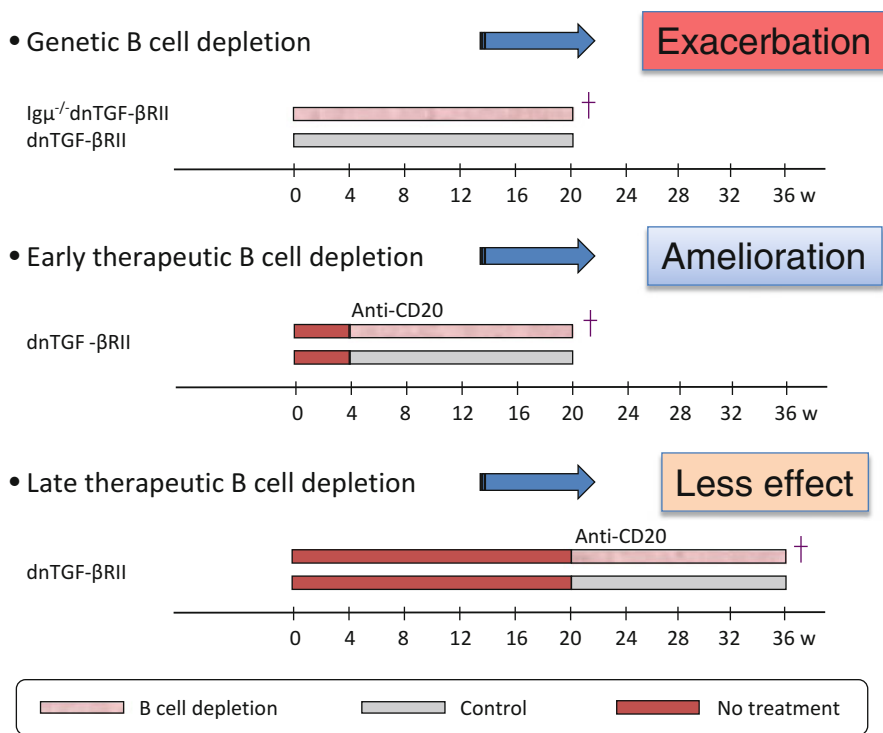
OT-I/Rag-1<sup>-/-</sup> mice induced cholangitis in Rag-1<sup>-/-</sup> recipients, confirming that the cholangiopathy is not secondarily due to nonspecifically activated CD8<sup>+</sup> T cells in dnTGF- $\beta$ RII mice.

### 14.2.2 B Cell Contribution in dnTGF- $\beta$ RII Mice

Despite the nearly universal occurrence of serum AMA as well as accumulation of B cells among liver infiltrates [62], the contribution of B cells to the pathogenesis of human PBC had remained unclear [63]. Similarly, B cell self-tolerance is abrogated in dnTGF- $\beta$ RII mice [5, 41]. Thus, an expectation seemed valid that B cell deficiency should ameliorate liver disease, and dnTGF- $\beta$ RII mice were crossed with B cell deficient mice (Ig $\mu$ <sup>-/-</sup>) and explored for liver inflammation as well as accompanying colitis. Contrarily, genetic B cell deprivation exacerbated both the PBC-like liver disease and colitis [34]. Also, B cell deletion enhanced the expansion of the CD8<sup>+</sup> T cell population compared to CD4<sup>+</sup> T cells and diminished the hepatic regulatory T cell (Treg) frequency in the CD4<sup>+</sup> T cell population. A putative regulatory B cell (Breg) population produces anti-inflammatory cytokine, especially IL-10 [64]; however, dnTGF- $\beta$ RII hepatic B cells indicated comparable levels of IL-10 mRNA expression to those in B6 mice (Moritoki Y, unpublished data), suggesting that Breg population exists outside of the liver and suppresses liver inflammation. Two major B cell pools, the peritoneal cavity and spleen, were chosen for the B cell sources to examine the B cell suppressive function in the CD8<sup>+</sup> T cell adoptive transfer model [33]. B cells from the peritoneal cavity, but not from the spleen of dnTGF- $\beta$ RII mice, were sufficient to regulate CD8<sup>+</sup> T cell induced PBC-like liver disease. These findings revealed the existence of a suppressive/regulatory B cell subset on autoimmune cholangitis in the dnTGF- $\beta$ RII mice and raised a new concept of regulatory B cells in liver inflammation.

However, since the role of B cells is still controversial and disease phase dependent in autoimmunity [65], the regulatory function may not be universal in B cells for liver inflammation in dnTGF- $\beta$ RII mice. Hence, to examine the effects of therapeutic B cell depletion, dnTGF- $\beta$ RII mice were treated by intraperitoneal injection of anti-mouse CD20 antibodies (Abs) every 2 weeks from young (4–6 weeks) and old (20–22 weeks) age and subjected to a comparison with control Ab-treated mice. Sixteen-week anti-CD20 treatment initiated from young age demonstrated a fully depleted serum AMA, a significantly lower incidence of liver inflammation, and a fewer number of activated hepatic CD8<sup>+</sup> T cells in dnTGF- $\beta$ RII mice [35]. However, colitis was significantly exacerbated in anti-CD20-treated mice. In contrast, in the aged mice treated from 20 to 22 weeks of age, anti-CD20 treatment was less effective on either liver or colon inflammation.

To summarize B cell functions in dnTGF- $\beta$ RII mice, in contrast to genetic B cell depletion to exacerbate liver inflammation, anti-CD20 treatment demonstrated therapeutic efficacy to regulate liver inflammation in young but not in old dnTGF- $\beta$ RII mice, suggesting time- and disease phase-dependent B cell function



**Fig. 14.1** Effect of B cell depletion in dnTGF- $\beta$ RII autoimmune cholangitis. Genetic B cell depletion exacerbated autoimmune cholangitis. However, in contrast, early therapeutic B cell depletion using anti-mouse CD20 monoclonal antibodies ameliorated liver pathology. Further, late B cell depletion exhibits no significant therapeutic efficacy in liver inflammation

in autoimmune cholangitis in this strain (Fig. 14.1). Also, B cell functions for liver inflammation observed in dnTGF- $\beta$ RII mice are quite similar to those recently reported in the other autoimmune disease [65]; however, B cells are collectively a suppressor population for colitis regardless of disease phase or age in dnTGF- $\beta$ RII mice. These data suggest that B cells separately contribute to each target organ in autoimmunity. In addition, B cell depletion using anti-CD20 can be a possible therapeutic option for human PBC treatment due to the rare concomitance of inflammatory bowel disease. Several reports for B cell depletion using anti-CD20 rituximab in human PBC patients emerged recently [29, 30, 66, 67]. In an open-labeled study comprised of six patients with PBC, those who were refractory to UDCA treatment and administered rituximab 1,000 mg intravenous infusion on day 1 and 15 demonstrated transient decreases in memory B and T cells, an increase of the CD25<sup>+</sup> regulatory subset, an increase in mRNA levels of FoxP-3 and TGF- $\beta$ , and a decrease in TNF- $\alpha$  in CD4<sup>+</sup> T cells. Serum levels of ALP and plasma levels of AMA, IgM, IgA, and IgG were also transiently reduced around 6 months after treatment [29]. Similarly to this, the other study with 14 PBC patients refractory to UDCA indicated a significant reduction of ALP, AMA, and IgM at 6 months after

rituximab treatment [30]. However, a case report demonstrated rapid progress in a PBC patient accompanied with coincidental gastric diffuse large B cell lymphoma (DLBCL) after eight doses of rituximab as a part of R-CHOP chemotherapy coupled with 40 Gy radiotherapy, although the patient showed biochemical, immunological, and histological improvements after rituximab treatment [67]. The other case report demonstrated persistent liver dysfunction in a PBC patient where concomitant rheumatoid arthritis was improved after treatment with 7.5 mg/week methotrexate and two doses of 1,000 mg rituximab at 2 weeks apart [66]. Taken together, the therapeutic usage of anti-CD20 seems potent to induce biochemical and immunological responses in PBC, but novel biomarkers are feasible to select adequate patients to be treated with rituximab.

### ***14.2.3 Natural Killer T (NKT) Cell Contribution in dnTGF- $\beta$ RII Mice***

Natural killer T (NKT) cells bridge innate and adoptive immunity and exhibit immunoregulatory function in some autoimmune diseases [68, 69]. NKT cells are primed for proinflammatory and anti-inflammatory phenotypes under DC-derived cytokine environment such as IL-12 and IL-10, respectively [70].

The activation of invariant NKT cells is a critical factor to accelerate cholangiopathy in PBC [19, 71]. In dnTGF- $\beta$ RII mice, since the absolute number and activation marker expression are augmented in TGF- $\beta$  signaling-deprived CD1d-restricted NKT cells, CD1d<sup>-/-</sup>-dnTGF- $\beta$ RII mice were generated to deplete CD1d-restricted NKT cells [32]. CD1d<sup>-/-</sup>-dnTGF- $\beta$ RII mice exhibited decreased lymphoid cell infiltrates, milder biliary damage, and higher levels of serum IFN- $\gamma$  compared to those of the control NKT cell sufficient CD1d<sup>+/-</sup>-dnTGF- $\beta$ RII mice. In vivo injection with  $\alpha$ -galactosylceramide ( $\alpha$ -GalCer) induced increases in liver cell infiltrates and serum IFN- $\gamma$  in dnTGF- $\beta$ RII mice. These results suggested CD1d-restricted NKT cells are a primarily proinflammatory subset inducing Th1 cytokine bias in dnTGF- $\beta$ RII mice [32]. Despite the milder liver inflammation, CD1d deletion did not affect the AMA titer in this strain [32].

In the other study, anti-CD40L treatment for 8 weeks demonstrated a reduction in hepatic NKT cells and activated CD8<sup>+</sup> T cells, milder portal inflammation, and diminution of bile duct destruction in dnTGF- $\beta$ RII mice [44].

### ***14.2.4 Cytokine/Chemokine Contribution in dnTGF- $\beta$ RII Mice***

Since dnTGF- $\beta$ RII mice demonstrated an increase in serum levels of proinflammatory cytokines TNF- $\alpha$ , IFN- $\gamma$ , IL-12p40, and IL-6, partly due to the overexpression of microRNA-21 [5, 40], germline deletion of genes encoding



those and related cytokines was extensively performed in dnTGF- $\beta$ RII mice. All four IL-12 family cytokines, IL-12, IL-23, IL-27, and IL-35, have heterodimeric constructs with  $\alpha$  and  $\beta$  chains, and each cytokine shares at least one chain with another member of the family [72]. The IL-12p40 subunit is a common component of both IL-12 and IL-23, which are deprived by the deletion of the IL-12p40 subunit. IL-12p40 deletion demonstrated a marked diminution in the levels of proinflammatory Th1 cytokines including IFN- $\gamma$  in livers of dnTGF- $\beta$ RII mice accompanied with reductions in cellular infiltrates around portal areas and bile duct damage [36]. In contrast, IFN- $\gamma$  depletion indicated no significant effect on the immunopathology of autoimmune cholangitis and colitis in this strain [36] (Yoshida K, unpublished data). These data suggest that IL-12p40 contributing signaling pathways, rather than one of its downstream cytokines IFN- $\gamma$ , is a major determinant of the autoimmune cholangitis that affects dnTGF- $\beta$ RII mice [36].

IL-23 comprised of IL-12p40 and IL-23p19 subunits enhances Th17 polarization in autoimmunity [73]. IL-23p19 depletion led to IL-23 deprivation, which improved colitis but not cholangitis in dnTGF- $\beta$ RII mice [39]. IL-23 supports the differentiation of naïve CD4<sup>+</sup> T cells into highly pathogenic Th17 cells to produce IL-17A; however, IL-17A deletion did not affect either cholangitis or colitis in this strain [39]. IL-6 coupled with TGF- $\beta$  induces Th17 cells from naïve CD4<sup>+</sup> T cells. Genetical IL-6 depletion in dnTGF- $\beta$ RII mice led to a marked improvement in inflammatory bowel disease, but deteriorated biliary pathology [38]. Hepatic levels of IFN- $\gamma$  and TNF- $\alpha$  were significantly elevated in IL-6-deficient dnTGF- $\beta$ RII mice while hepatic IL-12p40 was comparable to that of IL-6-sufficient controls [38]. Taken together, the IL-12/Th1 pathway, but not coupled with IL-23/Th17 axis, is essential and sufficient to develop autoimmune cholangitis in dnTGF- $\beta$ RII mice.

IL-12 heterodimeric cytokine is comprised of IL-12p40 and IL-12p35 subunits. In other words, the IL-12p35 subunit is shared in IL-12 and IL-35 coupled with an IL-12p40 subunit and an Epstein–Barr virus-induced gene 3 (EBI3) glycoprotein, respectively. IL-12p35 deletion in dnTGF- $\beta$ RII mice induced a similar degree of cholangitis with delayed onset, but not colitis, while AMA titer significantly increased at 6 months of age [43]. The deficiency of IL-12 and IL-35 in the presence of IL-23 did not inhibit but delayed the development of biliary disease, suggesting that the pathogenic role of IL-23 can be enhanced in the absence of a presumably immunoregulatory cytokine IL-35 secreted from Treg [72]. Importantly, IL-12p35 deprivation led to Th1 to Th17 shift with a higher production of IL-6 and IL-17 and reduced mRNA expression of both IFN- $\gamma$ /STAT1 signaling and an antifibrotic factor HGF, resulting in the development of liver fibrosis in 7 out of 13 examined mice at 24 weeks of age while none of the 14 control dnTGF- $\beta$ RII mice did so. Of note, liver fibrosis is the essential feature in advanced human PBC livers but has not been previously found in spontaneous PBC mouse models [43]. IL-35 deficiency might hamper the Treg functions including the regulation of both liver pathogenesis and fibrosis. Th17 cells are developed from naïve CD4<sup>+</sup> T cells through IL-6 and TGF- $\beta$  signaling; however, recent studies demonstrate that also

**Table 14.2** Effects on AMA, ANA, cholangitis, and colitis in cytokine-deleted dnTGF- $\beta$ RII mice

Deleted cytokine	AMA	ANA	Cholangitis	Colitis	References
Nil	++	++	+---	+---	[5, 36, 38, 39, 41, 43, 57]
IL-12p40 <sup>-/-</sup>	++	+	--+	(-) <sup>a</sup>	[36, 41, 43], <sup>a</sup> (Yoshida K, unpublished data)
L-12p35 <sup>-/-</sup>	+++	++	+---, delayed	-	[41, 43]
IFN- $\gamma$ <sup>-/-</sup>	++	++	+---	(+---) <sup>a</sup>	[36, 41], <sup>a</sup> (Yoshida K, unpublished data)
IL-6 <sup>-/-</sup>	+	±	++---	-	[38, 41]
IL-23p19 <sup>-/-</sup>	+++	+	+---	±-+	[39, 41]
IL-17 <sup>-/-</sup>	++	+	+---	+---	[39, 41]
TNF- $\alpha$ <sup>-/-</sup>	++	±	(+---) <sup>b</sup>	(+++) <sup>b</sup>	[41], <sup>b</sup> (Yang GX, unpublished data)

*dnTGF- $\beta$ RII* dominant-negative form of transforming growth factor (TGF)-beta receptor type II, *IFN* interferon, *IL* interleukin, *TNF* tumor necrosis factor

Th17 cells can be induced alternatively by way of the effects of IL-6, IL-1 $\beta$ , and IL-23 in the absence of TGF- $\beta$  [74, 75]. These “alternative” Th17 cells are thought to be more pathogenic than the “classical” Th17 cells [76]. Since the TGF- $\beta$  signaling pathway is obstructed in dnTGF- $\beta$ RII T cells, IL-12p35 deprivation is likely to induce more pathogenic Th17 cells.

A genome-wide association study (GWAS) identified that IL-12 $\alpha$  (IL-12p35 subunit) and IL-12 receptor  $\beta$ 2 gene variants were strongly associated with risk for human PBC [20, 77]. Human liver samples obtained from the patients with PBC ( $n = 51$ ) and non-PBC ( $n = 80$ ) control liver diseases were examined immunohistochemically for localized expression of cytokines, their subunits, and corresponding receptors with an extensive panel of antibodies directed to IL-12p70, IL-12p35, IFN- $\gamma$ , IL-12RB2, IL-23p40, IL-23p19, IL-17, and IL-23R [25]. The expression of IL-12RB2 and the presence of IFN- $\gamma$ -positive mononuclear cells were both observed around the damaged interlobular bile ducts in PBC livers [25].

Again, IL-12p40 depletion demonstrated significant amelioration of cholangitis in dnTGF- $\beta$ RII mice, suggesting that IL-12p40 suppression serves as a novel therapeutic option for human PBC. IL-12p40 suppression mediated by monoclonal antibodies or transcription inhibitors has been clinically studied to provide therapeutic benefit in other autoimmune diseases such as psoriasis [78] and Crohn’s disease [79–80].

Detection of antinuclear antibodies (ANAs) was first reported in paternal dnTGF- $\beta$ RII mice [57] and, thence, extensively studied in dnTGF- $\beta$ RII mice with concurrent deletions of IL-12p35, IL-12p40, IL-23p19, IL-17, IL-6, IFN- $\gamma$ , and TNF- $\alpha$  [41]. The changes of autoantibody titers, autoimmune cholangitis and colitis are summarized in Table 14.2. IL-12p40 depletion did not affect serum levels of AMA and anti-SP100, but diminished anti-GP210, suggesting that synthesis and upregulation of IL-12p40 is not essential for the production of AMA in this model [36, 41]. In contrast, AMA and anti-SP100 were significantly

higher, but anti-GP210 was lower in sera of IL-23p19-deleted dnTGF- $\beta$ RII mice [39, 41]. IL-6 depletion reduced both AMA and anti-GP210 without any change of anti-SP100 titers in dnTGF- $\beta$ RII mice [38, 41]. Further, higher levels of AMA and similar levels of anti-GP210 and anti-SP100 were detected in IL-12p35-deficient dnTGF- $\beta$ RII mice [41, 43]. IL-17 depletion did not affect AMA or anti-SP100, but reduced anti-GP210 in dnTGF- $\beta$ RII mice [39, 41]. IFN- $\gamma$  deletion did not change titers of any AMA, anti-GP210, or anti-SP100 antibodies [41]. TNF- $\alpha$  depletion did not affect the degree of bile duct damage (Yang GX, unpublished data) or the titers of AMA or anti-SP100, but reduced anti-GP210 antibodies [41]. Deterioration of colitis led to shorter survival in TNF- $\alpha$ -deprived model mice than that of parental dnTGF- $\beta$ RII mice, resulting in the difficulty of reproduction, and thus the study was discontinued (Yang GX, unpublished data). These results suggest that autoantibody-dependent cytotoxicity is not the major mechanism to induce and promote bile duct damage in the absence of these cytokines.

#### **14.2.5 Therapeutic Immunomodulation in dnTGF- $\beta$ RII Mice**

Other than B cell depletion, novel therapeutic immunomodulation has been performed in dnTGF- $\beta$ RII mice. First,  $\beta$ -glucosylceramide (GC) administration for 18 weeks from 6 weeks of age ameliorates liver inflammation and alleviated cholangitis accompanied with significant reduction of hepatic CD8<sup>+</sup> memory T cells in dnTGF- $\beta$ RII mice [37]. GC is a naturally occurring glycosphingolipid and has been shown to function as a “fine-tuning factor” in several mouse models of immune-mediated disorders [81–84]. AMA is not significantly reduced in GC-treated dnTGF- $\beta$ RII mice.

Second, therapeutic intraperitoneal administration of hamster anti-mouse CD40L antibodies was examined in dnTGF- $\beta$ RII mice. Although anti-CD40L treatment reduced AMA titer and delayed the development of cholangitis at 12 weeks of age (after 8 weeks of treatment), however, the severity of which was not affected at 24 weeks of age (after 20 weeks of treatment) [44]. Changes in serum IgM were not indicated in anti-CD40L-treated mice while CD40L promoter methylation inversely correlates with IgM levels in patients with PBC [85]. LPS and IFN- $\gamma$  stimulated human liver-derived macrophages induced apoptosis of cholangiocytes through a CD154 (CD40L)-dependent manner, which was attenuated by the antagonistic antibodies against CD154 [86]. The efficacy of blocking CD40L has been reported in the other models of systemic and organ-specific autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis [87–90].

### 14.3 IL-2R $\alpha$ <sup>-/-</sup> Mice

Interleukin-2 receptor  $\alpha$  (IL-2R $\alpha$ )<sup>-/-</sup> mice demonstrate low frequency but normal suppressive function of Treg [91], similar to the fact that the lower frequency and absolute number of Treg have been observed in PBC patients [92]. Also, PBC-like liver disease was observed in a child with homozygous IL-2R $\alpha$  deficiency [93]. IL-2R $\alpha$ <sup>-/-</sup> mice with C57BL/6 background show 100 % of AMA positivity against PDC-E2, 80 % of ANA positivity, lymphocyte infiltration around portal tracts, and cholangiocyte damage accompanied with intraepithelial CD8<sup>+</sup> cells in interlobular bile ducts [6]. Serum levels of IgA were markedly elevated in IL-2R $\alpha$ <sup>-/-</sup> mice compared to those of controls [6, 46]. Serum cytokine profiles are quite similar to those of dnTGF- $\beta$ RII mice, showing elevation of serum levels of TNF- $\alpha$ , IFN- $\gamma$ , IL-12p40, IL-6, as well as IL-2. Also, similar to liver immunopathology in dnTGF- $\beta$ RII mice, IL-2R $\alpha$ <sup>-/-</sup> mice indicate an increased number and frequency of the CD44<sup>+</sup> memory phenotype of CD4<sup>+</sup> and CD8<sup>+</sup> T cells and decreased CD4/CD8 ratio of liver cellular infiltrates [6].

#### 14.3.1 T Cell Contribution in IL-2R $\alpha$ <sup>-/-</sup> Mice

The role of the interleukin-2 receptor  $\alpha$  (IL-2R $\alpha$ , CD25) and its relationship with Treg has been well demonstrated in murine and human autoimmune diseases such as inflammatory bowel disease as well as PBC [92–94]. Although IL-2R $\alpha$ <sup>-/-</sup> mice develop autoimmune cholangitis concomitant with intestinal inflammation while colitis rarely coexists in patients with PBC, it was hypothesized that distinct effector mechanisms would work in selective targeting of autoimmune diseases in the colon and bile duct, and the effects of germline depletion of genes encoding CD4, CD8, or TCR (T cell receptor)- $\beta$  were evaluated for the severity of colitis and autoimmune cholangitis in IL-2R $\alpha$ <sup>-/-</sup> mice. IL-2R $\alpha$ <sup>-/-</sup>-CD4<sup>-/-</sup> mice, in which CD8<sup>+</sup> cells are the unique population in TCR<sup>+</sup> cells, demonstrated augmented intrahepatic biliary ductular destruction but diminished colitis. In contrast, IL-2R $\alpha$ <sup>-/-</sup>-CD8<sup>-/-</sup> mice lacked biliary ductular destruction but deteriorated colitis, compared with IL-2R $\alpha$ <sup>-/-</sup> mice [46]. These results are quite similarly observed in adoptive transfer studies in dnTGF- $\beta$ RII mice, where CD8<sup>+</sup> but not CD4<sup>+</sup> T cells are major contributors for biliary damage [33]. Of note, the lack of pathological changes in IL-2R $\alpha$ <sup>-/-</sup> TCR- $\beta$ <sup>-/-</sup> mice revealed the pivotal role of T cells to induce liver inflammation in IL-2R $\alpha$ <sup>-/-</sup> mice, closely similar to the absence of inflammation in Rag-1<sup>-/-</sup>-dnTGF- $\beta$ RII mice [5]. Taken together, these results further argue for the key role of CD8<sup>+</sup> T cells in the pathogenesis of autoimmune cholangitis. In human PBC, precursors of PDC-E2-specific CTL, recognizing amino acids 159–167 of PDC-E2, are tenfold more frequent in the liver than those in the blood, produce IFN- $\gamma$  in response to the PDC-E2, and provide cytotoxicity to PDC-E2 peptide-expressing cells [18].

### 14.3.2 B Cell Contribution in $IL-2R\alpha^{-/-}$ Mice

To date, B cell function has not been thoroughly examined in  $IL-2R\alpha^{-/-}$  mice although which sera demonstrated reactivity to both PDC-E2 and nuclear components [6, 46]. Serum reactivity against PDC-E2 was determined in  $IL-2R\alpha^{-/-}$  mice and CD4/CD8-deleted strains and compared to those of control C57BL/6 J mice. Frequencies of serum AMA positivity were varied: 50 % (4/8) in  $IL-2R\alpha^{-/-}$  mice; 0 % (0/8) in  $IL-2R\alpha^{-/-}$ -CD4<sup>-/-</sup> mice; 75 % (6/8) in  $IL-2R\alpha^{-/-}$ -CD8<sup>-/-</sup> mice. Of note, bile duct damage was apparent in all (8/8) in  $IL-2R\alpha^{-/-}$ -CD4<sup>-/-</sup> mice that were all negative for AMA but had an increase in hepatic B cells compared to control mice. In contrast,  $IL-2R\alpha^{-/-}$ -CD8<sup>-/-</sup> mice demonstrated higher positivity for AMA but lacked any liver inflammation. These results suggest again that CD8<sup>+</sup> T cells provide the primary contribution while AMAs themselves are not sufficient to initiate biliary disease in this strain.

Serum levels of IgA were significantly increased in both  $IL-2R\alpha^{-/-}$  and  $IL-2R\alpha^{-/-}$ -CD8<sup>-/-</sup> mice, possibly corresponding with the degree of colonic inflammation accompanied with an increase in B cell infiltration within the colonic epithelia. IgM levels were reduced in  $IL-2R\alpha^{-/-}$ -CD4<sup>-/-</sup> mice that had minimal colonic inflammation compared to those in control mice although hyper IgM is one of the features in human PBC patients [46].

### 14.3.3 Cytokine/Chemokine Contribution in $IL-2R\alpha^{-/-}$ Mice

In addition to the severe cellular infiltration in the portal tract, both proinflammatory and T helper 1 (Th1) cytokines, TNF- $\alpha$ , IFN- $\gamma$ , IL-12p40, IL-2, and IL-17, were elevated in sera from  $IL-2R\alpha^{-/-}$  and  $IL-2R\alpha^{-/-}$ -CD8<sup>-/-</sup> mice. In contrast,  $IL-2R\alpha^{-/-}$ -CD4<sup>-/-</sup> mice indicated elevation of those cytokines except IL-17 [46]. In  $IL-2R\alpha^{-/-}$  mice, serum levels of IL-17 peaked around 8–13 weeks of age and hepatic CD4<sup>+</sup>-positive cells grasped Th17 bias compared to Th1, which was greater than that in splenocytes [47]. Liver non-parenchymal cells supported splenic CD4<sup>+</sup> T cells to secrete IL-17 with a tenfold increase than that in CD4<sup>+</sup> T cell alone culture [47], suggesting a Th17 induction role in the liver microenvironment in cases of liver autoimmunity and other liver inflammatory diseases.

On the other hand, the CD45RB<sup>hi</sup> transfer model of colitis has demonstrated that IL-17A is a negative regulator of Th1 development [95]. The barrier function of tight junction in mouse cholangiocytes was disrupted by Th1 and proinflammatory cytokines IFN- $\gamma$  and TNF- $\alpha$  [96]. These results suggest that Th17 cell population is not only dispensable to develop autoimmune cholangitis, but also may reduce liver damage by inhibiting Th1 shift in autoimmune cholangitis in this model. In humans, Th17-related cytokines IL-23p19 and IL-17 were significantly elevated in sera of patients with PBC compared to those in healthy and chronic hepatitis B-affected subjects [28]. Also, IL-17-positive mononuclear cells are significantly increased in

portal tracts in diseased human livers affected by PBC, autoimmune hepatitis, nonalcoholic steatohepatitis, and chronic hepatitis C compared to those with normal livers [47]. IL-12RB2 and IL-23R were intensively expressed in cholangiocytes of PBC patients [25]. Th1/Th17 imbalance was slightly prone to Th17 in inflamed PBC livers while the ratio of Th1 and Th17, balanced in the early stage of PBC, grew into a Th17-weighted shift in advanced stage [25]. The expression of IL-6 and IL-1 $\beta$  was enhanced in damaged bile ducts in PBC patients [27], and IL-6, IL-1 $\beta$ , IL-23p19, and IL-23/IL-12p40 mRNAs were upregulated by toll-like receptor (TLR) ligand stimulation in human cholangiocytes where IL-17 induced the production of IL-6, IL-1 $\beta$ , IL-23p19, and chemokines (CXCL1, CXCL2, CXCL3, CXCL6, and CXCL8 and CCL2 and CCL20) [27]. These results suggest that biliary epithelia support Th17 cell development on the site of inflammation and enhance Th17 cell induced biliary damage in the advanced stage of PBC livers.

#### 14.4 NOD.c3c4 and NOD.ABD Mice

A congenic variant of the nonobese diabetic (NOD) strain NOD.c3c4 mice has B6- and B10-derived introgressed intervals on chromosome 3 and 4 and is fully protected from diabetes [7, 48]. NOD.c3c4 mice were reported as the first spontaneous model animal developing AMA and liver pathology similar to some features of PBC [7]. These mice demonstrated serum positivity to PDC-E2 up to 50–60 %, ANA positivity of 80–90 %, and autoimmune biliary disease (ABD) comprised of lymphocyte infiltration around portal tracts with nonsuppurative destructive cholangitis and epithelioid granuloma as seen in human PBC livers [7, 19, 48]. However, the morphological feature of biliary ducts is distinct from that of human PBC because intrahepatic and common bile ducts in this strain develop biliary cysts and ductal dilatation, respectively. The biliary cyst formation was affected by T cells since pathological changes of the liver and the common bile duct were ameliorated with a 50 % reduction of liver disease penetrance by single-dose anti-CD3 treatment in NOD.c3c4 mice around 6–10 weeks of age [7]. Also, impaired Fas expression on biliary epithelial cells contributed to the development of the biliary cysts [97].

NOD.c3c4-*scid* mice, lacking T and B lymphocytes, indicated minimal biliary disease, significantly milder than those of NOD.ABD mice, which was generated from the NOD.c3c4 strain following an intercross with the NOD.B6 *Idd10/18* congenic strain, and demonstrate ABD similar to that of NOD.c3c4 mice [7, 49]. NOD.c3c4 and NOD.ABD mice were both examined for adoptive transfer of autoimmune effector mechanisms [7, 49]. ABD was reproducible in both NOD.c3c4-*scid* and NOD-*scid* mice by transfer of whole splenocytes or splenic CD4<sup>+</sup> T cells from ABD-developed NOD.c3c4 mice [7, 48]. Also, NOD.ABD splenocytes transferred ABD except biliary cyst formation into NOD.c3c4-*scid* mice, but not into NOD-*scid* mice [49], suggesting that the primary lesions mediated by the adaptive immune system are biliary duct damage and nonsuppurative destructive

cholangitis, and the genetic background of NOD.c3c4-*scid* mice is indispensable to induce ABD [49]. NOD.ABD splenic CD8<sup>+</sup> T cell alone transfer was sufficient to develop ABD, and the degree of which was comparable to that by the CD8<sup>+</sup> T cell cotransfer with CD4<sup>+</sup>CD25<sup>-</sup> T cells, but ameliorated with CD4<sup>+</sup>CD25<sup>+</sup> Treg.

Autoantibody production is a distinctive feature between NOD.ABD and NOD.c3c4 mice possibly due to the difference of *Idd.9.3* allele origins derived from NOD and B10 mice in NOD.ABD and NOD.c3c4 mice, respectively. ANA was detectable in sera of NOD.c3c4 mice but, however, rarely (1/30) detected in NOD.ABD mice although all mice indicated liver inflammation. In contrast, the incidence of serum AMA increased with age and liver disease severity in NOD.ABD mice (up to 44 %) and NOD.c3c4 mice (up to 50 %) [49, 50], suggesting AMA occurrence is secondary to the biliary damage in these strains.

Similar to frequent concomitance of sicca syndrome in PBC, salivary gland inflammation coexists in NOD.c3c4 mice [50]. Genetic B cell depletion ameliorated inflammation in both liver and salivary tissues at 24 weeks, while no difference of which was observed regardless of B cell presence in 8 weeks of age in NOD.c3c4 mice [50], suggesting B cells do not contribute to the initiation of the biliary and salivary diseases in this model.

## 14.5 Scurfy Mice

In the context of decreased frequency of CD4<sup>+</sup>CD25<sup>high</sup> Treg in human PBC [92], also studied were Scurfy (Sf) mice, which have a *Foxp3* gene mutation that results in a deficiency of functional Treg [98, 99]. Sf mice demonstrated serological, histological, and cytokine features characteristic of autoimmune cholangitis as seen in the other mouse models, including expanded CD8<sup>+</sup> T cell population, similar to patients with PBC [9]. This report further emphasizes to argue universal Treg requirement to suppress autoimmune diseases including autoimmune cholangitis. Of note, Sf mice develop severe immune dysregulation, as observed in human immune dysregulation, polyendocrinopathy and enteropathy, and X-linked (IPEX) syndrome which affects humans that have mutations in the *Foxp3* gene [100]. One out of 12 patients with IPEX syndrome demonstrated enteropathy, skin disease, and elevation of serum total IgM, IgA, and IgA-AMA, but had no signs of cholestasis [101], although IgA and IgA-AMA have been implicated in cholangiocyte apoptosis in PBC livers [102, 103].

## 14.6 *Ae2<sub>a,b</sub>*<sup>-/-</sup> Mice

Antigen presenting requires costimulatory signals through CD80/86; however, human and mouse normal biliary epithelial cells (cholangiocytes) lack the ability to express these molecules [104]. It has not been addressed whether cholangiocytes

present specific antigen in spontaneous PBC mouse models described above. Since human and mouse cholangiocytes express various toll-like receptors (TLRs) [105], mouse cholangiocytes are involved in immune responses as a first-line defense for microbial infection. Cholangiocytes in patients with PBC expressed similar levels of TLR subtypes, CD40, and human leukocyte antigen DR $\alpha$  (HLA-DR $\alpha$ ) and secreted equivalent amounts of chemokines. Cholangiocyte-expressed chemokines enhanced transmigration of liver-infiltrating mononuclear cells (LMNCs) in PBC. Autologous LMNC cocultured with cholangiocytes from patients with PBC produced higher levels of chemokines and enhanced the expression of CD40 and HLA-DR $\alpha$  [60]. Cholangiocytes produced CX3CL1 in the presence of autologous LMNC, TNF- $\alpha$ , and a TLR3 ligand [106]. Unmodified PDC-E2 was localized in apoptotic cholangiocytes, which combined with AMA enhanced production of proinflammatory cytokines including IL-6, TNF- $\alpha$ , and IL-12p40 from monocyte-derived macrophages in PBC patients [107, 108]. These results suggest cholangiocytes are easily targeted on the site of inflammation by PDC-E2-reactive autoimmune cells in PBC livers.

Genetic modification of Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> anion exchanger 2 (AE2), primarily functioning in cholangiocytes, enabled to develop the other mouse model for human PBC. AE2 is involved in intracellular pH (pH<sub>i</sub>) regulation and transepithelial acid–base transport including secretin-stimulated biliary bicarbonate excretion. Combination therapy of UDCA and corticosteroid enhanced AE2 gene expression in normal human cholangiocytes [109]. The expression of AE2 gene was attenuated in liver tissues and blood mononuclear cells in patients with PBC [110–112], possibly due to the upregulated expression of microRNA506 to interfere with AE2 mRNA in PBC livers and cholangiocytes compared to those in normal humans [31]. Ae2 gene-disrupted mice (Ae2<sub>a,b</sub><sup>-/-</sup> mice) demonstrated similar features seen in human PBC: enhanced production of IL-12 and IFN- $\gamma$ , an expansion of CD8<sup>+</sup> T cell, and a reduction in Treg populations [8]. Since the major histocompatibility complex class I molecule H2-D1 is markedly upregulated in cholangiocytes, Ae2-deleted cholangiocytes may attract CD8<sup>+</sup> T cells and promote their attacks to biliary ducts. Of note, separately from the features of human PBC, Ae2<sub>a,b</sub><sup>-/-</sup> mice exhibit azoospermia, reduced gastric acid secretion, growth retardation, bone abnormalities, and deafness [113].

## 14.7 2-Octynoic Acid-Conjugated BSA (2OA-BSA)-Immunized Mice

The serological hallmark of PBC is the presence of antibodies to mitochondrial antigens identified as the E2 subunits of the pyruvate dehydrogenase complex (PDC) and related enzymes [63, 114], leading an expectation that PDC-E2 immunization develops mouse models for PBC; however, previous findings had been controversial [115–117]. Nonetheless, since PBC sera demonstrated high reactivity against 2-octynoic acid (OA)-modified PDC-E2 peptide [3, 118], 2-OA conjugated



bovine serum albumin (BSA) was immunized into B6 mice and a congenic variant of the nonobese diabetic (NOD) strain, NOD1101 mice. 2-OA conjugated BSA (2OA-BSA) was a potent immunodominant epitope in both strains to break down B cell tolerance against PDC-E2 and induce PBC-like liver disease, whose pathology was characterized by an abundance of lymphocytes in B6 mice and neutrophils in NOD1101 mice [10, 11]. In this model, B cells were the most dominant population in hepatic mononuclear cells and demonstrated an approximately four- to fivefold increase compared to CD4<sup>+</sup> and CD8<sup>+</sup> T cells [52]. 2OA-BSA immunization combined with either  $\alpha$ -GalCer or a TLR-3 ligand polyinosinic-polycytidylic acid (Poly I:C) induced liver fibrosis [53, 56].

### ***14.7.1 B Cells in 2OA-BSA-Immunized Mice***

Since autoantibody-producing B cells had been believed as contributors in autoimmune cholangitis, it was a surprising fact that B cells negatively suppressed liver inflammation in dnTGF- $\beta$ RII mice, confirmed in the genetic B cell depletion and CD8<sup>+</sup> T cell transfer autoimmune cholangitis model [33, 34]. Thereafter, B cell function has been examined in a few studies using anti-CD20-treated dnTGF- $\beta$ RII and genetic B cell depleted NOD.c3c4 mice demonstrating amelioration of liver disease [35, 50]. In addition, anti-CD20 and CD79b pretreatment was examined in 2OA-BSA-immunized mice. B cell depleting antibodies were administered intraperitoneally 1 week before the initiation of 2OA-BSA immunization. Bile duct damage, portal inflammation, and granuloma formation were deteriorated in B cell depleted 2OA-BSA-immunized mice, accompanied with elevation of proinflammatory IFN- $\gamma$  and MCP-1 in sera [54].

### ***14.7.2 NK and NKT Cells in 2OA-BSA-Immunized Mice***

NK and NKT cell depletion using NK1.1 antibodies demonstrated their roles to enhance AMA and cytokine production from T cells [51]. Enzyme-linked immunospot (ELISPOT) assay in response to pyruvate dehydrogenase complex (PDC)-E2 synthetic peptides revealed the reduction of serum AMA- and IFN- $\gamma$ -producing splenic T cells in anti-NK1.1-treated mice at 6 and 12 weeks after 2OA-BSA immunization. Both anti-NK1.1- and control antibody-treated groups demonstrated mild infiltration of lymphocytes in portal tracts; however, no histological difference was detected [51]. NK and NKT cell contribution is possibly limited to the induction phase of autoimmune cholangitis in the 2OA-BSA-immunized mice model.

Invariant NKT (iNKT) cells are efficiently activated by  $\alpha$ -GalCer, resulting in increases of IFN- $\gamma$  and IL-4 in sera, productions in hepatic iNKT cells, and maturation of DCs in the liver and spleen of C57BL/6 mice [53]. Intravenous

administration of  $\alpha$ -GalCer prior to 2OA-BSA immunization led to a profound deterioration of liver disease in 2-OA-BSA-immunized mice, including increments in AMA production, CD8<sup>+</sup> T cell biliary infiltration, portal inflammation, granuloma formation, bile duct damage, and liver fibrosis, suggesting iNKT cell activation is a critical factor to exacerbate the manifestation of PBC. It is noteworthy again that iNKT cell activation promoted liver fibrosis with higher incidence (10/13) after a 12-week 2OA-BSA immunization compared to that (1/9) of controls.

### ***14.7.3 Cytokine/Chemokine Contribution in 2OA-BSA-Immunized Mice***

2OA-BSA immunization was also examined in C57BL/6 background mice with deletion of cytokines IL-12p40, IL-12p35, IL-23p19, IL-17A, IL-17F, IL-22, and IFN- $\gamma$ . The Th1/Th17 signaling pathway was examined using gene deletion of IL-12p40 (Th1/Th17 deprivation), IL-12-p35 (Th1 deprivation), and IL-23p19 (Th17 deprivation), revealing that both Th1 and Th17 cytokines are involved, and IL-12p40 is indispensable in the development of autoimmune cholangitis in 2OA-BSA-immunized mice. IL-23p19 depletion did not affect IFN- $\gamma$  levels in peripheral blood as well as its secretion in mononuclear cells isolated from both the spleen and liver. However, IFN- $\gamma$  levels of liver-extracted protein were diminished by IL-23p19 deletion.

Bile duct damage and portal inflammation were ameliorated by deletion of IL-17A and IL-22, but not by IL-17F deprivation. Consistently, hepatic levels of inflammatory cytokines IFN- $\gamma$ , TNF- $\alpha$ , and IL-6 were significantly reduced in IL-17A- and IL-22-depleted mice, but not in IL-17F-deprived mice, suggesting Th17 cytokines IL-17A and IL-22 promote migration and accumulation of proinflammatory Th1 cells into biliary diseased livers. Serum reactivity against PDC-E2 was also reduced in IL-17A-deficient, but not in IL-22-depleted, mice. In contrast, IFN- $\gamma$  deletion completely abrogated the development of autoimmune cholangitis and significantly decreased serum levels of AMA, suggesting a pivotal role of IFN- $\gamma$  for the induction of biliary damage in this model [52].

### ***14.7.4 Therapeutic Immunomodulation in 2OA-BSA-Immunized Mice***

Coinhibitory immunoreceptor cytotoxic T lymphocyte antigen 4 (CTLA-4) has been remarked in recent PBC studies [119–122]. CTLA-4 is expressed in T cells and negatively regulates T cell activation through higher-affinity binding to CD80/CD86 than CD28 to inhibit costimulation and transmit inhibitory signals [123, 124]. CTLA-4 immunoglobulin (Ig) is one of the effective treatment options

in autoimmune diseases such as rheumatoid arthritis [125] and juvenile idiopathic arthritis [126]. CTLA-4-Ig comprised of the extracellular domain of human CTLA-4 linked to a modified portion of the Fc domain of human IgG-1 and inhibits T cell activation.

Since CTLA-4-Ig binds to both human and mouse CD80/86, its therapeutic effect was examined in 2OA-BSA mice [55]. CTLA-4-Ig pretreatment initiated 1 day before 2OA-BSA immunization completely abrogated liver inflammation, bile duct damage, and development of anti-PDC-E2 autoantibodies. Therapeutic efficacy of CTLA-4-Ig was examined on developed autoimmune cholangitis at 8 weeks after 2OA-BSA immunization. Four weeks of treatment with CTLA-4-Ig significantly diminished bile duct damage and frequency of effector T cells, but did not affect AMA titer in diseased mice, suggesting that an optimized regimen with CTLA-4-Ig is a potential therapeutic candidate for the treatment of PBC patients [55].

## 14.8 Summary

The spontaneous model mice reported herein have been developed by single factor disruption using gene modification primarily affecting the immune cells or the target biliary epithelia, except NOD.c3.c4 and NOD.ABD strains, demonstrating certain similarities of immunological, serological, biochemical, and histological features of human PBC, especially hepatic CD8<sup>+</sup> T cell expansion. However, of note, since these mice also have some disadvantages of complications distinct to human PBC (Table 14.1), a research objective demands a deliberate selection of a suitable mouse model.

These emerging models have enabled researchers to address issues residing on human PBC, contribute to elucidate pathogenic factors, and ultimately may propose novel therapeutic options for human PBC. Recent works imply potential usages of some biologics such as anti-CD20 (rituximab), anti-IL-12/23p40 (ustekinumab), and CTLA-4-Ig (abatacept) for human PBC.

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## Abbreviations

2OA-BSA	2-Octynoic acid-conjugated BSA
ABD	Autoimmune biliary disease
Breg	Regulatory B cells
BSA	Bovine serum albumin

CTLA-4	Cytotoxic T lymphocyte antigen 4
IFN	Interferon
IL	Interleukin
PBC	Primary biliary cirrhosis
Poly I:C	Polyinosinic-polycytidylic acid
TCR	T cell receptor
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	Regulatory T cells

## References

1. Ahrens EH Jr, Payne MA, Kunkel HG, Eisenmenger WJ, Blondheim SH (1950) Primary biliary cirrhosis. *Medicine (Baltimore)* 29(4):299–364
2. Kaplan MM, Gershwin ME (2005) Primary biliary cirrhosis. *N Engl J Med* 353(12):1261–1273
3. Gershwin ME, Mackay IR (2008) The causes of primary biliary cirrhosis: convenient and inconvenient truths. *Hepatology* 47(2):737–745. doi:[10.1002/hep.22042](https://doi.org/10.1002/hep.22042)
4. Selmi C, Zuin M, Gershwin ME (2008) The unfinished business of primary biliary cirrhosis. *J Hepatol* 49(3):451–460. doi:[10.1016/j.jhep.2008.06.006](https://doi.org/10.1016/j.jhep.2008.06.006)
5. Oertelt S, Lian ZX, Cheng CM, Chuang YH, Padgett KA, He XS, Ridgway WM, Ansari AA, Coppel RL, Li MO, Flavell RA, Kronenberg M, Mackay IR, Gershwin ME (2006) Anti-mitochondrial antibodies and primary biliary cirrhosis in TGF-beta receptor II dominant-negative mice. *J Immunol* 177(3):1655–1660
6. Wakabayashi K, Lian ZX, Moritoki Y, Lan RY, Tsuneyama K, Chuang YH, Yang GX, Ridgway W, Ueno Y, Ansari AA, Coppel RL, Mackay IR, Gershwin ME (2006) IL-2 receptor alpha(-/-) mice and the development of primary biliary cirrhosis. *Hepatology* 44(5):1240–1249
7. Irie J, Wu Y, Wicker LS, Rainbow D, Nalesnik MA, Hirsch R, Peterson LB, Leung PS, Cheng C, Mackay IR, Gershwin ME, Ridgway WM (2006) NOD.c3c4 congenic mice develop autoimmune biliary disease that serologically and pathogenetically models human primary biliary cirrhosis. *J Exp Med* 203(5):1209–1219
8. Salas JT, Banales JM, Sarvide S, Recalde S, Ferrer A, Uriarte I, Oude Elferink RP, Prieto J, Medina JF (2008) Ae2a, b-deficient mice develop antimitochondrial antibodies and other features resembling primary biliary cirrhosis. *Gastroenterology* 134(5):1482–1493. doi:[10.1053/j.gastro.2008.02.020](https://doi.org/10.1053/j.gastro.2008.02.020)
9. Zhang W, Sharma R, Ju ST, He XS, Tao Y, Tsuneyama K, Tian Z, Lian ZX, Fu SM, Gershwin ME (2009) Deficiency in regulatory T cells results in development of antimitochondrial antibodies and autoimmune cholangitis. *Hepatology* 49(2):545–552. doi:[10.1002/hep.22651](https://doi.org/10.1002/hep.22651)
10. Wakabayashi K, Lian ZX, Leung PS, Moritoki Y, Tsuneyama K, Kurth MJ, Lam KS, Yoshida K, Yang GX, Hibi T, Ansari AA, Ridgway WM, Coppel RL, Mackay IR, Gershwin ME (2008) Loss of tolerance in C57BL/6 mice to the autoantigen E2 subunit of pyruvate dehydrogenase by a xenobiotic with ensuing biliary ductular disease. *Hepatology* 48(2):531–540. doi:[10.1002/hep.22390](https://doi.org/10.1002/hep.22390)

11. Wakabayashi K, Yoshida K, Leung PS, Moritoki Y, Yang GX, Tsuneyama K, Lian ZX, Hibi T, Ansari AA, Wicker LS, Ridgway WM, Coppel RL, Mackay IR, Gershwin ME (2009) Induction of autoimmune cholangitis in non-obese diabetic (NOD).1101 mice following a chemical xenobiotic immunization. *Clin Exp Immunol* 155(3):577–586. doi:[10.1111/j.1365-2249.2008.03837.x](https://doi.org/10.1111/j.1365-2249.2008.03837.x)
12. Kamihira T, Shimoda S, Harada K, Kawano A, Handa M, Baba E, Tsuneyama K, Nakamura M, Ishibashi H, Nakanuma Y, Gershwin ME, Harada M (2003) Distinct costimulation dependent and independent autoreactive T-cell clones in primary biliary cirrhosis. *Gastroenterology* 125(5):1379–1387
13. Kamihira T, Shimoda S, Nakamura M, Yokoyama T, Takii Y, Kawano A, Handa M, Ishibashi H, Gershwin ME, Harada M (2005) Biliary epithelial cells regulate autoreactive T cells: implications for biliary-specific diseases. *Hepatology* 41(1):151–159. doi:[10.1002/hep.20494](https://doi.org/10.1002/hep.20494)
14. Shigematsu H, Shimoda S, Nakamura M, Matsushita S, Nishimura Y, Sakamoto N, Ichiki Y, Niho Y, Gershwin ME, Ishibashi H (2000) Fine specificity of T cells reactive to human PDC-E2 163–176 peptide, the immunodominant autoantigen in primary biliary cirrhosis: implications for molecular mimicry and cross-recognition among mitochondrial autoantigens. *Hepatology* 32(5):901–909
15. Shimoda S, Ishikawa F, Kamihira T, Komori A, Niho H, Baba E, Harada K, Isse K, Nakanuma Y, Ishibashi H, Gershwin ME, Harada M (2006) Autoreactive T-cell responses in primary biliary cirrhosis are proinflammatory whereas those of controls are regulatory. *Gastroenterology* 131(2):606–618
16. Shimoda S, Nakamura M, Ishibashi H, Kawano A, Kamihira T, Sakamoto N, Matsushita S, Tanaka A, Worman HJ, Gershwin ME, Harada M (2003) Molecular mimicry of mitochondrial and nuclear autoantigens in primary biliary cirrhosis. *Gastroenterology* 124(7):1915–1925
17. Shimoda S, Nakamura M, Shigematsu H, Tanimoto H, Gushima T, Gershwin ME, Ishibashi H (2000) Mimicry peptides of human PDC-E2 163–176 peptide, the immunodominant T-cell epitope of primary biliary cirrhosis. *Hepatology* 31(6):1212–1216
18. Kita H, Matsumura S, He XS, Ansari AA, Lian ZX, Van de Water J, Coppel RL, Kaplan MM, Gershwin ME (2002) Quantitative and functional analysis of PDC-E2-specific autoreactive cytotoxic T lymphocytes in primary biliary cirrhosis. *J Clin Invest* 109(9):1231–1240
19. Tsuneyama K, Yasoshima M, Harada K, Hiramatsu K, Gershwin ME, Nakanuma Y (1998) Increased CD11d expression on small bile duct epithelium and epithelioid granuloma in livers in primary biliary cirrhosis. *Hepatology* 28(3):620–623
20. Hirschfield GM, Liu X, Xu C, Lu Y, Xie G, Gu X, Walker EJ, Jing K, Juran BD, Mason AL, Myers RP, Peltekian KM, Ghent CN, Coltescu C, Atkinson EJ, Heathcote EJ, Lazaridis KN, Amos CI, Siminovitch KA (2009) Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N Engl J Med* 360(24):2544–2555. doi:[10.1056/NEJMoa0810440](https://doi.org/10.1056/NEJMoa0810440)
21. Chuang YH, Lian ZX, Cheng CM, Lan RY, Yang GX, Moritoki Y, Chiang BL, Ansari AA, Tsuneyama K, Coppel RL, Gershwin ME (2005) Increased levels of chemokine receptor CXCR3 and chemokines IP-10 and MIG in patients with primary biliary cirrhosis and their first degree relatives. *J Autoimmun* 25(2):126–132
22. Barak V, Selmi C, Schlesinger M, Blank M, Agmon-Levin N, Kalickman I, Gershwin ME, Shoenfeld Y (2009) Serum inflammatory cytokines, complement components, and soluble interleukin 2 receptor in primary biliary cirrhosis. *J Autoimmun* 33(3–4):178–182. doi:[10.1016/j.jaut.2009.09.010](https://doi.org/10.1016/j.jaut.2009.09.010)
23. Nagano T, Yamamoto K, Matsumoto S, Okamoto R, Tagashira M, Ibuki N, Matsumura S, Yabushita K, Okano N, Tsuji T (1999) Cytokine profile in the liver of primary biliary cirrhosis. *J Clin Immunol* 19(6):422–427
24. Takii Y, Nakamura M, Ito M, Yokoyama T, Komori A, Shimizu-Yoshida Y, Nakao R, Kusumoto K, Nagaoka S, Yano K, Abiru S, Ueki T, Matsumoto T, Daikoku M, Taniguchi K, Fujioka H, Migita K, Yatsushashi H, Nakashima M, Harada M, Ishibashi H

- (2005) Enhanced expression of type I interferon and toll-like receptor-3 in primary biliary cirrhosis. *Lab Invest* 85(7):908–920. doi:[10.1038/labinvest.3700285](https://doi.org/10.1038/labinvest.3700285)
25. Yang CY, Ma X, Tsuneyama K, Huang S, Takahashi T, Chalasani NP, Bowlus CL, Yang GX, Leung PS, Ansari AA, Wu L, Coppel R, Gershwin ME (2013) IL-12/Th1 and IL-23/Th17 biliary microenvironment in primary biliary cirrhosis: implications for therapy. *Hepatology*. doi:[10.1002/hep.26979](https://doi.org/10.1002/hep.26979)
26. Qian C, Jiang T, Zhang W, Ren C, Wang Q, Qin Q, Chen J, Deng A, Zhong R (2013) Increased IL-23 and IL-17 expression by peripheral blood cells of patients with primary biliary cirrhosis. *Cytokine* 64(1):172–180. doi:[10.1016/j.cyto.2013.07.005](https://doi.org/10.1016/j.cyto.2013.07.005)
27. Harada K, Shimoda S, Sato Y, Isse K, Ikeda H, Nakanuma Y (2009) Periductal interleukin-17 production in association with biliary innate immunity contributes to the pathogenesis of cholangiopathy in primary biliary cirrhosis. *Clin Exp Immunol* 157(2):261–270. doi:[10.1111/j.1365-2249.2009.03947.x](https://doi.org/10.1111/j.1365-2249.2009.03947.x)
28. Rong G, Zhou Y, Xiong Y, Zhou L, Geng H, Jiang T, Zhu Y, Lu H, Zhang S, Wang P, Zhang B, Zhong R (2009) Imbalance between T helper type 17 and T regulatory cells in patients with primary biliary cirrhosis: the serum cytokine profile and peripheral cell population. *Clin Exp Immunol* 156(2):217–225. doi:[10.1111/j.1365-2249.2009.03898.x](https://doi.org/10.1111/j.1365-2249.2009.03898.x)
29. Tsuda M, Moritoki Y, Lian ZX, Zhang W, Yoshida K, Wakabayashi K, Yang GX, Nakatani T, Vierling J, Lindor K, Gershwin ME, Bowlus CL (2012) Biochemical and immunologic effects of Rituximab in patients with primary biliary cirrhosis and an incomplete response to ursodeoxycholic acid. *Hepatology* 55(2):512–521. doi:[10.1002/hep.24748](https://doi.org/10.1002/hep.24748)
30. Myers RP, Swain MG, Lee SS, Shaheen AA, Burak KW (2013) B-cell depletion with Rituximab in patients with primary biliary cirrhosis refractory to ursodeoxycholic acid. *Am J Gastroenterol* 108(6):933–941. doi:[10.1038/ajg.2013.51](https://doi.org/10.1038/ajg.2013.51)
31. Banales JM, Saez E, Uriz M, Sarvide S, Urribarri AD, Splinter P, Tietz Bogert PS, Bujanda L, Prieto J, Medina JF, LaRusso NF (2012) Up-regulation of microRNA 506 leads to decreased Cl-/HCO3- anion exchanger 2 expression in biliary epithelium of patients with primary biliary cirrhosis. *Hepatology* 56(2):687–697. doi:[10.1002/hep.25691](https://doi.org/10.1002/hep.25691)
32. Chuang YH, Lian ZX, Yang GX, Shu SA, Moritoki Y, Ridgway WM, Ansari AA, Kronenberg M, Flavell RA, Gao B, Gershwin ME (2008) Natural killer T cells exacerbate liver injury in a transforming growth factor beta receptor II dominant-negative mouse model of primary biliary cirrhosis. *Hepatology* 47(2):571–580. doi:[10.1002/hep.22052](https://doi.org/10.1002/hep.22052)
33. Yang GX, Lian ZX, Chuang YH, Moritoki Y, Lan RY, Wakabayashi K, Ansari AA, Flavell RA, Ridgway WM, Coppel RL, Tsuneyama K, Mackay IR, Gershwin ME (2008) Adoptive transfer of CD8(+) T cells from transforming growth factor beta receptor type II (dominant negative form) induces autoimmune cholangitis in mice. *Hepatology* 47(6):1974–1982. doi:[10.1002/hep.22226](https://doi.org/10.1002/hep.22226)
34. Moritoki Y, Zhang W, Tsuneyama K, Yoshida K, Wakabayashi K, Yang GX, Bowlus C, Ridgway WM, Ueno Y, Ansari AA, Coppel RL, Mackay IR, Flavell RA, Gershwin ME, Lian ZX (2009) B cells suppress the inflammatory response in a mouse model of primary biliary cirrhosis. *Gastroenterology* 136(3):1037–1047. doi:[10.1053/j.gastro.2008.11.035](https://doi.org/10.1053/j.gastro.2008.11.035)
35. Moritoki Y, Lian ZX, Lindor K, Tuscano J, Tsuneyama K, Zhang W, Ueno Y, Dunn R, Kehry M, Coppel RL, Mackay IR, Gershwin ME (2009) B-cell depletion with anti-CD20 ameliorates autoimmune cholangitis but exacerbates colitis in transforming growth factor-beta receptor II dominant negative mice. *Hepatology* 50(6):1893–1903. doi:[10.1002/hep.23238](https://doi.org/10.1002/hep.23238)
36. Yoshida K, Yang GX, Zhang W, Tsuda M, Tsuneyama K, Moritoki Y, Ansari AA, Okazaki K, Lian ZX, Coppel RL, Mackay IR, Gershwin ME (2009) Deletion of interleukin-12p40 suppresses autoimmune cholangitis in dominant negative transforming growth factor beta receptor type II mice. *Hepatology* 50(5):1494–1500. doi:[10.1002/hep.23132](https://doi.org/10.1002/hep.23132)

37. Zhang W, Moritoki Y, Tsuneyama K, Yang GX, Ilan Y, Lian ZX, Gershwin ME (2009) Beta-glucosylceramide ameliorates liver inflammation in murine autoimmune cholangitis. *Clin Exp Immunol* 157(3):359–364. doi:[10.1111/j.1365-2249.2009.03971.x](https://doi.org/10.1111/j.1365-2249.2009.03971.x)
38. Zhang W, Tsuda M, Yang GX, Tsuneyama K, Rong G, Ridgway WM, Ansari AA, Flavell RA, Coppel RL, Lian ZX, Gershwin ME (2010) Deletion of interleukin-6 in mice with the dominant negative form of transforming growth factor beta receptor II improves colitis but exacerbates autoimmune cholangitis. *Hepatology* 52(1):215–222. doi:[10.1002/hep.23664](https://doi.org/10.1002/hep.23664)
39. Ando Y, Yang GX, Tsuda M, Kawata K, Zhang W, Nakajima T, Tsuneyama K, Leung P, Lian ZX, Okazaki K, Ridgway WM, Norman GL, Ansari AA, He XS, Coppel RL, Gershwin ME (2012) The immunobiology of colitis and cholangitis in interleukin-23p19 and interleukin-17A deleted dominant negative form of transforming growth factor beta receptor type II mice. *Hepatology* 56(4):1418–1426. doi:[10.1002/hep.25803](https://doi.org/10.1002/hep.25803)
40. Ando Y, Yang GX, Kenny TP, Kawata K, Zhang W, Huang W, Leung PS, Lian ZX, Okazaki K, Ansari AA, He XS, Invernizzi P, Ridgway WM, Lu Q, Gershwin ME (2013) Overexpression of microRNA-21 is associated with elevated pro-inflammatory cytokines in dominant-negative TGF-beta receptor type II mouse. *J Autoimmun* 41:111–119. doi:[10.1016/j.jaut.2012.12.013](https://doi.org/10.1016/j.jaut.2012.12.013)
41. Yang CY, Leung PS, Yang GX, Kenny TP, Zhang W, Coppel R, Norman GL, Ansari AA, Mackay IR, Worman HJ, Gershwin ME (2012) Epitope-specific anti-nuclear antibodies are expressed in a mouse model of primary biliary cirrhosis and are cytokine-dependent. *Clin Exp Immunol* 168(3):261–267. doi:[10.1111/j.1365-2249.2012.04577.x](https://doi.org/10.1111/j.1365-2249.2012.04577.x)
42. Kawata K, Yang GX, Ando Y, Tanaka H, Zhang W, Kobayashi Y, Tsuneyama K, Leung PS, Lian ZX, Ridgway WM, Ansari AA, He XS, Gershwin ME (2013) Clonality, activated antigen-specific CD8(+) T cells, and development of autoimmune cholangitis in dnTGFbetaRII mice. *Hepatology* 58(3):1094–1104. doi:[10.1002/hep.26418](https://doi.org/10.1002/hep.26418)
43. Tsuda M, Zhang W, Yang GX, Tsuneyama K, Ando Y, Kawata K, Park O, Leung PS, Coppel RL, Ansari AA, Ridgway WM, Gao B, Lian ZX, Flavell R, He XS, Gershwin ME (2013) Deletion of interleukin (IL)-12p35 induces liver fibrosis in dominant-negative TGFbeta receptor type II mice. *Hepatology* 57(2):806–816. doi:[10.1002/hep.25829](https://doi.org/10.1002/hep.25829)
44. Tanaka H, Yang GX, Iwakoshi N, Knechtle SJ, Kawata K, Tsuneyama K, Leung P, Coppel RL, Ansari AA, Joh T, Bowlus C, Gershwin ME (2013) Anti-CD40 ligand monoclonal antibody delays the progression of murine autoimmune cholangitis. *Clin Exp Immunol* 174(3):364–371. doi:[10.1111/cei.12193](https://doi.org/10.1111/cei.12193)
45. Tsuneyama K, Moritoki Y, Kikuchi K, Nakanuma Y (2012) Pathological features of new animal models for primary biliary cirrhosis. *Int J Hepatol* 2012:403954. doi:[10.1155/2012/403954](https://doi.org/10.1155/2012/403954)
46. Hsu W, Zhang W, Tsuneyama K, Moritoki Y, Ridgway WM, Ansari AA, Coppel RL, Lian ZX, Mackay I, Gershwin ME (2009) Differential mechanisms in the pathogenesis of autoimmune cholangitis versus inflammatory bowel disease in interleukin-2Ralpha(-/-) mice. *Hepatology* 49(1):133–140. doi:[10.1002/hep.22591](https://doi.org/10.1002/hep.22591)
47. Lan RY, Salunga TL, Tsuneyama K, Lian ZX, Yang GX, Hsu W, Moritoki Y, Ansari AA, Kemper C, Price J, Atkinson JP, Coppel RL, Gershwin ME (2009) Hepatic IL-17 responses in human and murine primary biliary cirrhosis. *J Autoimmun* 32(1):43–51. doi:[10.1016/j.jaut.2008.11.001](https://doi.org/10.1016/j.jaut.2008.11.001)
48. Koarada S, Wu Y, Fertig N, Sass DA, Nalesnik M, Todd JA, Lyons PA, Fenyk-Melody J, Rainbow DB, Wicker LS, Peterson LB, Ridgway WM (2004) Genetic control of autoimmunity: protection from diabetes, but spontaneous autoimmune biliary disease in a nonobese diabetic congenic strain. *J Immunol* 173(4):2315–2323
49. Yang GX, Wu Y, Tsukamoto H, Leung PS, Lian ZX, Rainbow DB, Hunter KM, Morris GA, Lyons PA, Peterson LB, Wicker LS, Gershwin ME, Ridgway WM (2011) CD8 T cells mediate direct biliary ductule damage in nonobese diabetic autoimmune biliary disease. *J Immunol* 186(2):1259–1267. doi:[10.4049/jimmunol.1001597](https://doi.org/10.4049/jimmunol.1001597)

50. Moritoki Y, Tsuda M, Tsuneyama K, Zhang W, Yoshida K, Lian ZX, Yang GX, Ridgway WM, Wicker LS, Ansari AA, Gershwin ME (2011) B cells promote hepatic inflammation, biliary cyst formation, and salivary gland inflammation in the NOD.c3c4 model of autoimmune cholangitis. *Cell Immunol* 268(1):16–23. doi:[10.1016/j.cellimm.2011.01.005](https://doi.org/10.1016/j.cellimm.2011.01.005)
51. Shimoda S, Tsuneyama K, Kikuchi K, Harada K, Nakanuma Y, Nakamura M, Ishibashi H, Hisamoto S, Niiro H, Leung PS, Ansari AA, Gershwin ME, Akashi K (2012) The role of natural killer (NK) and NK T cells in the loss of tolerance in murine primary biliary cirrhosis. *Clin Exp Immunol* 168(3):279–284. doi:[10.1111/j.1365-2249.2012.04581.x](https://doi.org/10.1111/j.1365-2249.2012.04581.x)
52. Kawata K, Tsuda M, Yang GX, Zhang W, Tanaka H, Tsuneyama K, Leung P, He XS, Knechtle S, Ansari AA, Coppel RL, Gershwin ME (2013) Identification of potential cytokine pathways for therapeutic intervention in murine primary biliary cirrhosis. *PLoS One* 8(9): e74225. doi:[10.1371/journal.pone.0074225](https://doi.org/10.1371/journal.pone.0074225)
53. Wu SJ, Yang YH, Tsuneyama K, Leung PS, Illarionov P, Gershwin ME, Chuang YH (2011) Innate immunity and primary biliary cirrhosis: activated invariant natural killer T cells exacerbate murine autoimmune cholangitis and fibrosis. *Hepatology* 53(3):915–925. doi:[10.1002/hep.24113](https://doi.org/10.1002/hep.24113)
54. Dhirapong A, Lleo A, Yang GX, Tsuneyama K, Dunn R, Kehry M, Packard TA, Cambier JC, Liu FT, Lindor K, Coppel RL, Ansari AA, Gershwin ME (2011) B cell depletion therapy exacerbates murine primary biliary cirrhosis. *Hepatology* 53(2):527–535. doi:[10.1002/hep.24044](https://doi.org/10.1002/hep.24044)
55. Dhirapong A, Yang GX, Nadler S, Zhang W, Tsuneyama K, Leung P, Knechtle S, Ansari AA, Coppel RL, Liu FT, He XS, Gershwin ME (2013) Therapeutic effect of cytotoxic T lymphocyte antigen 4/immunoglobulin on a murine model of primary biliary cirrhosis. *Hepatology* 57(2):708–715. doi:[10.1002/hep.26067](https://doi.org/10.1002/hep.26067)
56. Ambrosini YM, Yang GX, Zhang W, Tsuda M, Shu S, Tsuneyama K, Leung PS, Ansari AA, Coppel RL, Gershwin ME (2011) The multi-hit hypothesis of primary biliary cirrhosis: polyinosinic-polycytidylic acid (poly I:C) and murine autoimmune cholangitis. *Clin Exp Immunol* 166(1):110–120. doi:[10.1111/j.1365-2249.2011.04453.x](https://doi.org/10.1111/j.1365-2249.2011.04453.x)
57. Gorelik L, Flavell RA (2000) Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* 12(2):171–181
58. Gershwin ME, Leung PS, Ridgway WM, Coppel RL, Ansari AA (2013) Reply: to PMID 22996325. *Hepatology* 58(2):830. doi:[10.1002/hep.26242](https://doi.org/10.1002/hep.26242)
59. Hohenester S, Beuers U, Medina JF, Elferink RP (2013) Antimitochondrial antibodies may be insufficiently specific to define primary biliary cirrhosis-like disease in mouse models. *Hepatology* 58(2):828–830. doi:[10.1002/hep.26243](https://doi.org/10.1002/hep.26243)
60. Shimoda S, Harada K, Niiro H, Yoshizumi T, Soejima Y, Taketomi A, Maehara Y, Tsuneyama K, Nakamura M, Komori A, Migita K, Nakanuma Y, Ishibashi H, Selmi C, Gershwin ME (2008) Biliary epithelial cells and primary biliary cirrhosis: the role of liver-infiltrating mononuclear cells. *Hepatology* 47(3):958–965. doi:[10.1002/hep.22102](https://doi.org/10.1002/hep.22102)
61. Kita H, Lian ZX, Van de Water J, He XS, Matsumura S, Kaplan M, Luketic V, Coppel RL, Ansari AA, Gershwin ME (2002) Identification of HLA-A2-restricted CD8(+) cytotoxic T cell responses in primary biliary cirrhosis: T cell activation is augmented by immune complexes cross-presented by dendritic cells. *J Exp Med* 195(1):113–123
62. Nakanuma Y (1993) Distribution of B lymphocytes in nonsuppurative cholangitis in primary biliary cirrhosis. *Hepatology* 18(3):570–575
63. He XS, Ansari AA, Ridgway WM, Coppel RL, Gershwin ME (2006) New insights to the immunopathology and autoimmune responses in primary biliary cirrhosis. *Cell Immunol* 239(1):1–13
64. Mizoguchi A, Bhan AK (2006) A case for regulatory B cells. *J Immunol* 176(2):705–710
65. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF (2008) Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J Clin Invest* 118(10):3420–3430. doi:[10.1172/JCI36030](https://doi.org/10.1172/JCI36030)



66. Lazrak F, Abourazzak FE, Berrada K, Kadi N, Manssouri S, Harzy T (2013) A rare association of rheumatoid arthritis and primary biliary cirrhosis treated with Rituximab: a case report. *J Med Case Rep* 7(1):99. doi:[10.1186/1752-1947-7-99](https://doi.org/10.1186/1752-1947-7-99)
67. Tajiri K, Tsuneyama K, Miyazono T, Kawai K, Minemura M, Sugiyama T (2013) A case of primary biliary cirrhosis that progressed rapidly after treatment involving Rituximab. *Case Rep Gastroenterol* 7(1):195–201. doi:[10.1159/000351173](https://doi.org/10.1159/000351173)
68. Sharif S, Arreaza GA, Zucker P, Mi QS, Sondhi J, Naidenko OV, Kronenberg M, Koezuka Y, Delovitch TL, Gombert JM, Leite-De-Moraes M, Gouarin C, Zhu R, Hameg A, Nakayama T, Taniguchi M, Lepault F, Lehuen A, Bach JF, Herbelin A (2001) Activation of natural killer T cells by alpha-galactosylceramide treatment prevents the onset and recurrence of autoimmune type 1 diabetes. *Nat Med* 7(9):1057–1062. doi:[10.1038/nm0901-1057](https://doi.org/10.1038/nm0901-1057)
69. Furlan R, Bergami A, Cantarella D, Brambilla E, Taniguchi M, Dellabona P, Casorati G, Martino G (2003) Activation of invariant NKT cells by alphaGalCer administration protects mice from MOG35-55-induced EAE: critical roles for administration route and IFN-gamma. *Eur J Immunol* 33(7):1830–1838. doi:[10.1002/eji.200323885](https://doi.org/10.1002/eji.200323885)
70. Taniguchi M, Tashiro T, Dashtsoodol N, Hongo N, Watarai H (2009) The specialized iNKT cell system recognizes glycolipid antigens and bridges the innate and acquired immune systems with potential applications for cancer therapy. *Int Immunol*. doi:[10.1093/intimm/dxp104](https://doi.org/10.1093/intimm/dxp104)
71. Kita H, Naidenko OV, Kronenberg M, Ansari AA, Rogers P, He XS, Koning F, Mikayama T, Van De Water J, Coppel RL, Kaplan M, Gershwin ME (2002) Quantitation and phenotypic analysis of natural killer T cells in primary biliary cirrhosis using a human CD1d tetramer. *Gastroenterology* 123(4):1031–1043
72. Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA (2007) The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 450(7169):566–569. doi:[10.1038/nature06306](https://doi.org/10.1038/nature06306)
73. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 201(2):233–240. doi:[10.1084/jem.20041257](https://doi.org/10.1084/jem.20041257)
74. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441(7090):235–238. doi:[10.1038/nature04753](https://doi.org/10.1038/nature04753)
75. Ghoreschi K, Laurence A, Yang XP, Tato CM, McGeachy MJ, Konkel JE, Ramos HL, Wei L, Davidson TS, Bouladoux N, Grainger JR, Chen Q, Kanno Y, Watford WT, Sun HW, Eberl G, Shevach EM, Belkaid Y, Cua DJ, Chen W, O’Shea JJ (2010) Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature* 467(7318):967–971. doi:[10.1038/nature09447](https://doi.org/10.1038/nature09447)
76. Peters A, Lee Y, Kuchroo VK (2011) The many faces of Th17 cells. *Curr Opin Immunol* 23(6):702–706. doi:[10.1016/j.coi.2011.08.007](https://doi.org/10.1016/j.coi.2011.08.007)
77. Liu X, Invernizzi P, Lu Y, Kosoy R, Bianchi I, Podda M, Xu C, Xie G, Macciardi F, Selmi C, Lupoli S, Shigeta R, Ransom M, Lleo A, Lee AT, Mason AL, Myers RP, Peltekian KM, Ghent CN, Bernuzzi F, Zuin M, Rosina F, Borghesio E, Floreani A, Lazzari R, Niro G, Andriulli A, Muratori L, Muratori P, Almasio PL, Andreone P, Margotti M, Brunetto M, Coco B, Alvaro D, Bragazzi MC, Marra F, Pisano A, Rigamonti C, Colombo M, Marzioni M, Benedetti A, Fabris L, Strazzabosco M, Portincasa P, Palmieri VO, Tiribelli C, Croce L, Bruno S, Rossi S, Vinci M, Prisco C, Mattalia A, Toniutto P, Picciotto A, Galli A, Ferrari C, Colombo S, Casella G, Morini L, Caporaso N, Colli A, Spinzi G, Montanari R, Gregersen PK, Heathcote EJ, Hirschfield GM, Siminovitch KA, Amos CI, Gershwin ME, Seldin MF (2010) Genome-wide meta-analysis identify three loci associated with primary biliary cirrhosis. *Nat Genet* 42(8):658–660. doi:[10.1038/ng.627](https://doi.org/10.1038/ng.627)
78. Leonardi CL, Kimball AB, Papp KA, Yeilding N, Guzzo C, Wang Y, Li S, Dooley LT, Gordon KB (2008) Efficacy and safety of ustekinumab, a human interleukin-12/23

- monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). *Lancet* 371(9625):1665–1674. doi:[10.1016/S0140-6736\(08\)60725-4](https://doi.org/10.1016/S0140-6736(08)60725-4)
79. Mannon PJ, Fuss IJ, Mayer L, Elson CO, Sandborn WJ, Present D, Dolin B, Goodman N, Groden C, Hornung RL, Quezado M, Yang Z, Neurath MF, Salfeld J, Veldman GM, Schwertschlag U, Strober W (2004) Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med* 351(20):2069–2079. doi:[10.1056/NEJMoa033402](https://doi.org/10.1056/NEJMoa033402)
  80. Burakoff R, Barish CF, Riff D, Pruitt R, Chey WY, Farraye FA, Shafran I, Katz S, Krone CL, Vander Vliet M, Stevens C, Sherman ML, Jacobson E, Bleday R (2006) A phase 1/2A trial of STA 5326, an oral interleukin-12/23 inhibitor, in patients with active moderate to severe Crohn's disease. *Inflamm Bowel Dis* 12(7):558–565. doi:[10.1097/01.ibd.0000225337.14356.31](https://doi.org/10.1097/01.ibd.0000225337.14356.31)
  81. Zigmund E, Preston S, Pappo O, Lalazar G, Margalit M, Shalev Z, Zolotarov L, Friedman D, Alper R, Ilan Y (2007) Beta-glucosylceramide: a novel method for enhancement of natural killer T lymphocyte plasticity in murine models of immune-mediated disorders. *Gut* 56(1):82–89. doi:[10.1136/gut.2006.095497](https://doi.org/10.1136/gut.2006.095497)
  82. Zigmund E, Zangen SW, Pappo O, Sklair-Levy M, Lalazar G, Zolotaryova L, Raz I, Ilan Y (2009) Beta-glycosphingolipids improve glucose intolerance and hepatic steatosis of the Cohen diabetic rat. *Am J Physiol Endocrinol Metab* 296(1):E72–E78. doi:[10.1152/ajpendo.90634.2008](https://doi.org/10.1152/ajpendo.90634.2008)
  83. Ilan Y, Ohana M, Pappo O, Margalit M, Lalazar G, Engelhardt D, Rabbani E, Nagler A (2007) Alleviation of acute and chronic graft-versus-host disease in a murine model is associated with glucocerebroside-enhanced natural killer T lymphocyte plasticity. *Transplantation* 83(4):458–467. doi:[10.1097/01.tp.0000252783.66886.f3](https://doi.org/10.1097/01.tp.0000252783.66886.f3)
  84. Lalazar G, Ben Ya'acov A, Eliakim-Raz N, Livovsky DM, Pappo O, Preston S, Zolotarov L, Ilan Y (2008) Beta-glycosphingolipids-mediated lipid raft alteration is associated with redistribution of NKT cells and increased intrahepatic CD8+ T lymphocyte trapping. *J Lipid Res* 49(9):1884–1893. doi:[10.1194/jlr.M800113-JLR200](https://doi.org/10.1194/jlr.M800113-JLR200)
  85. Lleo A, Liao J, Invernizzi P, Zhao M, Bernuzzi F, Ma L, Lanzi G, Ansari AA, Coppel RL, Zhang P, Li Y, Zhou Z, Lu Q, Gershwin ME (2012) Immunoglobulin M levels inversely correlate with CD40 ligand promoter methylation in patients with primary biliary cirrhosis. *Hepatology* 55(1):153–160. doi:[10.1002/hep.24630](https://doi.org/10.1002/hep.24630)
  86. Alabraba EB, Lai V, Boon L, Wigmore SJ, Adams DH, Afford SC (2008) Coculture of human liver macrophages and cholangiocytes leads to CD40-dependent apoptosis and cytokine secretion. *Hepatology* 47(2):552–562. doi:[10.1002/hep.22011](https://doi.org/10.1002/hep.22011)
  87. Gerritse K, Laman JD, Noelle RJ, Aruffo A, Ledbetter JA, Boersma WJ, Claassen E (1996) CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. *Proc Natl Acad Sci U S A* 93(6):2499–2504
  88. Durie FH, Fava RA, Foy TM, Aruffo A, Ledbetter JA, Noelle RJ (1993) Prevention of collagen-induced arthritis with an antibody to gp39, the ligand for CD40. *Science* 261(5126):1328–1330
  89. Mohan C, Shi Y, Laman JD, Datta SK (1995) Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. *J Immunol* 154(3):1470–1480
  90. Ma J, Xu J, Madaio MP, Peng Q, Zhang J, Grewal IS, Flavell RA, Craft J (1996) Autoimmune lpr/lpr mice deficient in CD40 ligand: spontaneous Ig class switching with dichotomy of autoantibody responses. *J Immunol* 157(1):417–426
  91. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY (2005) A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat Immunol* 6(11):1142–1151. doi:[10.1038/ni1263](https://doi.org/10.1038/ni1263)
  92. Lan RY, Cheng C, Lian ZX, Tsuneyama K, Yang GX, Moritoki Y, Chuang YH, Nakamura T, Saito S, Shimoda S, Tanaka A, Bowlus CL, Takano Y, Ansari AA, Coppel RL, Gershwin ME (2006) Liver-targeted and peripheral blood alterations of regulatory T cells in primary biliary cirrhosis. *Hepatology* 43(4):729–737

93. Aoki CA, Roifman CM, Lian ZX, Bowlus CL, Norman GL, Shoenfeld Y, Mackay IR, Gershwin ME (2006) IL-2 receptor alpha deficiency and features of primary biliary cirrhosis. *J Autoimmun* 27(1):50–53
94. Lan RY, Selmi C, Gershwin ME (2008) The regulatory, inflammatory, and T cell programming roles of interleukin-2 (IL-2). *J Autoimmun* 31(1):7–12. doi:[10.1016/j.jaut.2008.03.002](https://doi.org/10.1016/j.jaut.2008.03.002)
95. O'Connor W Jr, Kamanaka M, Booth CJ, Town T, Nakae S, Iwakura Y, Kolls JK, Flavell RA (2009) A protective function for interleukin 17A in T cell-mediated intestinal inflammation. *Nat Immunol* 10(6):603–609. doi:[10.1038/ni.1736](https://doi.org/10.1038/ni.1736)
96. Hanada S, Harada M, Koga H, Kawaguchi T, Taniguchi E, Kumashiro R, Ueno T, Ueno Y, Ishii M, Sakisaka S, Sata M (2003) Tumor necrosis factor-alpha and interferon-gamma directly impair epithelial barrier function in cultured mouse cholangiocytes. *Liver Int* 23(1):3–11
97. Nakagome Y, Ueno Y, Kogure T, Fukushima K, Moritoki Y, Ridgway WM, Eric Gershwin M, Shimosegawa T (2007) Autoimmune cholangitis in NOD.c3c4 mice is associated with cholangiocyte-specific Fas antigen deficiency. *J Autoimmun* 29(1):20–29. doi:[10.1016/j.jaut.2007.03.004](https://doi.org/10.1016/j.jaut.2007.03.004)
98. Godfrey VL, Wilkinson JE, Russell LB (1991) X-linked lymphoreticular disease in the scurfy (sf) mutant mouse. *Am J Pathol* 138(6):1379–1387
99. Brunkow ME, Jeffery EW, Hjerrild KA, Paepfer B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F (2001) Disruption of a new forkhead/winged-helix protein, scurfy, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 27(1):68–73. doi:[10.1038/83784](https://doi.org/10.1038/83784)
100. Ochs HD, Gambineri E, Torgerson TR (2007) IPEX, FOXP3 and regulatory T-cells: a model for autoimmunity. *Immunol Res* 38(1–3):112–121
101. Tsuda M, Torgerson TR, Selmi C, Gambineri E, Carneiro-Sampaio M, Mannurita SC, Leung PS, Norman GL, Gershwin ME (2010) The spectrum of autoantibodies in IPEX syndrome is broad and includes anti-mitochondrial autoantibodies. *J Autoimmun* 35(3):265–268. doi:[10.1016/j.jaut.2010.06.017](https://doi.org/10.1016/j.jaut.2010.06.017)
102. Matsumura S, Van De Water J, Leung P, Odin JA, Yamamoto K, Gores GJ, Mostov K, Ansari AA, Coppel RL, Shiratori Y, Gershwin ME (2004) Caspase induction by IgA antimitochondrial antibody: IgA-mediated biliary injury in primary biliary cirrhosis. *Hepatology* 39(5):1415–1422
103. Salunga TL, Cui ZG, Shimoda S, Zheng HC, Nomoto K, Kondo T, Takano Y, Selmi C, Alpini G, Gershwin ME, Tsuneyama K (2007) Oxidative stress-induced apoptosis of bile duct cells in primary biliary cirrhosis. *J Autoimmun* 29(2–3):78–86. doi:[10.1016/j.jaut.2007.04.002](https://doi.org/10.1016/j.jaut.2007.04.002)
104. Yahagi K, Ishii M, Kobayashi K, Ueno Y, Mano Y, Niitsuma H, Igarashi T, Toyota T (1998) Primary culture of cholangiocytes from normal mouse liver. *In Vitro Cell Dev Biol Anim* 34(7):512–514. doi:[10.1007/s11626-998-0106-x](https://doi.org/10.1007/s11626-998-0106-x)
105. Moritoki Y, Lian ZX, Ohsugi Y, Ueno Y, Gershwin ME (2006) B cells and autoimmune liver diseases. *Autoimmun Rev* 5(7):449–457
106. Shimoda S, Harada K, Niuro H, Taketomi A, Maehara Y, Tsuneyama K, Kikuchi K, Nakanuma Y, Mackay IR, Gershwin ME, Akashi K (2009) CX3CL1 (fractalkine): a signpost for biliary inflammation in primary biliary cirrhosis. *Hepatology*. doi:[10.1002/hep.23318](https://doi.org/10.1002/hep.23318)
107. Lleo A, Bowlus CL, Yang GX, Invernizzi P, Podda M, Van de Water J, Ansari AA, Coppel RL, Worman HJ, Gores GJ, Gershwin ME (2010) Biliary apoptoses and anti-mitochondrial antibodies activate innate immune responses in primary biliary cirrhosis. *Hepatology* 52(3):987–998. doi:[10.1002/hep.23783](https://doi.org/10.1002/hep.23783)
108. Lleo A, Selmi C, Invernizzi P, Podda M, Coppel RL, Mackay IR, Gores GJ, Ansari AA, Van de Water J, Gershwin ME (2009) Apoptoses and the biliary specificity of primary biliary cirrhosis. *Hepatology* 49(3):871–879. doi:[10.1002/hep.22736](https://doi.org/10.1002/hep.22736)

109. Arenas F, Hervias I, Uriz M, Joplin R, Prieto J, Medina JF (2008) Combination of ursodeoxycholic acid and glucocorticoids upregulates the AE2 alternate promoter in human liver cells. *J Clin Invest* 118(2):695–709. doi:[10.1172/JCI33156](https://doi.org/10.1172/JCI33156)
110. Medina JF, Martinez A, Vazquez JJ, Prieto J (1997) Decreased anion exchanger 2 immunoreactivity in the liver of patients with primary biliary cirrhosis. *Hepatology* 25(1):12–17. doi:[10.1002/hep.510250104](https://doi.org/10.1002/hep.510250104)
111. Melero S, Spirli C, Zsembery A, Medina JF, Joplin RE, Duner E, Zuin M, Neuberger JM, Prieto J, Strazzabosco M (2002) Defective regulation of cholangiocyte Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> and Na<sup>+</sup>/H<sup>+</sup> exchanger activities in primary biliary cirrhosis. *Hepatology* 35(6):1513–1521. doi:[10.1053/jhep.2002.33634](https://doi.org/10.1053/jhep.2002.33634)
112. Prieto J, Qian C, Garcia N, Diez J, Medina JF (1993) Abnormal expression of anion exchanger genes in primary biliary cirrhosis. *Gastroenterology* 105(2):572–578
113. Recalde S, Muruzabal F, Looije N, Kunne C, Burrell MA, Saez E, Martinez-Anso E, Salas JT, Mardones P, Prieto J, Medina JF, Elferink RP (2006) Inefficient chronic activation of parietal cells in Ae2a, b(–/–) mice. *Am J Pathol* 169(1):165–176
114. Gershwin ME, Coppel RL, Mackay IR (1988) Primary biliary cirrhosis and mitochondrial autoantigens—insights from molecular biology. *Hepatology* 8(1):147–151
115. Sasaki M, Long SA, Van De Water J, He XS, Shultz L, Coppel RL, Ansari A, Nakanuma Y, Gershwin ME (2002) The SJL/J mouse is not a model for PBC. *Hepatology* 35(5):1284–1286. doi:[10.1053/jhep.2002.32540](https://doi.org/10.1053/jhep.2002.32540)
116. Jones DE, Palmer JM, Yeaman SJ, Kirby JA, Bassendine MF (1999) Breakdown of tolerance to pyruvate dehydrogenase complex in experimental autoimmune cholangitis: a mouse model of primary biliary cirrhosis. *Hepatology* 30(1):65–70. doi:[10.1002/hep.510300123](https://doi.org/10.1002/hep.510300123)
117. Jones DE, Palmer JM, Kirby JA, De Cruz DJ, McCaughan GW, Sedgwick JD, Yeaman SJ, Burt AD, Bassendine MF (2000) Experimental autoimmune cholangitis: a mouse model of immune-mediated cholangiopathy. *Liver* 20(5):351–356
118. Amano K, Leung PS, Rieger R, Quan C, Wang X, Marik J, Suen YF, Kurth MJ, Nantz MH, Ansari AA, Lam KS, Zeniya M, Matsuura E, Coppel RL, Gershwin ME (2005) Chemical xenobiotics and mitochondrial autoantigens in primary biliary cirrhosis: identification of antibodies against a common environmental, cosmetic, and food additive, 2-octynoic acid. *J Immunol* 174(9):5874–5883
119. Juran BD, Atkinson EJ, Larson JJ, Schlicht EM, Liu X, Heathcote EJ, Hirschfield GM, Siminovich KA, Lazaridis KN (2010) Carriage of a tumor necrosis factor polymorphism amplifies the cytotoxic T-lymphocyte antigen 4 attributed risk of primary biliary cirrhosis: evidence for a gene-gene interaction. *Hepatology* 52(1):223–229. doi:[10.1002/hep.23667](https://doi.org/10.1002/hep.23667)
120. Juran BD, Atkinson EJ, Schlicht EM, Fridley BL, Lazaridis KN (2008) Primary biliary cirrhosis is associated with a genetic variant in the 3′ flanking region of the CTLA4 gene. *Gastroenterology* 135(4):1200–1206. doi:[10.1053/j.gastro.2008.06.077](https://doi.org/10.1053/j.gastro.2008.06.077)
121. Juran BD, Atkinson EJ, Schlicht EM, Fridley BL, Petersen GM, Lazaridis KN (2008) Interacting alleles of the coinhibitory immunoreceptor genes cytotoxic T-lymphocyte antigen 4 and programmed cell-death 1 influence risk and features of primary biliary cirrhosis. *Hepatology* 47(2):563–570. doi:[10.1002/hep.22048](https://doi.org/10.1002/hep.22048)
122. Aiba Y, Nakamura M, Joshita S, Inamine T, Komori A, Yoshizawa K, Umemura T, Horie H, Migita K, Yatsuhashi H, Nakamura M, Fukushima N, Saoshiro T, Hayashi S, Kouno H, Ota H, Muro T, Watanabe Y, Nakamura Y, Komeda T, Shimada M, Masaki N, Komatsu T, Yagura M, Sugi K, Koga M, Tsukamoto K, Tanaka E, Ishibashi H (2011) Genetic polymorphisms in CTLA4 and SLC4A2 are differentially associated with the pathogenesis of primary biliary cirrhosis in Japanese patients. *J Gastroenterol* 46(10):1203–1212. doi:[10.1007/s00535-011-0417-7](https://doi.org/10.1007/s00535-011-0417-7)
123. Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, Mattei MG, Golstein P (1987) A new member of the immunoglobulin superfamily—CTLA-4. *Nature* 328(6127):267–270. doi:[10.1038/328267a0](https://doi.org/10.1038/328267a0)

124. Alegre ML, Frauwirth KA, Thompson CB (2001) T-cell regulation by CD28 and CTLA-4. *Nat Rev Immunol* 1(3):220–228. doi:[10.1038/35105024](https://doi.org/10.1038/35105024)
125. Caporali R, Bugatti S, Cavagna L, Antivalle M, Sarzi-Puttini P (2014) Modulating the co-stimulatory signal for T cell activation in rheumatoid arthritis: could it be the first step of the treatment? *Autoimmun Rev* 13(1):49–53. doi:[10.1016/j.autrev.2013.06.008](https://doi.org/10.1016/j.autrev.2013.06.008)
126. Ruperto N, Lovell DJ, Quartier P, Paz E, Rubio-Perez N, Silva CA, Abud-Mendoza C, Burgos-Vargas R, Gerloni V, Melo-Gomes JA, Saad-Magalhaes C, Chavez-Corrales J, Huemer C, Kivitz A, Blanco FJ, Foeldvari I, Hofer M, Horneff G, Huppertz HI, Job-Deslandre C, Loy A, Minden K, Punaro M, Nunez AF, Sigal LH, Block AJ, Nys M, Martini A, Giannini EH (2010) Long-term safety and efficacy of abatacept in children with juvenile idiopathic arthritis. *Arthritis Rheum* 62(6):1792–1802. doi:[10.1002/art.27431](https://doi.org/10.1002/art.27431)