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Animal models of primary biliary cirrhosis

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Abstract

Primary biliary cirrhosis (PBC) is a liver specific autoimmune disease characterized by antimitochondrial autoantibodies (AMAs) and progressive destruction of intrahepatic bile ducts. The elucidation of early events in the induction of tissue inflammation and autoimmunity in PBC has been hampered by the cryptic onset of the disease, the practical limitations in accessing human liver and the lack of a suitable animal model. Three spontaneous autoimmune biliary disease (ABD) mouse models, including NOD.c3c4 and NOD.c3c4-derived, dominant negative TGF- β receptor II (dnTGF β RII), and IL-2R $\alpha^{-/-}$ mouse models have been reported recently. All of these spontaneous ABD animal models for PBC develop features characteristic of human PBC, including biliary lymphocytic infiltrates, AMAs directed against the inner lipoyl domain of the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), and elevated serum levels of IFN- γ and TNF- α . Although some differences exist, these models provide important tools to study the genetic basis and the role of immunoregulation in autoimmune cholangitis and also to allow observation of the earliest stages of loss of tolerance.

Keywords

autoimmune biliary disease; NODc3c4, IL-2 $\alpha^{-/-}$; Dominant negative TGF- β receptor II; xenobiotics

Introduction

Primary biliary cirrhosis (PBC) is a liver specific autoimmune disease characterized by antimitochondrial autoantibodies (AMAs) and progressive destruction of intrahepatic bile ducts. Over the past two decades, the target mitochondrial autoantigens identifying the T and

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B cell epitopes, the innate and adaptive immune responses, the immunobiology of biliary epithelium, and the mechanisms of tissue destruction in PBC have been studied [1,2]. While this work has illuminated many of the late stage events, it has been extremely difficult to study initiating events because of the cryptic nature of disease onset which prevents identification of patients in the earliest stages. In other autoimmune diseases, understanding the immune process has been markedly enhanced by the availability of informative animal models, such as the NZB $\times$ NZW F1 and MRL/lpr mouse for systemic lupus erythematosus (SLE) and NOD models of type 1 diabetes (T1D). Until recently there has been no such model in PBC. Herein, we will review three spontaneous mouse models and two induced models of PBC._

Clinical features and the immune profile of patients with PBC

PBC is an autoimmune liver disease which predominantly affects middle-aged women, characterized by the presence of AMAs, infiltration of lymphocytes in portal tracts and destruction of intrahepatic small bile ducts, causing liver fibrosis and eventually liver failure [1–3]. Lymphocytic infiltration and granuloma formation are often seen in the hepatic lesions of PBC. The presence of AMAs is the hallmark of PBC and can be detected years before disease onset [4]. The autoantigens recognized by AMAs are located on the inner mitochondrial membrane and identified as members of the 2-oxo-acid dehydrogenase enzyme complexes (2-OADC) which include the E2 subunit of the pyruvate dehydrogenase complex (PDC), the branched chain 2-oxo-acid dehydrogenase complex (BCOADC), the α -ketoglutaric acid dehydrogenase complex (OGDC), and the dihydrolipoamide dehydrogenase binding protein [5]. Among them, the major autoantigen in PBC is the E2 subunit of PDC (PDC-E2) [6]. The major epitope of PDC-E2 has been mapped to its inner lipoyl domain [7]. Interestingly, the dominant autoreactive B cell epitope (peptides 167–186) and the T cell epitopes (CD4⁺ T cell: peptides 163–176; CD8⁺ T cell: peptides 159–167) from patients with PBC all appear to localize to the inner lipoyl domain of the PDC-E2 [7–10].

The presence of T lymphocytes infiltrating portal tracts and the detectable expression of class II MHC on biliary epithelium [11] suggests that liver-infiltrating autoreactive T cells mediate the destruction of biliary epithelial cells. Autoreactive CD4⁺ and CD8⁺ T cells against PDC-E2 in the periphery are accompanied by a disease specific 100–150 fold increase in the precursor frequency of PDC-E2 specific CD4⁺ T cells in the hilar lymph nodes and liver from the same PBC patients [7], and a 10–15 fold enrichment of PDC-E2 specific CD8⁺ T cells in liver compared to PBMC [9]. In addition, CD4⁺ T cells are more frequently found in inflamed portal tracts compared to CD8⁺ T cells; however, CD8⁺ T cells are more dominant in areas of bile duct destruction [12], which is suggestive of their active participation in tissue damage. Infiltrating T cells appear to be polyclonal, and skewed towards a Th1 profile with high IFN- γ production and low levels of IL-4 [13].

Defects of regulatory T cells (Tregs) may also accelerate T cell autoimmunity in PBC [14]. PBC patients have been found to have fewer peripheral CD4⁺CD25⁺ Tregs than healthy controls. Moreover, the number of Foxp3-expressing Tregs in portal tracts affected by PBC is markedly lower compared with other liver diseases including chronic hepatitis C and autoimmune hepatitis. Interestingly, healthy daughters and sisters of patients with PBC also exhibit a reduced peripheral Treg frequency, implicating genetic factors in the relative Treg deficiency observed in PBC. In addition, the presence of AMAs and a PBC-like liver disease has been observed in a child with an IL-2R α (CD25) homozygous deficiency [15]. These findings suggest that Treg deficiency may play a role in the loss of tolerance in PBC.

Patients with PBC also manifest elevated sera levels of IgM [16]. This elevation of sera IgM is associated with an increased response to CpGB, but not CpGA, in patients with PBC, with a corresponding upregulation of TLR9. This increased responsiveness is found primarily in

CD27⁺ memory B cells and requires the presence of T cells as well as plasmacytoid dendritic cells. Interestingly, this elevation of sera IgM precedes the onset of disease and is also found in AMA negative PBC [17]. In addition, CpGB-stimulated B cells from patients with PBC produce AMA and express TLR9, CD86 and one of the potassium channels, KCa3.1 [18].

There is a female predominance in PBC and it is 50–100 fold higher frequency in first degree relatives having a member with PBC than the general population [19]. There is no particular HLA haplotype associated with the occurrence of PBC, even in familial cases. The involvement of an abnormal X chromosome in the female susceptibility to PBC has been suggested [20] and the frequency of X monosomy in peripheral blood cells from female PBC patients is significantly higher than in chronic hepatitis C and healthy controls using fluorescence *in situ* hybridization. In addition, the concordance rate of PBC in identical twins is approximately 60%, the highest of any autoimmune disease other than celiac sprue [21]. The importance of a genetic background for the development of PBC is further demonstrated by studying first degree relatives of PBC. As above mentioned, healthy daughters and sisters of PBC patients also exhibited a reduced peripheral Treg frequency. Furthermore, increased plasma levels of interferon- γ -inducible protein 10 (IP-10) and monokine induced by gamma interferon (MIG) were observed not only in patients with PBC but also their healthy daughters and sisters compared to unrelated healthy controls [22].

The early histopathology of PBC involves damage to biliary epithelium, presumably mediated by lymphocytes that surround and often infiltrate the duct [23]. Epithelial cells may be vacuolated, shrunken, and pyknotic. Necrotic bile ducts are often located at the center of large granuloma-like lesions that consist of histiocytes, lymphocytes, plasma cells, eosinophils, and occasionally true giant cells [24]. At this stage, inflammation remains confined to the portal triads. The lymphocytes that infiltrate the ductular epithelial cells directly and presumably destroy them are CD8 cells [9]. The majority of lymphocytes in the portal triads are CD4 cells (helper-inducer cells) [25] although B cells are also seen. As the disease progresses to stage II, many portal triads become scarred, inflammatory cells spill out of the triads into the surrounding periportal parenchyma, normal bile ducts cut in cross section disappear including atypical, poorly formed, tortuous bile ducts with no obvious lumens [26]. Periportal hepatocytes become vacuolated and surrounded by foamy macrophages, a process termed biliary piecemeal necrosis [27]. In stage III, scarring progresses to the point at which fibrous septa link many adjoining portal triads. Stage IV is frank cirrhosis [28].

NOD.c3c4 and NOD.c3c4-derived mouse lines

NOD.c3c4

The NOD mouse is a well-characterized spontaneous model of autoimmune type 1 diabetes (T1D) with immune-mediated pancreatic islet destruction. NOD mice exhibit multiple immune similarities with patients with T1D, including the presence of pancreas-specific autoantibodies and autoreactive CD4⁺ and CD8⁺ T cells, functional abnormalities of T cells, NK cells and perhaps B cells, deficiencies in NKT cells and CD4⁺CD25⁺ Tregs [29–31]. In addition to autoimmune diabetes, NOD and NOD-related strains are prone to develop other autoimmune syndromes, including autoimmune sialitis [32], autoimmune thyroiditis [33], autoimmune peripheral polyneuropathy [34], and Sjogren's-like syndrome [35]. Thus, similar to some humans with T1D, the NOD mouse combines an overall genetic propensity for multi-organ autoimmunity with specific targets not limited to organs of the endocrine system.

The NOD.c3c4 mouse, the first spontaneous mouse model of ABD, was discovered unexpectedly. It was originally constructed to demonstrate that complete protection from diabetes ensued when B6/B10 insulin dependent diabetes (*Idd*) resistance loci (from chromosomes 3 and 4) were placed on the NOD background [36]. However, while resistant to

diabetes, it develops biliary lymphocytic infiltrates, autoantibodies, and progressive, often fatal, biliary obstruction [37]. Moreover, the disease can be transferred by splenocytes to naïve mice, proving an autoimmune etiology [37].

The NOD.c3c4 mouse and patients with PBC share several comparable clinical and autoimmune phenotypes. Histopathological examination demonstrates that NOD.c3c4 mice develop extensive peri-biliary lymphocytic infiltrates, granuloma-like lesions, and chronic nonsuppurative destructive cholangitis in the liver [38]. Furthermore, 50–60% of the NOD.c3c4 mice produce AMAs that react with the inner lipoyl domain of PDC-E2 and inhibit PDC enzyme function. Immunohistochemistry clearly demonstrates that CD3⁺ cells infiltrate areas of active cyst formation. Moreover, the CD3⁺ cells are found directly infiltrating biliary epithelial cells. In addition, administration of CD3⁺ T cell depleting anti-CD3 antibodies in young NOD.c3c4 mice significantly reduces disease onset in these mice [38]. These results suggest an active role for T cells in the PBC disease process.

However, distinct from human PBC, in NOD.c3c4 mice, common bile duct dilation is observed as early as 3 weeks of age and up to 90% of mice develop progressive intra-hepatic biliary cyst formation accompanied by adjacent lymphocytic infiltration by the age of 30 weeks [37,38]. A follow-up study shows that Fas expression on cholangiocytes in NOD.c3c4 mice is significantly reduced compared to control mice. In addition, cholangiocytes in NOD.c3c4 mice are proliferative cellular nuclear antigen (PCNA) positive but TUNEL negative [39]. These results suggest that the abnormal cell proliferation and absence of apoptosis contribute to biliary cyst formation in NOD.c3c4 mice and this defect accelerates an autoimmune response in mice genetically susceptible to loss of tolerance.

NOD.c3c4-derived mice: a congenic mapping approach

Multiple loci control genetic susceptibility to diabetes in NOD mice. The strongest genetic contribution is the MHC class II molecule, I-A^{g7}; this allele is necessary but not sufficient for disease [29,30]. In addition to the MHC locus, other loci contribute to disease development and are termed insulin dependent diabetes (*Idd*) loci. At present at least 20 potential *Idd* loci have been identified using congenic mice [40]. This congenic strategy has been applied to define the underlying immunogenetic basis of the Autoimmune biliary disease (ABD) phenotype. The construction of *Idd* congenic mice demonstrates that several genes are critical for causing liver diseases in NOD.c3c4 mice [37]. For example, neither the NOD.c3 nor the NOD.c4 substrains develop ABD. However, NOD.c3 mice develop liver lymphocytic infiltrates without autoantibody production, while NOD.c4 mice develop antinuclear antibodies (ANAs) but neither AMAs nor liver pathology. When combined in the NOD.c3c4 mouse, these genetic intervals interact synergistically to produce both ABD and AMAs [37]. The critical genetic regions causing liver disease in ABD mice will eventually be identified using the “congenic dissection” strategy, which has involved producing progressively smaller intervals from the critical regions on chromosomes 3 and 4 by selecting recombination events during a backcross to the NOD strain (Figure 1).

Dominant negative TGF- β receptor II (dnTGF β RII) mouse

TGF- β is a well established immune regulatory cytokine with pleiotropic effects on cell proliferation, differentiation, migration, and survival that affect multiple biological processes, including carcinogenesis, fibrosis, wound healing, and immune responses. Importantly, it exerts suppressive effects on the survival, activation, proliferation, and effector functions of several key immune players such as T cells, B cells, natural killer cells, macrophages and dendritic cells [41,42]. The generation of TGF- β 1^{-/-} mice demonstrated a critical role for this cytokine in inhibiting inflammation and autoimmune diseases [43]. TGF- β produced by Th3 cells in mucosal tissues, such as the gut, also plays a critical role in mucosal tolerance [44]. In

addition, TGF- β has been demonstrated to promote the development of peripheral FoxP3⁺ regulatory T cells [45,46]. The association with a decrease in peripheral Tregs in PBC patients [14], the description of PBC as a mucosal disease [47], and the role of TGF- β in immune modulation, make TGF β deficient mouse an interesting model for PBC. However, generic TGF- $\beta^{-/-}$ mice have a life span of 3–4 weeks [43] and are not ideal for study of chronic disease.

In contrast, dnTGF β RII mice have an over-expression of a dominant-negative form of TGF β receptor type II under the control of the CD4 promoter and spontaneously develops features characteristic of PBC [48]. These features include spontaneous production of AMAs reacting to the mitochondrial autoantigens, PDC-E2, BCOADC-E2 and OGDC-E2, and these AMAs inhibit PDC-E2 activity and reactivity with the inner lipoyl domain of the respective E2 subunits. Moreover, lymphocytic infiltration in the liver with periportal inflammation is similar to the histological profile in human PBC. Serum levels of cytokines such as TNF α , IFN γ , IL-12p40 and IL-6 in dnTGF β RII mice are higher than control mice. In the liver, both the frequency and the total number of CD3⁺ cells among the total lymphoid cell infiltrate are significantly increased in dnTGF β RII mice. In addition, the absolute number of hepatic invariant NKT (iNKT) cells is significantly increased compared to control mice.

While the total number of intrahepatic CD4⁺ lymphocytes is increased, the percentage of CD4⁺ cells within the CD3⁺ population does not increase. In contrast, the CD8⁺ population is significantly amplified in total number as well as percentage in the CD3⁺ population compared with controls. These features result in a change in the ratio of CD4⁺ to CD8⁺ T cells biased toward the CD8⁺ population [48]. This finding is particularly interesting, considering previous reports of an increase in precursors of CTLs in the blood of patients in the early stages of PBC versus advanced stages and the 10-fold increase of autoantigen-specific CTLs within the liver as compared with the circulating pool [9]. The biased ratio of CD4 to CD8 in the liver of dnTGF β RII mice is due to the dominant migration of CD8⁺ T cells. Rag-1^{-/-} mice adoptively transferred with CD8⁺ T cells from dnTGF β RII mice demonstrate a significant expansion of T cells and the presence of portal tract infiltrates. In contrast, Rag-1^{-/-} mice adoptively transferred with CD4⁺ T cells from dnTGF β RII mice also demonstrate an increased number of T cells but they do not home or focus within the portal tracts. The different homing pattern may explain the increased number of CD8⁺ T cells in liver of PBC patients (Figure 2).

While CD1d expression and the frequency of invariant NKT (iNKT) cells are both increased in PBC liver [49,50] the percentage as well as the absolute numbers of hepatic iNKT cells are augmented in this dnTGF β RII mouse model [48]. Furthermore, CD1d^{-/-}dnTGF β RII mice exhibit significantly decreased hepatic lymphoid cell infiltrates and milder cholangitis compared to CD1d^{+/-}dnTGF β RII mice. In addition, there is a significant increase in the production of IFN- γ in hepatic iNKT cells activated by α -galactosylceramide (α -GalCer) in young but not older dnTGF β RII mice. These results suggest iNKT cells could promote the development of PBC in early stages by secreting IFN- γ (Chuang, et al., *Hepatology*, in press; Figure 2).

Interleukin-2 receptor α (IL-2R α , CD25) ^{-/-} mice

IL-2 is critical for the development and peripheral expansion of CD4⁺CD25⁺ Tregs, which promote self-tolerance by suppressing T cell responses *in vivo* [51]. IL-2R $\alpha^{-/-}$ mice develop severe anemia and lymphoproliferative autoimmune disorders as well as inflammatory bowel disease, and between 8 and 20 weeks, 25–50% of IL-2R $\alpha^{-/-}$ mice die from severe hemolytic anemia [52].

IL-2R $\alpha^{-/-}$ mice develop portal inflammation and biliary ductular damage similar to human PBC [53]. CD4⁺ and CD8⁺ T cells predominate among portal cell infiltrates and significantly increased numbers of CD8⁺ cells are observed. Serum levels of cytokines such as TNF α ,

IFN γ , IL-12p40 and IL-6 in IL-2R $\alpha^{-/-}$ mice are higher than that of control mice. Importantly, IL-2R $\alpha^{-/-}$ mice produce AMAs against the inner lipoyl domain of PDC-E2 [53] similar to PBC patients and the other spontaneous ABD mouse models. What about the human model for IL-2 deficiency?

Induced models

The autoimmune liver disease PBC is characterized by the breakdown of self tolerance to self antigens such as PDC-E2. Administration of PDC-E2 with adjuvant to mice produces AMA but not antigen specific bile duct disease [54]. However, portal tract inflammatory infiltrates are seen in the covalent modified (biotinylated) PDC-sensitized mice [55]. Another approach of breakdown tolerance is xenobiotic immunization. Guinea pigs immunized with xenobiotics, such as 6-bromohexanoate, conjugated with a BSA not only develop AMA similar to human PBC, but also develop PBC-like liver lesions including significant lymphoid cell infiltrates surrounding damaged bile ducts, variable swollen lymphoplasmacytes surrounding interlobular bile duct and bile duct loss [56,57] unlike the animal models the biliary tract disease is confined to the interlobular bile ducts as in PBC. These models suggest that tolerance breakdown by either modification of the self antigens or molecular mimicry leads to the pathogenesis of PBC. These models also offer the potential to study several key mechanistic issues which relate to generic induction of autoimmunity, including the role of toll-like receptors, innate immunity, repertoire-dependent immunopathology, autoreactive B cells, T regs, naturally occurring autoantibodies, apoptosis, and the immunological homonculus [58–69].

Conclusion

The features of human and those of the mouse models are compared in Table 1. All of these spontaneous ABD animal models of PBC develop features similar to human PBC, i.e. lymphocytic infiltrates, decreased hepatic CD4/CD8 ratios with age, AMAs against inner lipoyl domain of PDC-E2, and elevated serum levels of IFN- γ and TNF- α . It is noted that no experimental model can provide a true facsimile of the counterpart in the genetically complex outbred human. Immunopathology develops equally in male and female mice of these animal models, compared to the female predominance in human PBC. NOD.c3c4 mice develop increased serum IgA and IgM, dnTGF β RII mouse develops increased serum IgA, IgM and IgG, and IL-2R $\alpha^{-/-}$ mouse develops increased serum IgA and IgG. However, elevated levels of total IgM in serum are noted in human PBC [17]. PBC is characterized histologically by the chronic destruction of small intrahepatic bile ducts with surrounding portal lymphocytic infiltration [2], features also seen in the liver of all of these mouse models which however develop disease in both the extrahepatic as well as the intrahepatic ducts. However, these mouse models develop additional features that are not found in human PBC. For example, NOD.c3c4 mice develop marked biliary cyst formation [37,38]. Clusters of macrophages and lymphocytes located in parenchyma and adjacent to the blood vessels are observed in the liver of dnTGF β RII mice [70]. IL-2R $\alpha^{-/-}$ mice develop an ulcerative colitis-like disease. Taken together, these models serve as important tools to study the genetics and immunoregulation of PBC in the earliest stage of disease (Figure 1 and Figure 2).

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Abbreviations

ABD, autoimmune biliary disease; AMA, antimitochondrial autoantibodies; ANA, antinuclear antibodies; dnTGFβRII, dominant negative TGF-β receptor II; Idd, insulin dependent diabetes; iNKT, invariant natural killer T; PBC, primary biliary cirrhosis; PDC-E2, the E2 subunits of the pyruvate dehydrogenase complex; Treg, regulatory T cell; T1D, type 1 diabetes..

Figure 1.

Genetic dissection of ABD in NOD.c3c4-related mice. The upper portion of the figure shows the B6/B10 regions introgressed onto the NOD background in seven congenic strains. The bottom portion of the figure indicates which regions are present in which strains (as marked by a “+”), and whether the strains develop ABD. Two genetic regions proven to be necessary for disease are marked by Abd1 and Abd2.

Figure 2.

Immune cells are involved in the early pathogenesis of PBC. T cells mediating the destruction of bile duct in PBC is documented in the dnTGF β RII model by adoptive transfer study. Rag-1^{-/-} mice transferred with CD8⁺ T cells from dnTGF β RII mice demonstrate a significant expansion of T cells and the presence of portal tract infiltrate. However, the specific homing receptors for CD4⁺ and CD8⁺ T cells have not been identified. iNKT cells promote the development of PBC in early stages by secreting IFN- γ . Anti-PDC-E2 autoantibodies are detected in NOD.c3c4, 2445, dnTGF β RII, and IL-2R $\alpha^{-/-}$ mouse models. The presence of liver disease similar to human PBC in dnTGF β RII and IL-2R $\alpha^{-/-}$ mouse models indicates the importance of Tregs in PBC.

Table 1
Human PBC vs. ABD mouse models: Clinical, histological and immunological observations.

	Human PBC	NOD.c3c4	2445	dnTGFβRII	IL-2Rα ^{-/-}
General feature					
Genetic factor	60% concordance in identical twin	100%	100%	100%	100%
Environment factor	Yes	?	?	?	?
Affected gender	mostly female	both sexes	both sexes	both sexes	both sexes
Age of onset	40–60 ys	9–10 wks	9–10 wks	5–6 wks	5–6 wks
Liver histology					
specific destruction of small bile ducts	Yes	Yes	Yes	Yes	Yes
periductal T cell infiltration	Yes	Yes	Yes	Yes	Yes
Granuloma formation	Yes	Yes	Yes	No	No
Eosinophilia	Yes	Yes	Yes	No	No
Fibrosis	Yes	No	No	No	No
Pathological role of lymphocytes	?	Yes*	?	Yes*	?
T cell immunity					
liver infiltration of T cells	Yes	Yes	Yes	Yes	Yes
autoreactive CD4 ⁺ T cell response	Yes	?	Yes	Yes	?
target of autoreactive CD4 ⁺ T cells	PDC-E2	?	PDC-E2	PDC-E2	?
reduced Treg frequency	Yes	?	?	?	Yes
increased iNKT frequency	Yes	?	?	Yes	No
B cell immunity					
Immunoglobulins	IgM, IgG	IgM, IgA	?	IgM, IgG, IgA	IgA, IgG
AMA	Yes	Yes	Yes	Yes	Yes
Dominant AMA target protein	PDC-E2	PDC-E2	PDC-E2	PDC-E2	PDC-E2
Dominant Epitope	Lipoyl domain	Lipoyl domain	Lipoyl domain	Lipoyl domain	Lipoyl domain
ANA	Yes	Yes	No	?	Yes
Serum biochemistry					
increased alkaline phosphatase	Yes	?	?	?	?
serum cytokines					
increased IFN-γ	Yes	Yes	?	Yes	Yes
increased TNF-α	Yes	Yes	?	Yes	Yes

* adoptive transfer of splenocytes causes disease.