Primary Biliary Cirrhosis: One Disease with Many Faces

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ABSTRACT: Primary biliary cirrhosis (PBC) is considered a model autoimmune disease because of the similarities between patients, their relative homogeneous presentation and natural history, and the presence of the signature autoantibody, the anti-mitochondrial antibodies. PBC also illustrates the potential role of genetic and environmental influence and is unique in having several well-defined animal models that recapitulate distinct features of the disease. The pathogenesis of the disease includes genetic predisposition, the production of both innate and adaptive immune responses, and cholangiocyte-specific biology that addresses the specificity of disease. In this review we highlight these features of PBC in comparison to other autoimmune diseases.

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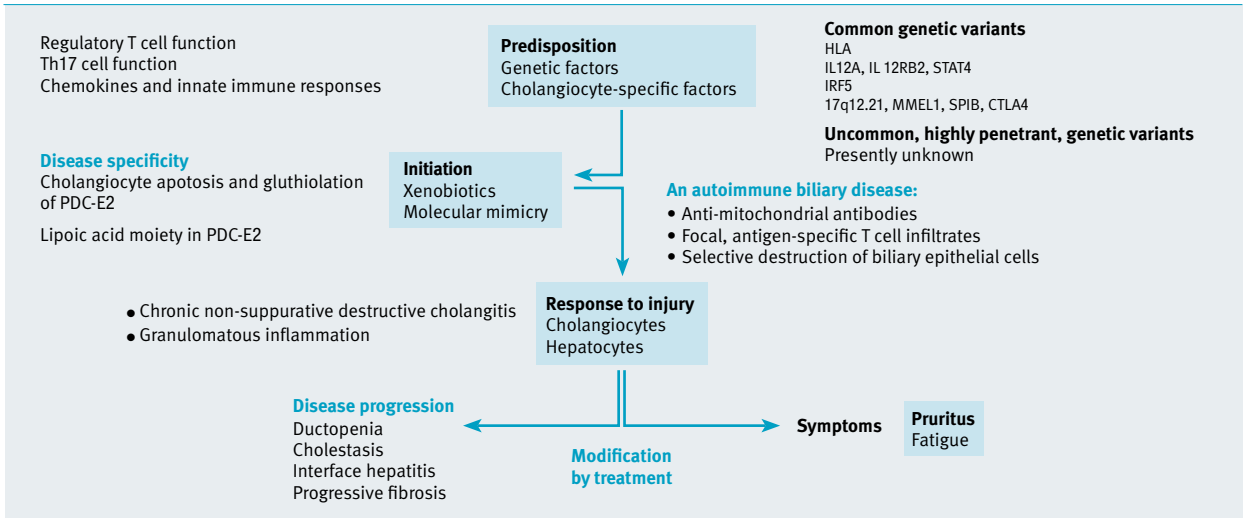
Primary biliary cirrhosis is the most common of the family of autoimmune liver diseases, and the prevalence in those populations that have been appropriately studied is estimated at 1 in 1000 women over the age of 40 [1]. A classic triad of features is used, by consensus, to diagnose patients and includes the presence of chronic biochemical cholestasis, demonstration of circulating specific anti-mitochondrial antibodies, and characteristic biopsy findings of destructive non-suppurative granulomatous/lymphocytic cholangitis [Figure 1]. Most patients now present without symptoms and are identified because routine biochemistry demonstrates anicteric cholestasis. This, however, is in striking contrast to the cohort described by Sheila Sherlock [2] in which only 4 of 100 patients between 1965 and 1972 were found because of incidental testing and 41% of patients underwent a peri-operative biopsy [2]. Since the positive predictive value of liver biochemistry and positive immune serology is so high in PBC, treatment with the choleretic bile acid ursodeoxycholic acid is currently used, in the correct clinical context, without recourse to diagnostic or staging histology. In this review we summarize some salient features of PBC biology and highlight some challenges in our understanding of the disease.

PBC = primary biliary cirrhosis

PBC: GENES AND ENVIRONMENT

Descriptive and epidemiological studies have confirmed two important distinct aspects that contribute to the genesis of this chronic immunologically driven biliary disease. Firstly,

Figure 1. The multi-step pathogenesis of primary biliary cirrhosis



PBC has an important genetic component, a concept clearly apparent to all autoimmune diseases. This is clear from the data showing an increased prevalence of other autoimmune diseases in patients and their families, namely, the presence – greater than predicted – of AMA in family members, and the strong disease concordance in identical twins, as well as raised prevalence of PBC itself in family members. Additionally, there are reported families with apparent PBC pedigrees [3-5]. The second epidemiological feature is the probable role of environmental triggers, which most likely lead to the specific loss of tolerance to the pyruvate dehydrogenase complex of mitochondrial enzymes, in those with an appropriate genetic predisposition. This is believed to occur either as a result of molecular mimicry or the chemical effects of xenobiotic exposure. Population level data report important associations for PBC that include smoking, recurrent urinary tract infections and possible chemical exposures [6], while time-space cluster analysis provides surrogate evidence of environmental challenges that presumably could be infective or toxic [7].

The diagnostic features of PBC are relatively homogeneous: cholestatic liver enzyme profile, AMA and characteristic lymphocytic/granulomatous small bile duct cholangitis. Biopsy is not generally needed for diagnosis

Genome-wide studies have now pinpointed the interleukin-12 axis as one key pathway in this disease, with genetic associations in particular with the *IL12A* and *IL12RB2* loci [8], which are as important as the previously well-reported, but poorly understood, human leukocyte antigen disease associations [9]. Further studies identified additional risk loci including genes or loci already implicated in associated autoimmune diseases, e.g., *IRF5*, *MMEL1*, *SPIB* and *17q12.21* [10,11]. For sure additional loci will be discovered as study cohorts grow in size and their power to distinguish associations strengthens. However, genome-wide studies can likely explain only a proportion of genetic risk, and the application of whole-genome sequencing aimed at identifying highly penetrant but low prevalence mutations will take center stage. Nevertheless, the present genetic risk loci have clearly suggested that immunoregulatory pathways are important in disease, something that has also become apparent from an increasing array of targeted animal models of autoimmune cholangitis. These include the transforming growth factor-beta receptor II dominant-negative mouse [12], the IL-2 receptor alpha-deleted mouse [13], scurfy mice that have a mutation in the gene encoding the Foxp3 transcription factor that results in a complete abolition of Foxp3(+) Tregs, and a congenically bred non-obese diabetic c3c4 strain [14,15]. These models collectively highlight important roles for regulatory T cells, autoreactive CD8(+) cells, and natural killer cells in experimental autoimmune cholangitis. Intriguingly, the AE2 (Cl^-/HCO_3^- anion exchange) knockout mouse also develops autoimmune cholangitis [16], although whether this really implicates biliary transporters in disease is not clear because the transporter is expressed in lymphocytes, and human studies have failed to show robust associations between AE2 and disease. Using murine models, investigators have also demonstrated the likely significance for chemical xenobiotics and bacteria in triggering characteristic disease-specific immunological signatures [17-19].

BILIARY SPECIFICITY

The biliary specificity of PBC is striking and not fully defined, but the evidence to date suggests this is driven by a specific facet of cholangiocyte apoptosis, in which the relevant autoantigen, pyruvate dehyrogenase E2, undergoes differential glutathiolation, increasing its immunogenicity. Biliary epithelial cells translocate immunologically intact PDC-E2 to apoptotic bodies and create apoptotic blebs, which present intact and immunoreactive antigen. In the context of a permissive immune system in which subtle changes in the checks and balances of normal immunoregulation are present, loss of tolerance occurs [20,21].

TOWARDS DISEASE PHENOTYPES

With so many new insights, much remains to be done to piece together the full biological process in our patients. Added to this is the need to address some of the differences seen in patients related to their clinical course and outcome, beyond the sole fact that they have PBC [Table 1]. In what some might perhaps argue is an over-simplistic view but which reflects simple day to day observations from the clinic, one can conceptualize a number of broad patient descriptors for those now living with PBC. Firstly there is the middle-aged woman with early disease and few if any symptoms, who has a good biochemical response to UDCA and an excellent and apparently benign outcome. Second are those who do not respond to treatment and in whom disease progresses slowly towards cholestatic liver failure. This group does seem distinct from a smaller cohort of patients who appear to have quite modest disease with reasonable biochemistry over many years or decades on treatment, but who in their seventies and eighties develop progressive portal hypertension, ascites and then liver failure. This itself is in further contrast to some very elderly patients who have PBC, usually with few symptoms or concerns, but

AMA = anti-mitochondrial antibodies
IL = interleukin
PDC-E2 = pyruvate dehyrogenase E2
UDCA = ursodeoxycholic acid

Table 1. Markers of possible disease phenotypes

Phenotype	Clinical overview	Comments
Symptoms	Symptomatic disease is often considered to follow from asymptomatic disease and to be associated with a poorer prognosis	Symptoms are hard to define and are not specific to PBC. Asymptomatic patients can have significant liver disease. A linear progression from asymptomatic to symptomatic disease is not always seen
Anti-mitochondrial antibody status	~5% of patients have no circulating AMA; these patients may have lower IgM levels	Clinically and immunologically there is no difference in outcome. AMA can be seen in acute liver failure and other autoimmune liver diseases
Antinuclear antibody status	More than of 50% of patients have disease-specific ANA, e.g., gp210 or sp100; gp210-seropositive patients are predicted to fare worse. Patients positive for anti-centromere antibody are predicted to develop more portal hypertension	No prospective studies have shown whether treatment response differs according to ANA status. Specific ELISA assays for gp210 and sp100 are not widely available
Overlap features	The severity of interface hepatitis is reportedly associated with the severity of liver fibrosis	Adequate and validated criteria for evaluation of hepatitic activity clinically and histologically are needed. Drug injury can mimic "autoimmune" activity
Ductopenia	The severity of ductopenia correlates with symptoms, particularly itch, and with treatment efficacy	Evaluation requires liver biopsy, which is subject to sampling variability, but no validated means exists to quantify ductopenia reproducibly
Presence of other autoimmune disease	Many patients have celiac disease, scleroderma, thyroid disease and Sjogrens in addition to PBC	There is a lack of large prospective studies of other autoimmune diseases to determine if the disease course is distinguishable
Biochemical response to treatment	During treatment biochemical responses to UDCA therapy are associated with improved clinical outcomes	Most outcome data are relatively short term (10 year follow-up). As a surrogate of treatment efficacy it cannot be assumed that biochemical response to an alternative therapy other than UDCA equates positively with a good outcome. Mild disease with predetermined good outcomes may appear to respond better to UDCA
Liver transplant/ cirrhosis	Hard end-points (death, liver transplant, cirrhosis) are relatively objective markers of a severe clinical phenotype	An apparently ever-decreasing proportion of patients need liver transplantation. Many non-disease factors can contribute to delayed diagnosis and inadequate treatment and thus cirrhosis. Many patients live with cirrhosis without ever succumbing to decompensation. Liver transplantation may not be offered to every patient, especially those over 70

AMA = anti-mitochondrial antibodies, ANA = antinuclear antibody, IgM = immunoglobulin M, ELISA = enzyme-linked immunosorbent assay, UDCA = ursodeoxycholic acid

according to imaging and biopsy are clearly cirrhotic. A final and starkly contrasting group, fortunately representing only a small number of patients, are younger women driven to total distraction with intractable itch and marked devastating ductopenia on histology. These unfortunate patients do not respond to UDCA and progress to transplantation at a relatively young age.

If one keeps these very broad and sweeping descriptions in mind, it becomes clear that even "simple" markers of disease severity, such as cirrhosis or transplant, are imperfect if we wish to understand the biology of disease progression, more so if one were able to adequately correct for timely, appropriate and compliant therapy. To further understand such clinical distinctions and dissect the biological facets of disease as a whole, we need to be able to phenotype our patients more robustly. This represents the first step in identifying who might benefit from new and improved therapies, which preferably – unlike UDCA – will be disease-specific.

The clinical features of disease are heterogeneous with varying clinical outcomes and response to treatment. A biochemical response to UDCA predicts a favorable long term outcome

The presence of symptoms has traditionally been one arbitary phenotype. However, most symptoms are not specific to PBC itself (particularly fatigue), and symptom severity, such as itch for example, is often clinically also multifactoral. Some patient-based laboratory distinctions do stand out, albeit without adequate biological explanation. Thus, even if one uses the most sophisticated immunotesting, around 5% of patients remain AMA-negative [22]. Clinically (and at the "microscopic" immunologic level [23]) these patients appear to fare the same as AMA-positive PBC patients, but historically they were identified as they appeared to have lower immunoglobulin M values and were usually positive to antinuclear antibody. The ANA patterns seen in PBC appear to represent a potential distinguishing biomarker, which seemingly may flag differing disease courses. Unlike autoimmune hepatitis, in PBC the ANA immunofluorescence is highly specific and is characterized when present (~50% of patients) as anti-gp210 (nuclear membrane pattern) or anti-sp100 (multiple nuclear dot pattern). It appears clinically that patients who are anti-gp210 positive have a greater propensity to progress to liver failure, while biologically we noted that anti-sp100 patients had a potentially distinct HLA profile. Another ANA pattern commonly seen in PBC, but which is not disease-specific, is anti-centromere staining, and some report that this ANA pattern predicts a portal hypertensive

ANA = antinuclear antibody
HLA = human leukocyte antigen

disease course. Patients with scleroderma/systemic sclerosis and PBC are indeed reported to have a slower disease progression as well. The detailed surveillance of immune serology in patients with PBC may be insightful mechanistically and may prove to be a way of identifying patient subtypes [24].

Histologically, PBC transpires to be more than just a duct disease, and interface hepatitis can be present and quite marked, even sometimes misleading pathologists to a mistaken diagnosis of autoimmune hepatitis [25]. The factors that determine why some patients have more inflammatory activity histologically are unknown, but it seems there are two processes that are distinct: so-called piecemeal necrosis (inflammatory destruction of hepatocytes similar to AIH) and biliary piecemeal necrosis in association with ductopenia (increase in the frequency of ductular profiles extending periportally). It has been argued that the degree of interface hepatitis drives the progression to cirrhosis. Overall estimates also suggest that 5–10% of patients with PBC have features of AIH, and regardless of whether one calls this an overlap syndrome or just a florid hepatitic PBC, greater understanding of this and other "overlap" presentations might be insightful regarding all patients with PBC [26].

It has become clearer that the biochemical response to UDCA treatment is a useful clinical means to evaluate outcome, but universally applicable and well-validated definitions are lacking and predictors of response are not adequately understood. Whether a reflection of disease heterogeneity (including the possibility that some disease is always destined to be mild and non-progressive) or treatment efficacy, it nevertheless appears to distinguish patients with more benign outcomes from those on a trajectory to liver failure. Disease severity, including stage of fibrosis and degree of ductopenia, appear relevant but other factors are likely at play. Examples of treatment response algorithms, in practice usually applied at varying time points after treatment with UDCA, include the Mayo criteria (alkaline phosphatase < 2 times the upper limits of normal) [27], the French criteria (ALP < 3 x ULN, and aspartate aminotransferase < 2 x ULN, and bilirubin < 17.1 μmol/L), the Spanish criteria (decline in ALP of more than 40% of baseline or to a normal value) [28], and our Toronto criteria (patients whose ALP is < 1.67 x ULN after 2 years of UDCA treatment have less than one stage of fibrosis progression at 10 years, or those whose ALP is < 1.76 x ULN have less than two stages of fibrosis progression at 10 years) [29]. Patients meeting the Spanish criteria had a similar survival to that of the matched control population, while those meeting the French criteria are predicted to have a 10 year transplant-free survival rate of 90% (compared with 51% for those who did not). Understanding further the basis of treatment response, not just to UDCA but to other drugs such as fenofibrate, will aid disease classification.

AIH = autoimmune hepatitis
ALP = alkaline phosphatase
ULN = upper limits of normal

PBC: CONTRASTS AND COMPARISONS

PBC is considered a model autoimmune disease. For one thing, there are greater similarities in the onset and natural history of disease, unlike other organ-specific autoimmune diseases [30]. Moreover, PBC illustrates the clustering and commonalities found among other autoimmune diseases, including some of the common environmental triggers that have been postulated [31,32]. In contrast, however, PBC does not respond well, if at all, to immunosuppressive agents. This is in sharp distinction to several other autoimmune diseases and raises the possibility that some of the pathology is mediated other than by an adaptive response [33-35]. Finally, the genetics of PBC, including genome-wide and microRNA experimental studies, are yielding exciting data pointing to potential therapeutic targets [8,11,36]. In this respect we note the increasing emphasis on the molecular consequences of apoptosis and the use of epigenetics and proteomics in autoimmunity and suggest that this will become a focus for further study not only in PBC but other autoimmune diseases as well [37-40].

The biologic processes are increasingly being revealed by genomic testing and development of targeted animal models: subtle immunoregulatory changes appear likely to drive this classic autoimmune disease

CONCLUSIONS

PBC, like all diseases, has a lot more to it than meets the eye, and while three cardinal features may be relevant for diagnosis, there remain many other clinical and laboratory aspects of disease that need more extensive definition. With improved methods of patient phenotyping, greater progress will follow biologically, and hopefully translate into new, effective and specific therapies.

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