Late-Onset Autoimmunity: The Paradigm of Primary Biliary Cirrhosis – A Mini-Review

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Key Words

Tolerance · Autoreactivity · Antimitochondrial antibody · Immunosenescence

Abstract

The incidence of autoimmune diseases appears to be increasing in virtually every population. This is well illustrated by primary biliary cirrhosis, a disease characterized by an immune response to small bile ducts and an incidence increasing with age. The etiology of primary biliary cirrhosis remains enigmatic, although there is clearly an interplay of genetics and environment with a possible role for immunosenescence. The increasing usage of serologic tests for the hallmark of primary biliary cirrhosis, the antimitochondrial antibody, has dramatically changed the spectrum of disease presentation and natural history. In the past, data were predominantly based on the greater prevalence of advanced cases, whereas now most patients are detected earlier through the use of multi-phasic chemistry panels. In this review, we will place the epidemiology of primary biliary cirrhosis in the context of its clinical features and treatment as well as emphasize the future directions to further understand the immunobiology of this disease.

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Is There a Link between Autoimmunity and Immunosenescence?

The majority of autoimmune diseases manifest incidence rates increasing with age as well as a striking predominance in the female sex. These features are quite consistently encountered in patient series with either organ-specific or systemic conditions, thus raising the possibility that these observations may hold the key to the remaining major issues in the development of autoimmunity. Indeed, immunosenescence (i.e. the aging of the immune system) begins approximately in the 55–60 year age range and implies a reduced immune response, as manifest, for example, by a relative inability of senior citizens to produce a protective response following vaccination. These observations are prompted by the age-dependent progressive reduction in the general immune competence [1]. Immunosenescence manifests in all aspects of the immune response by affecting both the innate and acquired immune system compartments and it has been studied particularly in neoplasia where it may constitute a possible therapeutic target [2]. The correlation between immunosenescence and autoimmunity is currently based on several experimental observations but remains inconclusive. In particular, the age at diagnosis of the major

Fig. 1. Age at the time of diagnosis for the major autoimmune diseases. To compute these values, only population-based studies were included; mean results are represented when more than one study is available.

autoimmune diseases varies widely and is illustrated in figure 1. These can be summarized in the hypothesis that the thymus involution related to age may cause a decline in naïve T cells and the accumulation of T-cell clones reacting to a growing number of neoantigens [3], but there are no data on the link between these findings and the development of autoimmune diseases.

A Paradigmatic Disease

Primary biliary cirrhosis (PBC) is an uncommon chronic cholestatic liver disease that is characterized by highly variable rates of progressive destruction of the intrahepatic bile ducts resulting in fibrosis, cirrhosis, and – ultimately – liver failure [4]. The etiology of PBC is unknown but, due to the presence of serum antimitochondrial antibodies (AMAs) and autoreactive T cells in the vast majority of cases, it is currently believed to be of autoimmune pathogenesis, with the E2 subunit of the pyruvate dehydrogenase complex being the primary target of the inflammatory response [5]. Similar to most autoimmune diseases, PBC manifests a striking female predominance [6].

The increasing availability of serological tests for routine AMAs has significantly changed the spectrum of disease presentation. In fact, earlier reports were based on a large preponderance of advanced cases with jaundice while patients are now diagnosed most frequently at asymptomatic and early stages [7]. Other classically accepted symptoms at presentation include fatigue and pruritus, but the true impact and prevalence of these

symptoms remain to be determined. The diagnosis is based on the presence of at least two of three internationally accepted criteria: i.e. serum AMAs at titers >1:40; increased serum alkaline phosphatase levels for at least 6 months, and/or a compatible liver histology. The need for a liver biopsy at the time of PBC diagnosis remains debated and it is currently indicated only in patients lacking one of the other criteria, patients requiring accurate staging (although the possibility of sampling errors should be accounted), or in patients enrolled in clinical trials. Discriminating PBC from other autoimmune or inflammatory liver diseases is usually easy, mostly based on serum autoantibody profiles (table 1).

Epidemiology

As observed for most autoimmune diseases, women with PBC are found to outnumber men by as much as 9:1, although such gender ratio varies widely in different epidemiological studies [6]. These changing numbers may reflect true differences in gender distribution of PBC or may be related to differences in case-finding methods. The largest non-population-based study reported an 8:1 female to male ratio among 1,032 PBC cases [8]. Table 2 summarizes the available data on PBC epidemiology.

Descriptive epidemiology suggests the existence of a geo-epidemiological pattern with the disease being more prevalent in northern European and American areas [6]. Indeed, the highest prevalence and incidence rates have been reported in Scandinavian countries and Great Britain, although the lack of uniform case-finding methods

Table 1. Autoantibody patterns encountered in liver autoimmunity, including PBC, autoimmune hepatitis (AIH, types 1 and 2), and primary sclerosing cholangitis (PSC)

Autoantibody	Molecular targets	Disease (diagnosis)	Other liver diseases
AMA	E2 subunits of 2-oxo-acid dehydrogenase complexes, mainly PDC-E2	PBC (95%)	–
ANA	Multiple nuclear targets Sp100, PML, SUMO gp210.NUP62	AIH-1 (70–80%) PBC (30–50%) PBC (30–50%)	Drug/alcohol hepatitis, HCV, PSC, PBC – –
SMA	Multiple targets: F-actin (most specific) and non-actin components	AIH-1 (80%)	HCV, advanced liver diseases, PBC
Anti-LKM-1 Atypical pANCA or pANNA	CYP2D6 50-kDa neutrophil-specific nuclear pore complex protein	AIH-2 (100%) PSC (88%)	HCV AIH (70%) PBC (5%)
Anti-SLA	UGA-suppressor serine tRNA-associated protein	AIH-1, (10–50%), AIH negative for other Ab, AIH-2	–

AMA = Antimitochondrial antibody; ANA = antinuclear antibody; SMA = anti-smooth muscle antibody; ANCA = antineutrophil cytoplasmic antibody; ANNA = antineuronal nuclear antibody; SLA = anti-soluble liver antigen antibody; HCV = hepatitis C virus; LKM = anti-liver/kidney microsomal antibody; PDC = pyruvate dehydrogenase complex; CYP = cytochrome P450; SUMO = small ubiquitin-like molecule; PML = promyelocytic leukemia protein; gp210 = glycoprotein 210.

Table 2. Reported PBC incidence and prevalence rates and sex ratios [6]

Year	Location	Number of cases	Annual incidence (per million)	Prevalence (per million)	Gender ratio (M/F)
1980	Sheffield, UK	34	5.8	54	1:16
1980	Dundee, UK	21	10.6	40.2	1:9.5
1983	Newcastle, UK	117	10	37–144	1:14
1984	Malmö, Sweden	33	4–24	28–92	1:3
1984	Western Europe	569	4	23 (5–75)	1:10
1985	Orebro, Sweden	18	14	128	1:3.5
1987	Glasgow, UK	373	11–15	70–93	–
1990	Umea, Sweden	111	13.3	151	1:6
1990	Ontario, Canada	225	3.26	22.4	1:13
1990	Northern England	347	19	129–154	1:9
1995	Victoria, Australia	84	–	19.1	1:11
1995	Estonia	69	2.27	26.9	1:22
1997	Newcastle, UK	160	14–32	240	1:10
2000	Olmsted County, Minn., USA	46	27	402	1:8

and the need for population-based studies militate against this hypothesis. The highest prevalence rate described thus far, which was obtained through a retrospective analysis in a Minnesota county, was 40/100,000 [7]. Serological studies performed in large cohorts of healthy subjects have led to the assumption that AMA prevalence in the general population is approximately 0.5%; these stud-

ies have been performed using indirect immunofluorescence while newer methods with recombinant AMA antigens are awaited to produce more representative data. Lastly, it should be noted that anecdotal reports seem to support a clustering of cases in limited areas [9, 10], although the implications of these observations remain unclear.

Table 3. Risk factors for primary biliary cirrhosis observed in our recently completed US-based epidemiological study on 1,032 patients with PBC and 1,041 controls [8]

	β	OR	95% CI	p
Family history of PBC	1.1868	10.736	4.227–27.268	<0.0001
Family history of SLE	0.4019	2.234	1.261–3.957	0.0059
History of urinary tract infections	0.2065	1.511	1.192–1.915	0.0006
Ever smoked during life	0.2252	1.569	1.292–1.905	<0.0001
Ever used hormonal replacement	0.2185	1.548	1.273–1.882	<0.0001
Age at first pregnancy	–0.0470	0.9541	0.9331–0.9755	<0.0001

Several studies have attempted to determine the factors influencing the risk of developing PBC and have been concordant on the association between the disease and a history of recurrent urinary tract infections or a positive family history. Our recently completed epidemiological study (table 3) has demonstrated that a high risk of developing PBC is associated with a positive family history for PBC, a history of urinary or vaginal infections, comorbidity with other autoimmune diseases, lifestyle factors such as smoking, and previous pregnancies. Furthermore, we observed that the frequent use of nail polish also slightly increased the risk of having PBC [8]. This observation has obvious implications based on the xenobiotic theory for PBC occurrence [11], yet we are convinced that further mechanistic evidence is needed before proposing a causative effect of cosmetic products.

Clinical Features

The symptoms accompanying early-stage PBC at presentation are classically recorded as fatigue and pruritus while symptoms in patients with advanced disease are similar to those encountered in cirrhosis cases of other etiologies and include ascites, jaundice, hepatic encephalopathy, and upper digestive bleeding.

Fatigue is an incompletely defined, nonspecific symptom that is reported to affect as many as 70% of patients with PBC while being often overlooked by patients, particularly middle-aged women. The severity of fatigue is independent of the stage of PBC or its other features (pruritus or severe cholestasis) nor does it depend on psychiatric factors [12].

Pruritus is reported by as many as 70% of patients with advanced-stage PBC, often including patients with jaundice. However, the vast majority of patients will eventually experience this symptom during their disease history

and the appearance of pruritus might precede the onset of jaundice. Typically, pruritus secondary to cholestasis is felt to worsen at night, following contact with wool, or in warm climates. It has been suggested that more than one third of all patients manifest skin lesions, but this observation requires confirmation [13].

Portal hypertension is frequently found in patients with PBC and, importantly, may not imply the presence of liver cirrhosis and may be observed long before any clinical or imaging sign of advanced liver disease. Over one half of untreated patients eventually develop portal hypertension over a 4-year period. The prevention and treatment of PBC-associated portal hypertension is not different from other chronic liver diseases.

Accelerated bone loss, often found in postmenopausal women with long-standing cholestasis, is considered a metabolic bone disease. It is not clear whether this is ultimately different when compared to sex- and age-matched healthy individuals [14]. Current treatment of this condition is similar to other cases of osteopenia and osteoporosis and includes oral calcium supplementation and vitamin D replacement. Patients with PBC are commonly characterized by hyperlipidemia with high levels of both serum cholesterol and triglycerides. From a clinical standpoint, these disorders do not increase the incidence of cardiovascular events or atherosclerosis and do not correlate with disease stage [15]. Treatment with bile acid helps to reduce blood lipid levels via unknown mechanisms.

Several autoimmune diseases are more frequently diagnosed in patients with PBC. Our study of 1,032 patients with PBC has demonstrated that one third of cases are also affected by another autoimmune disease, most commonly Sjögren's syndrome, Raynaud's phenomenon, and autoimmune thyroid disease [8]. Interestingly, recent data demonstrated that patients affected by both PBC and scleroderma manifest a less aggressive liver disease,

thus suggesting an active interaction between the two conditions [16]. PBC at cirrhotic stages is a risk factor for the development of hepatocellular carcinoma and patients with intense nodular liver structure at ultrasound should be monitored by computed tomography.

Blood Tests for PBC

From a biochemical standpoint, PBC often presents with a cholestatic pattern represented by persistently elevated plasma alkaline phosphatase and/or γ -glutamyl-transferase levels. Such an increase is typically not accompanied by a similar increase in plasma aminotransferase levels and a careful history allows discrimination from metabolic causes of abnormal liver chemistries. Immunoglobulin M levels are typically elevated in PBC without being correlated with AMA levels, while bilirubin remains within the normal range until the late stages of disease when it becomes an important prognostic factor.

A positive serum AMA is highly specific for PBC and is detected in approximately 90% of patients when sensitive diagnostic methodologies based on recombinant antigens are used [17], although the lack of a detectable serum AMA does not have significance in the disease natural history [18]. In clinical settings, indirect immunofluorescence techniques are used for initial screening of cases and might provide falsely positive and negative results. A recent important addition to PBC clinical management is based on the observation that up to 50% of patients with PBC have detectable serum antinuclear antibodies (ANAs), most commonly producing 'nuclear rim' or 'multiple nuclear dots' patterns [19]. Further, ANA positivity is associated with a worse prognosis.

Liver Histology

Liver histology is not conclusive in several cases in which PBC is suspected. When allowing a diagnosis, Ludwig's classification [20] is used to identify four PBC stages, ranging from portal tract inflammation with predominantly lymphoplasmacytic infiltrates, resulting in vanishing septal and interlobular bile ducts (stage 1) to frank cirrhosis (stage 4). Eosinophil infiltration and non-suppurative granulomas are not uncommon. In cases in which findings are consistent with more than one stage, the disease is defined according to the more advanced one. Stage 1 is characterized by lymphocyte

infiltration of the portal tracts, duct destruction and granular infiltration; stage 2 by the extension of the inflammatory components to the periportal areas or around the bile ductules; stage 3 by septal fibrosis with bridges connecting portal triads, while stage 4 includes frank cirrhosis.

Liver fibrosis observed in PBC is similar to the process found in all evolving chronic liver diseases. Briefly, fibrosis is a wound-healing response to a variety of chronic stimuli and the basis of liver damage is the excessive deposition of extracellular matrix proteins, especially type I collagen on liver tissue [21]. This kind of alteration modifies the structure of the liver ultimately causing organ dysfunction; the activation of hepatic stellate cells (HSCs) is one of the main symptoms primarily responsible for excess collagen deposition during liver fibrosis [22]. During the progression of PBC stages, indeed, HSCs become directly fibrogenic by synthesizing extracellular matrix proteins and HSC proliferation increases the fibrogenic response, in a sort of disease-enhancing self-perpetuating mechanism. The pathways regulating HSC activation/proliferation have not been clearly elucidated in PBC [23] or other chronic liver diseases, but recent evidence suggests an increase in HSC responsiveness to both proliferative and fibrogenic cytokine which then activates intracellular signaling cascades driving to cell cycle progression and collagen gene expression.

Medical Management of PBC

The management of patients with PBC differs significantly in early and advanced cases although symptoms can be encountered in both groups.

Fatigue

The impact and true significance of fatigue in PBC remains to be determined as well-controlled studies are still lacking to define the importance of chronic liver disease in the development of this symptom. Morphological abnormalities of the central nervous system due to accumulation of manganese have been postulated as putative causes of fatigue in PBC [24], but no medical treatment has been shown to be effective in alleviating this symptom. Of note, similar degrees of fatigue can be observed in other autoimmune conditions including systemic lupus erythematosus in which, however, it often correlates with depression rather than with immunological markers or inflammation.

Table 4. Comorbidities (%) observed in previous studies and in our study on 1,032 US patients with PBC

	Previous studies	Gershwin et al. [8]
Sjögren's syndrome	75	10
Raynaud's syndrome	32	12
Autoimmune thyroid disease	11	9
Systemic sclerosis	12	2

Pruritus

What causes PBC itching is not clear and two hypotheses have been proposed based on the retention of bile acids secondary to chronic cholestasis or, alternatively but not exclusively, an amplified release of endogenous opioids [25]. Treating this symptom is often a clinical challenge. Antihistamines have proven to be ineffective and their use should be discouraged particularly in patients with cirrhosis. Conversely, cholestyramine (4 g up to three times a day) significantly ameliorates pruritus. In selected cases poorly responsive to resins, rifampin can be used for short periods. The use of opiate antagonists such as naltrexone (50 mg/day) has limited adverse effects but debated efficacy [26]. Lastly, sertraline is promising in preliminary studies but requires further evaluation and should be considered as a last option. When intractable, pruritus is an indication for liver transplantation.

Portal Hypertension

Portal hypertension in PBC does not follow the development of liver cirrhosis but may precede any clinical or biochemical sign of advanced disease. The prevention and treatment of PBC-associated portal hypertension is not different from other chronic liver diseases [27].

Bone Density Loss

A metabolic bone disease has been found in PBC, with accelerated bone loss due to reduced bone deposition being noted in patients compared to sex- and age-matched healthy individuals. This finding has been inconsistently reproduced. Some degree of osteopenia is observed in 30% of PBC patients while frank osteoporosis is diagnosed in 10% of cases. Current treatment of bone loss is the same as in non-PBC cases, including oral calcium supplementation, weight-bearing activity, and oral vitamin D replacement (if a deficiency is present). Oral alendronate is a promising new treatment in these patients

[28]. Liver transplantation worsens the bone loss due to the use of immunosuppressive drugs and steroids.

Blood Lipid Profiles

As many as 85% of patients with PBC manifest hyperlipidemia, particularly hypercholesterolemia, independent of the disease stage. Interestingly, there seems to be no association with an increased cardiovascular risk or the onset of early atherosclerotic lesions [29]. The impact of ursodeoxycholic acid (UDCA) on blood lipid levels remains to be determined.

Associated Conditions

Approximately one third of patients with PBC manifest an additional autoimmune disease [8]. Table 4 illustrates the most commonly associated diseases and their prevalence in PBC according to our recent study. Raynaud's (12%) and Sjögren's syndrome (10%) are the most frequently encountered conditions, but also scleroderma comorbidity is common. The observed discrepancy between our recent data and previous figures is, in our opinion, to be explained with the large population included in our study or the self-referred nature of comorbidities.

Malignancies

PBC with cirrhosis should be monitored for the occurrence of hepatocellular carcinoma [30] using ultrasonography (and computed tomography in selected cases) twice a year. Previously suggested associations between PBC and cholangiocellular carcinoma or breast cancer have not been confirmed.

Treatment of PBC

UDCA is the only medication approved by the US Food and Drug Administration and worldwide for the treatment of PBC [4]. The mechanism of action of UDCA remains elusive and is considered to be at several levels. In fact, UDCA changes the bile acid pool by promoting its hydrophilic conditions and endogenous bile acid secretion [31]. Further, UDCA exerts in vitro anti-inflammatory and anti-apoptotic effects [31]. The currently recommended regimen is a total of 12–15 mg/kg divided into three daily administrations [4]. UDCA efficacy has been debated since conflicting data were provided by a comprehensive meta-analysis. This meta-analysis, however, took into account studies using suboptimal doses, small groups of patients, or insufficient follow-up duration [32]. Large studies with recommended doses have supported the benefits of UDCA treatment in early dis-

ease stages in terms of biochemical response [33] and disease progression [34, 35]. These benefits are virtually absent in patients who initiate the treatment when cirrhosis has already developed, while results are also debated in patients who manifest an incomplete biochemical response (i.e. do not normalize alkaline phosphatase levels).

In contrast to most autoimmune diseases, PBC does not respond to treatment with immunosuppressants, although the role of steroids may need reevaluation [36]. Similarly, the debated effects of methotrexate are complicated by severe side effects [37] while colchicine is ineffective [38].

Liver transplantation is the ultimate treatment for end-stage PBC. The timing and indications are similar to other advanced liver diseases. PBC recurs following transplant and favoring factors have been suggested [39]. We are convinced that PBC recurrence following allograft might constitute a major culprit to obtain definitive evidence on the disease mechanisms since it suggests that cholangiocytes might be an innocent victim of the autoimmune aggression. In this regard, the proposed mechanisms are of great interest [40].

Prognostic Factors in PBC

Several studies have consistently demonstrated that AMA titers or patterns do not correlate with PBC stage or prognosis [18, 41, 42] and their serial testing is not recommended. It is still debated if ANAs directed against antigens of the nuclear pore complex or 'nuclear dot' are associated with features of more severe disease. It has been recently demonstrated that PBC-specific ANAs are stable over time and may identify a subgroup of patients who will manifest a more aggressive disease [43]. The Mayo score is a validated prognostic index that takes into account age, serum bilirubin and albumin levels, prothrombin time, and the presence of edema (including ascites) [44], but its applicability seems limited to advanced cases.

PBC Etiology and Pathogenesis

Several features and observations strongly support an autoimmune pathogenesis for PBC while others make PBC a peculiar entity among autoimmune diseases [5]. Indeed, PBC is characterized by the presence of detectable and specific AMAs in approximately 90% of affected

individuals, although we note that patients lacking AMAs present a similar disease and progression compared to AMA-positive subjects, seemingly arguing against a pathogenic role for these autoantibodies. Autoreactive T cells, both CD4+ and CD8+, have been identified in PBC regardless of the AMA status and such lymphocytes and AMAs recognize overlapping epitopes within the mitochondrial autoantigens. No direct proof has been provided for a direct pathogenic role of AMAs or autoreactive cells in bile duct injury. In most cases, indirect immunofluorescence is used for AMA screening but novel methods have led to a reduction in the AMA-negative subgroup of patients [45]. Several studies have attempted to associate AMA patterns or titers with a number of variables, including disease severity, but have failed to clearly demonstrate any correlation. In a subset of patients with PBC (in some cases approaching 50%), serum ANAs are detected, more specifically producing 'nuclear rim' or 'multiple nuclear dots' staining patterns, based on the recognition by the autoantibodies of gp210 and nucleoporin 62 (within the nuclear pore complex) and nuclear body protein sp100 and PML [19]. Most recently, several comprehensive spontaneous animal models have been developed, including a NOD mouse variant, and TGF- β IR or IL2AR knockout mice [46].

We are convinced, based on numerous studies, that both genetic and environmental factors are necessary for the development of PBC. The importance of genetic susceptibility to PBC has been recently supported by the 63% pair-wise concordance among monozygotic twin sets compared to the null concordance among dizygotic ones [47]. In contrast to the majority of autoimmune diseases, there are no clear associations with human leukocyte antigen haplotypes, while several other genetic associations have been reported but independent confirmation is still awaited [6]. Most recently a role for sex chromosome abnormalities has been proposed [48]. Other mechanisms such as molecular mimicry by either microorganisms (most recently *Novosphingobium aromaticivorans*) [49] or xenobiotics [11] may also play a role.

Conclusions and Future Directions

As PBC suggests, the relationship between autoimmunity and immunosenescence should not be overlooked. Indeed, the observation that most autoimmune conditions share female predominance [50] and the incidence peak at older ages is of major support for this hypothesis and should be the focus of upcoming research. We believe

that autoimmunity should be regarded as a unique entity [51] and that solving one enigma will eventually solve others, regardless of the differences in organ specificity. In the past year, multiple lines of evidence have prompted us to look at new directions to solve the autoimmunity enigma, as well represented by the new experimental data on B [52] and T cells [53], or the solid epidemiology clus-

tering of autoimmune diseases [54], or their solid unsuspected associations [55]. For all the above reasons, only a strenuous coordinated effort of gerontologists, immunologists, and basic scientists with the aid of newer laboratory techniques (well represented by genome-wide multiplex platforms) will provide an answer to the several remaining questions in autoimmunity development.

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