Autoimmun Highlights (2012) 3:105–112
DOI 10.1007/s13317-012-0038-z

REVIEW ARTICLE

Predicting and preventing autoimmunity: the case of anti-mitochondrial antibodies

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Received: 22 September 2012 / Accepted: 3 October 2012 / Published online: 16 October 2012

Abstract To be able to predict who will develop autoimmune disease would allow for early treatment which may dramatically alter the course of the disease. In some cases, it may also lead to prevention of the disease development. The prediction of disease development is based on the analysis of risk factors which have been associated with the disease in question. These factors include genetic susceptibility, as well as immunological and environmental factors. One autoimmune disease that may serve as a model for disease prediction is primary biliary cirrhosis (PBC), an autoimmune liver disease affecting the small- and medium-sized bile ducts. PBC could be an ideal model due to recent advances in elucidating its genetic associations. As well, a variety of immunological and environmental risk factors have been well established. Indeed, the presence of PBC-specific antimitochondrial antibodies and/or antinuclear antibodies has been shown to be predictor of disease development and possibly prognosis. This review will examine the current evidence which suggests that we may potentially be able to predict the development of PBC in some individuals. These concepts may also be applied to autoimmune diseases in general.

Keywords Autoimmunity · Autoimmune disease · Genetics · Prevention · Prediction · Risk factor · Susceptibility

Abbreviations

AMA	Antimitochondrial antibodies
ANA	Antinuclear antibodies
E. coli	*Escherichia coli*
L. delbrueckii	*Lactobacillus delbrueckii*
PBC	Primary biliary cirrhosis
PDC-E2	Pyruvate dehydrogenase complex
UTI	Urinary tract infection

Introduction

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterised by immune-mediated destruction of the small- and medium-sized intrahepatic bile ducts, which in many individuals may progress to cirrhosis and liver failure [1, 2]. In advanced cases, transplantation may be required. At the time of diagnosis, patients may be symptomatic or asymptomatic, with asymptomatic patients having normal or abnormal biochemistry tests, possibly with cholestatic indices being raised [1–6]. Symptomatic patients typically present with non-specific symptoms such as fatigue, pruritus and arthralgias, with liver disease not being suspected initially

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[1–6]. It is not uncommon for PBC patients to have had several non-hepatological investigations and consultations for their symptomatology. In advanced stages of the disease, patients may present with complications from portal hypertension and hepatic decompensation, such as jaundice, ascites or variceal bleeding [1–6]. Although there may be great variation in the presenting signs and symptoms, the commonality found among patients are the presence of anti-mitochondrial (AMA) or disease-specific antinuclear antibody (ANA), which are present in almost all patients [7–9]. In order to establish the diagnosis a patient has to fulfil two from the three criteria mentioned below: elevated alkaline phosphatase (ALP), seropositivity for disease-specific AMA and/or ANA, as well as specific histological features of PBC [1, 5, 6]. Histological features of PBC are destruction of the biliary epithelial cells, ductopaenia, portal inflammatory cell infiltration, as well as granuloma formation [1, 2]. Cholestatic markers include increased levels of alkaline phosphatase and γGT [1–6, 10]. PBC is a slowly progressive disease, with the clinical course being well known as unpredictable [1–6, 10]. Medical treatment of PBC is with ursodeoxycholic acid (UDCA) administered at an appropriate dose (13–15 mg/kg/day) [1–6]. Early administration of UDCA slows the disease progression and has improved the quality of life in many patients [1–6, 11].

Epidemiological studies from the USA, UK and France have established several risk factors to be associated with PBC development [12–15]. Although risk factors vary among the studies, recurrent urinary tract infection, a history of smoking and oestrogen disturbance have been found to be risks in all studies. Female sex as well as being a first degree relative to a patient with PBC also increases the risk for disease development [12–15]. In recent years, genetic and genome wide association studies (GWAS) have identified several disease genes to be associated with PBC. A combination of genetic and environmental factors likely work together in the development of the disease.

Currently, no reliable prognostic index has been developed which can predict the course of the disease and its outcome over time in patients at early stages [1, 3–6]. However, given that several risk factors have been identified, the question remains as to whether we can predict the development of PBC in some individuals. Indeed, this question is raised not only with PBC, but with all autoimmune diseases in general. If we can predict the development of PBC, it may be possible to prevent disease development in some or detect the disease early in others. This review will examine the known risk factors for PBC development, in an attempt to analyse whether PBC development is predictable in certain individuals. This approach with PBC would perhaps be applied to other autoimmune diseases.

PBC-specific autoantibodies as prognosticators of disease's development

AMA are pathognomic for PBC as they are present in up to 95 % of PBC patients, and their presence in asymptomatic individuals is generally believed to precipitate eventual disease development [1–9, 16–18]. Several studies indicate that the prevalence of AMA in the general population is <1 % [19–21]. In PBC patients, AMA react against components of the 2-oxo-acid dehydrogenase complexes and mostly recognise the E2 subunit of the pyruvate dehydrogenase complex (PDC) [1, 2, 9, 16–18, 22–26]. AMA are reactive against PDC-E2 in more than 90 % of cases, and there is also cross-reactivity with the PDC-E3 binding protein (E3BP) [27–29]. Other targets (in 20–70 % of cases) include the E2 subunits of branched-chain 2-oxoacid dehydrogenase complex (BCOADC) and 2-oxoglutarate dehydrogenase complex (OGDC) and the E1α and E1β subunits of PDC [1, 7, 8, 24, 26–29].

The Newcastle group has studied the significance of AMA in detail, and they indicate that the majority of asymptomatic, non-cholestatic patients positive for AMA, have histological features of PBC [23, 30]. Their first study included 29 patients with positive AMA (greater than 1/40 titer by indirect IFL), who were asymptomatic for liver disease [23]. The average age of the cohort was 54.7 years, 28 patients were females, and one set consisted of a mother–daughter pair [23]. All patients had normal LFTs, although raised γ-GT levels were found in 14 individuals [23]. Ten patients had elevated IgM levels, and 2 had marginally raised IgG, with an IgG subclass distribution of the AMA being primarily IgG3 followed by IgG1 [23]. Histological examination of liver biopsies demonstrated that 12 individuals had changes compatible with PBC, including inflammatory cell infiltrates around damaged bile ducts with and without macrophage granulomata in the portal tracts [23]. Twelve had non-specific chronic hepatitis around bile ducts or lymphoid aggregates within the portal areas [23]. Mild fibrosis was found in seven biopsies, and only two biopsies in the entire cohort were completely normal [23]. After a mean 8.7-year follow-up, one patient had died of post-cricoid carcinoma, and five developed PBC symptoms (unexplained pruritus with malaise or malaise alone) [23]. Two of 13 patients followed-up for <4 years had developed pruritus, and 12 of 16 (all female) followed up for more than 4 years had developed abnormal LFTs [23]. These results led those authors to conclude that AMA positivity in the absence of symptoms, with or without normal liver disease-related biochemistry, is predictive of future PBC development, and many of these patients may already have histological evidence of early PBC [23]. The same group followed up the same cohort 10 years later [30]. The median follow-up of those patients

was 17.8 years, ranging from 11–23.9 years, although five of the original patients had died from non-liver-related causes [30]. PBC symptoms had developed in 22 of 24 patients (76 %), which included pruritus, ongoing fatigue, and chronic right upper quadrant pain [30]. Persistently cholestatic LFTs were reported in 24 patients (83 %) [30]. Repeat liver biopsy material was available in ten patients and showed fibrosis progression from grade 1 to 2 in two individuals, and progression from grade 2 to 3 in another two [30]. Cirrhosis had not developed in any of the patients [30]. ELISA of baseline serum samples demonstrated positive AMA in 21 of 27, all of which had initial biopsy results compatible with PBC, confirming the presence of PBC in asymptomatic, non-cholestatic patients with positive AMA [30].

A study of another cohort has produced similar results. Kisand and colleagues [19] conducted an ELISA-based study for antibodies to PDC-E2, the major PBC-specific autoantigen, in three groups of Estonian patients. The first group consisted of 1,461 individuals (ages 15–95 years, mean 41 years, 637 males and 824 females), the second 497 individuals (ages 50–91 years, mean 61 years, 189 males and 308 females), and a third of 104 volunteers from neighbouring small villages (mean age 42 years, 29 males and 75 females) [19]. Fourteen individuals were found to have antibodies to PDC-E2, all of which were asymptomatic [19]. These patients were followed-up at 2, 4, 7 and 9 years [19]. Eight of the 14 were available for follow-up, and 3 of the 8 had developed abnormal LFTs by the 9th year of follow-up [19]. These patients also had AMA detectable on indirect IFL, had inhibitory antibodies to PDC and had anti-PDC of the IgG and IgA subclass, although the IgG subclass was predominant [19]. The remaining five AMA positive patients had low titer positivity, remained positive for AMA on follow-up, but did not develop any evidence of cholestasis [19]. Unfortunately, liver biopsies were not performed in any patients, apparently due to lack of consent [19]. The above studies demonstrate the strong predictive value of AMA positivity for future PBC development.

ANA are also found in PBC, and their presence suggests a more progressive form of the disease [1, 7, 8, 24, 26–29, 31–41]. Several PBC-specific ANA patterns have been identified by indirect immunofluorescence (IFL). These include the "multiple nuclear dot" and "nuclear membrane/rim like" patterns [7, 8, 42, 43]. The "multiple nuclear dot" pattern relates to autoantibodies against Sp100, Sp140, promyelocytic leukaemia nuclear body proteins and small ubiquitin-like modifiers [1, 7, 8, 24, 26–29, 31–35]. The "nuclear envelope/rim like" pattern relates to reactivities specific for gp210 and nucleoporin p62 [42]. Both patterns show disease specificity and are present in approximately 30 % of patients. These ANA may also be present in patients who are AMA negative, in addition to asymptomatic individuals and family members of PBC patients [8, 17, 31–35, 44, 45].

The initiating events leading to AMA/ANA seropositivity, as well as the pathological role of these autoantibodies, remain unknown [1, 44, 46–49]. It has been suggested that impaired T-regulation, apoptosis-mediated autoimmune attack, xenobiotics-induced cellular destruction and molecular mimicry and immunological cross-reactivity may account for the development of autoantibodies [50–85]. Experimentally, numerical and/or functional impairment of regulatory T cells has been demonstrated in PBC [55, 65–67, 86–89]. Apoptotic blebs of biliary epithelial cells have been shown to contain intact immunoreactive mitochondrial autoantigens, indicating a role for apoptosis in mediating tissue-specific injury in PBC [1, 46, 47, 64]. The method with which xenobiotics may induce the formation of autoantibodies is similar to that seen in microbial/self molecular mimicry [1, 75, 76]. Molecular mimicry occurs when antigenic similarities between microbial or viral antigens and self-targets lead to the induction of cross-reactive immune responses targeting both microbial and self-epitopic regions [1, 7, 26, 46, 49–54, 56, 58, 59, 63, 68, 70–72, 83, 90]. The immune attack against self targets may initiate a self-perpetuating progressive inflammation and destruction of the biliary epithelial cells [1, 7, 26, 46, 49–54, 56, 58, 59, 63, 68, 70–72, 83, 91]. A variety of environmental and infectious agents, as well as immunological and genetic factors have been suggested as potential triggers for the induction of PBC-specific antibodies[50–54, 58, 59, 64, 73–83, 86–89].

The prevalence of AMA, and perhaps to a lesser extent PBC-specific ANA, may be useful in the identification of individuals at risk of PBC development. This is especially the case in first-degree relatives of PBC patients, with female relatives being at higher risk than others. Indeed, the most common familial pairing in PBC is seen in mother–daughter and sister pairs [92–95]. Therefore, the screening of these individuals for disease-specific autoantibodies may identify those asymptomatic ones who are likely to go on to develop PBC. Further screening of these patients for markers of cholestasis would be recommended, as would early treatment.

Genetics

Bianchi et al. in this special issue discusses in great detail the influence of environment and genetics in the development of PBC. It appears that female relatives of PBC patients have higher risk for PBC compared with demographically matched controls, which demonstrates the likelihood of genetic factors being involved in the disease

development [90, 96–101]. The female preponderance of the disease may be due to genetic factors affecting the X chromosome, as a higher frequency of monosomy X in peripheral leukocytes has been found in patients with PBC [62, 102–106]. A single study in twins demonstrated a high concordance among monozygotic twins and a low concordance among dizygotic twins [107]. Genetic association studies (GWAS) have identified multiple gene loci to be associated with PBC, such as HLA DQB1, IL12A, IL12RB2, STAT4, CTLA4, IRF5-TNPO3, 17q12-21, and MMEL1, 17q12-21, DENND1B, CD80, IL7R, CXCR5, TNFRSF1A, CLEC16A and NFKB1 [108, 109, 110–113]. Although GWAS and other genetic studies provide us with a list of genetic associations, it is not clear as to how many of these associations are required for disease development. As well, there is no prognostic significance attached to these genetic associations although it is likely that future research will elucidate those genes which are responsible for fast versus slow disease progression. Genetic associations may also assist in the identification of those at higher risk of disease development, not only in PBC, but also in other conditions in which there is a strong genetic influence. Patients with a particular genetic trait (likely in combination with other clinical and demographic data) may be monitored for biochemical, immunological and clinical indices of disease development. As well, patients who have a susceptible trait may be advised in regard to risk reduction, such as hormonal therapy or smoking cessation and the aggressive treatment of recurrent UTI. All of these have been shown to be risk factors in PBC development [12–15, 114].

Recurrent urinary tract infections

Recurrent UTI has been indicated as a risk factor for PBC development [12–15, 94]. UTI was first suspected as a risk factor for PBC when investigators noted a higher incidence of bacteriuria in PBC patients compared with controls [115]. A study by Gershwin et al. [13] demonstrated that 59 % of 1,032 PBC patients reported a history of UTI. Prince and colleagues [15] reported similar associations in a study involving one group of 318 PBC patients from a geographically defined area and another involving 2,258 patients from a PBC support group [15]. UTI and PBC were found to be associated in the multivariate analysis (in both PBC groups) but not in a cohort of 3,936 demographically matched controls [15].

As a major causative organism of UTI, *E. coli* has been identified as an organism of interest in PBC, and it has been suggested that molecular mimicry between human and *E. coli* PDC-E2 may be involved in the breakdown of tolerance to PDC-E2 [116, 117]. Molecular mimicry and immunological cross-reactivity between bacterial-self peptides has been investigated as a mechanism responsible for the induction of liver autoimmunity [49, 57, 61, 63, 68–70, 91, 118–123]. At the experimental level, sera from PBC patients react with both *E. coli* and human PDC-E2. Also, more than half of the patients with recurrent UTI cross-recognize human PDC-E2 [124]. The significance of the disease-specific presence of antibodies against an ATP-dependent Clp protease of *E. coli* remains unclear [125–127]. Amino acid homologies between *E. coli* and human PDC-E2 sequences required for T cell epitope recognition of PDC-E2 have also been reported and pathogenic scenarios involving these epitopes in the pathogenesis of PBC have been formulated [119, 128]. Elegant studies have demonstrated that the *E. coli* and human PDC-E2 homologues are targets of cross-reactive responses at the CD4 T cell level [119, 129].

Several other organisms have been associated with PBC via a link with recurrent UTI, including *Lactobacillus delbrueckii* (*L. delbrueckii*). Vaginitis and vaginal infections have been found to be prevalent among PBC patients, and the question arises as to whether these are due to *L. delbrueckii*, which is part of the normal flora of the vagina [13, 14]. A pathogenic scenario implies that infections with *L. delbrueckii* increase susceptibility to vaginal infections, leading to recurrent UTI and subsequently PBC [130]. However, this hypothesis lacks experimental validation. Lactobacilli have also been linked to PBC development a 39-year-old female who received Lactobacilli vaccination for recurrent vaginitis [131]. The authors of this study have speculated that an immune response against Lactobacilli initiated a cross-reactive response targeting human PDC-E2 via a mechanism of molecular mimicry. The sera of the patient tested positive for AMA and the AMA targeting epitope from beta-galactosidase of *Lactobacillus delbrueckii* [131]. This is an interesting finding taking into account a previous report demonstrating that *L. delbrueckii* and human PDC-E2 share sequences in common which are targeted by antibodies specifically found in approximately 50 % of patients with PBC [130]. The fact that *L. delbrueckii* is a cause of recurrent UTI in a considerable proportion of elderly women further supports the theory involving this infectious agent in the pathogenesis of PBC [132, 133].

Despite UTI being linked to PBC, it remained unclear as to whether this association was causal or casual, as changes in mucosal immunity may infer an increased susceptibility to UTI via alterations in vaginal flora. A recent study by Varyani and colleagues [134] found that UTI preceded the diagnosis of PBC in a cohort of PBC patients. That study involved 800 PBC patients, 7,991 matched controls and 12,137 patients with chronic liver disease as controls [134]. UTI had been diagnosed within 1 year prior to PBC

diagnosis in 29 % of PBC patients, in comparison with 22 % of healthy controls and 17 % of chronic liver disease controls [134]. UTI had been diagnosed in 19 % of PBC patients within a 5-year period prior to PBC diagnosis, compared with 14 % of matched controls and 11 % chronic liver disease controls [134]. That study has demonstrated that in a large cohort of PBC patients, UTI does precede PBC diagnosis and therefore adds further evidence towards a potential role for molecular mimicry and cross reactivity. It also highlights that recurrent UTI may be a predicting factor of PBC development and may prompt aggressive antibiotic therapy for those who have further risk factors (such as family history, AMA positivity, or genetic traits).

Conclusions

To predict whether one will develop an autoimmune disease is as complex matter as the aetiology of the diseases themselves. The most important factors which must be taken into account are risk factors which have been associated with the disease and their additive effect within the individual. As with PBC, it is unlikely that genetic associations can wholly predict the disease development, but a variety of other intrinsic and extrinsic factors must also be taken into account. AMA and/or ANA positivity, the disease-specific autoantibody markers, appear to have great predictive value in both disease development and prognosis, with a history of UTI adding to the likelihood of PBC development.

New high-throughput methods are now permitting rapid screening of hundreds of autoantibodies at an affordable cost. Screening of such autoantibodies with significant positive predictive value for specific autoimmune diseases, including those seen in patients with primary biliary cirrhosis, may become a routine part of medical management in the long run. The individuals screened for such autoantibodies will only be those who have HLA and non-HLA genes conferring susceptibility to specific autoimmune diseases, as detected by genetic screening. Close monitoring for evidence of exposure to specific environmental and infectious agents that increase the risk for the development of autoimmunity may assist in the clinical management of these cases. The benefit and cost savings of such practice will be tremendous once preventative methods and cost-effective, targeted therapies become available.

Conflict of interest None.

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