Infection as a risk factor in the pathogenesis of primary biliary cirrhosis: Pros and cons

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Abstract. Primary biliary cirrhosis (PBC) is a chronic and slowly progressive cholestatic liver disease of autoimmune etiology, characterized by injury of the intrahepatic bile ducts that may eventually lead to cirrhosis and liver failure. Evidence suggests cardinal roles for both environmental factors and genetic susceptibility. Nevertheless, the absolute etiology of PBC is unclear, despite recent well-designed case-control studies that reported environmental risk factors, including infectious agents, for PBC. Of the reported infectious agents, some of them are not reproducible and remain controversial. However, infection is no doubt one of the major risks among the environmental factors. This is supported by the fact that infectious agents in autoimmune diseases express antigens resulting in molecular mimicry and xenobiotics that play a role in breaking tolerance. Taken together, recent findings from genome wide assays as well as novel animal models may enable us to better understand the mechanism of pathogenesis responsible for this disease.

Keywords: Primary biliary cirrhosis, risk factors, infectious agents, xenobiotics

1. Introduction

Primary biliary cirrhosis (PBC) is an autoimmune liver disease that often affects middle-aged women. Anti-mitochondrial antibody (AMA), which is a hallmark of this disease, is present in approximately 90% of patients [1]. High association with other autoimmune diseases supports the concept of autoimmunity in PBC. Previous extensive evidence suggests a significant role for genetic susceptibility: family history [2–5], high concordance in monozygotic twins [6,7], HLA typing [8,9], SNP analysis [10–13] and, most strikingly, the results from recent genome wide assay [14–16]. Case-control studies support the idea that environmental factors may trigger the onset of the disease [17–25]. However, the environmental factors are less defined than genetic factors and the etiology of PBC is poorly understood.

In this review, we will overview the risk factors for PBC. Furthermore, we will summarize the current knowledge of infectious agents, one of the major etiologies among environmental factors, and xenobiotics in the pathogenesis of PBC, and discuss the directions for future research.

2. Overview of the risk factors in PBC

Until 2000, there were few published case-control studies of PBC that described some of the risk factors for the disease [17–20]. The first study, enrolling 38 patients with PBC by Baur G et al. in 1982, examined the high prevalence of miscarriage ($n = 11$, 29%) in hospital in-patients as compared to their counterpart controls (with no liver disease, chronic-aggressive hepatitis and other forms of liver disease) [17]. Two studies investigated the association of urinary tract infection (UTI) with other liver disease by using controls: one by Burroughs A et al. ($n = 89$) [18] and the other by Floreani A et al. ($n = 160$) [19]. However, these studies did not take an age-match control into consideration during their analysis. Moreover, all of these studies recruited

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less than 160 patients with PBC in their cohort, which is far less than a recent case-control study that included over 2,500 patients [21]. Nevertheless, an observation of significant bacteriuria, for instance, found in patients with PBC [report by Burroughs A et al. showing higher prevalence in PBC (19%) compared to other types of chronic liver disease (7%) and rheumatoid arthritis (8%)] contributed and led to designing a study in order to further investigate the association of UTI. The only study using healthy age-matched controls and PBC patients ($n = 87$) compared the frequency of hysterec-tomy, dilatation and curettage: the indications in both instances being menorrhagia mainly caused by high estrogen states such as endometriosis [20]. Again, this finding encouraged the researchers to further examine involvement of reproductive factors in female patients with PBC.

Since 2000, there have been five large case-control studies [21–25], all from Western countries, which looked into risk factors for PBC [Tables 1, 2]. The Newcastle group conducted an exploratory, population-based case-control study asking 123 patients to complete a questionnaire; 100 cases (81%) responded [22]. Although they did not use multivariate analysis in their study, they found an unexpected association with past smoking history (ever smoked: OR 2.4, 95% CI 1.4–4.1; smoked more than 20 years: OR 3.5, 95% CI 1.9–6.3) and psoriasis (OR 4.6, 95% CI 1.2–17.3) as risk factors, whereas eczema (OR 0.13, 95% CI 0.02–1.0) was found to be a “protective role”. However, they failed to show an association to PBC in surgical procedures such as appendectomy and tonsillectomy, events in pregnancy, past infections including UTI, vaccinations, drinking alcohol or taking medications. The family history of PBC and other autoimmune diseases showed an associative tendency but this was found to be less than had been previously reported.

A decade later, the same group has recently expanded the study, recruiting patients from a geographically defined epidemiology study (epidemiological cases, $n = 318$) and a survey of the National Patient Support Group (foundation cases, $n = 2,258$) within Northeast England [21]. In the univariate analysis, they showed a significant association with smoking, hair dye use, previous history of UTI, psoriasis, shingles and previous autoimmune diseases as risk factors for PBC in both epidemiological cases and foundation cases as compared to the controls. For the first time, they have also shown previous obstetric cholestasis as a risk factor. Finally, they have strictly analyzed the aforementioned risk factors by using multivariate analysis and found

a significant association to smoking, hair dye use and previous history of UTI in both epidemiological cases and foundation cases in comparison to controls, suggesting the strength of these three risk factors. Previous history of psoriasis, shingles, previous autoimmune diseases and previous obstetric cholestasis were significantly associated only in epidemiological cases or foundation cases but not in both. Additionally, they could not show the protective role of eczema in this study.

On the other hand, the first report from the United States in 2001 comparing PBC patients ($n = 199$) to their friends without PBC identified an association with other autoimmune diseases (OR 4.9, 95% CI 2.4–10.2), smoking (OR 2.0, 95% CI 1.1–3.8), tonsillectomies (OR 1.9, 95% CI 1.0–3.4) and UTI in females only (OR 2.1, 95% CI 1.1–4.1) [23]. These results of elevated ORs held true when compared between PBC patients and their siblings.

A nationwide controlled interview-based study from 23 tertiary referral centers for liver disease in the United States included 1032 patients [24]. Their data indicated that a first-degree relative with PBC (OR 10.8, 95% CI 4.2–27.3), systemic lupus erythematosus (OR 2.2, 95% CI 1.3–4.0), Sjogren syndrome (OR 5.8, 95% CI 1.3–26.4), history of UTI (OR 1.5, 95% CI 1.2–1.9), history of smoking (OR 1.6, 95% CI 1.3–1.9), and use of hormonal replacement (OR 1.5, 95% CI 1.3–1.9) were significantly associated with an increased risk of PBC. Interestingly, frequent use of nail polish was weakly associated with the risk of having PBC, indicating the involvement of cosmetics in the pathogenesis of PBC.

Another and most recent report from Europe but the first one from France confirmed some of the previously identified risk factors for PBC: family history of PBC (OR 6.8, 95% CI 2.8–16.4), history of smoking (OR 3.1, 95% CI 2.0–5.0) and UTI (OR 2.7, 95% CI 2.0–3.7) [25].

Taken together with the results from the previous case-control studies, it is likely that environmental factors that include infectious agents through UTI (*Escherichia coli* as a representative bacterium), chemicals contained in cigarette smoke, cosmetics (nail polish and hair dye) and exogenous estrogens in female may induce PBC in genetically susceptible subjects.

3. Infectious agents as an environmental factor in primary biliary cirrhosis

Infectious agents such as *Streptococcus* and *Campylobacter jejuni* can induce disorders such as rheumat-

Table 1
Risk factors for PBC derived from univariate analysis in major case-control studies since 2000

	Howel D [22]		Parikh-Patel A [23]		Gershwin ME [42]		Prince MI [21]*		Prince MI [21]**		Corpechot C [25]	
	Yes/No	OR (95% CI)	Yes/No	p-value	Yes/No	p-value	Yes/No	OR (95% CI)	Yes/No	OR (95% CI)	Yes/No	p-value
Life style factors												
Smoking history	Yes	2.4 (1.4–4.1)	Yes	< 0.01	Yes	< 0.005	Yes*	1.6 (1.3–2.1)*	Yes**	1.6 (1.4–1.8)**	Yes	< 0.0001
Hair dye					Yes	< 0.05	Yes*	1.3 (1.0–1.8)*	Yes**	1.3 (1.1–1.5)**	No	
Nail polish use					Yes	< 0.0001						
AIDs in study participants												
Rheumatoid arthritis	Yes	2.1 (1.0–4.2)			No		Yes*	1.5 (1.1–2.1)*	Yes**	1.2 (1.0–1.5)**	No	
Systemic lupus erythematosus			No		Yes	< 0.0001					No	
Autoimmune thyroiditis	No		Yes	< 0.0005	Yes	< 0.0001	Yes*	2.4 (1.8–3.1)*	Yes**	1.6 (1.4–1.9)**	Yes	< 0.0001
Celiac disease							No*	2.0 (0.9–4.3)*	Yes**	2.0 (1.3–3.1)**		
Raynaud syndrome			Yes	< 0.005	Yes	< 0.0001					Yes	< 0.0001
Scleroderma			No		Yes	< 0.0001					No	
Sjogren's syndrome			Yes	< 0.0001	Yes	< 0.0001					Yes	< 0.0001
Autoimmune hepatitis			Yes	< 0.0001	Yes	< 0.0001					Yes	< 0.0001
Psoriasis	Yes	4.6 (1.2–17.3)					Yes*	1.9 (1.2–2.9)*	Yes**	1.3 (1.0–1.7)**	No	
Family history of AIDs												
Primary biliary cirrhosis	No				Yes	< 0.0001					Yes	< 0.005
Rheumatoid arthritis	No				Yes	< 0.05					No	
Systemic lupus erythematosus					Yes	< 0.005					No	
Autoimmune thyroiditis	No				Yes	< 0.0001					Yes	< 0.0001
Raynaud syndrome					Yes	< 0.05					No	
Sjogren's syndrome					Yes	< 0.0005					No	
Polymyositis					Yes	< 0.005					No	
Other past history												
Urinary tract infection	No		Yes***	< 0.01	Yes	< 0.0005	Yes*	2.1 (1.6–2.7)*	Yes**	1.8 (1.5–2.1)**	Yes	< 0.0001
Eczema	Yes	0.13 (0.02–1.0)					No*	1.0 (0.6–1.5)*	Yes**	1.4 (1.0–1.6)**		
Shingles			No				Yes*	2.4 (1.8–3.1)*	Yes**	1.2 (1.0–1.4)**		
Tonsillectomy			Yes	< 0.01	Yes	< 0.005	No*	1.1 (0.8–1.5)*	Yes**	1.7 (1.5–1.9)**	No	
Appendectomy			No		No		No*	1.3 (0.9–1.8)*	Yes**	1.6 (1.4–1.7)**	No	
Cholecystectomy			Yes	< 0.01	Yes	< 0.0001					Yes	< 0.05
Colon polyp removal			No		Yes	< 0.0005					No	
Hysterectomy	No				No		No*	1.0 (0.8–1.4)*	Yes**	1.3 (1.1–1.5)**	No	
Reproductive history												
Hormone replacement	No				Yes	< 0.0001					No	
Birth control pill											Yes	< 0.05
Age at first pregnancy					Yes	< 0.05					Yes	< 0.01
Abortion and/or miscarriage											Yes	< 0.05
Pruritus during pregnancy	No						Yes*	2.1 (1.3–3.6)*	Yes**	2.2 (1.6–3.0)**	Yes	< 0.0005

*epidemiological cases vs. controls; **foundation cases vs. controls; ***female patients only; AIDs: autoimmune diseases; OR: odds ratio; CI: confidential interval.

Table 2
Risk factors for PBC derived from multivariate analysis in major case-control studies since 2000

	Parikh-Patel A [23]	Gershwin ME [24]	Prince MI [21]*	Prince MI [21]**	Corpechot C [25]
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Life style factors					
Smoking history	2.0 (1.1–3.8)	1.6 (1.3–1.9)	1.6 (1.2–2.3)*	1.5 (1.3–1.7)**	3.1 (2.0–5.0)
Nail polish		1.002 (1.000–1.003)			
Hair dye			1.8 (1.2–2.7)*	1.3 (1.0–1.5)**	
AIDs in Study Participants					
Other AIDs	4.9 (2.4–10.2)				
Autoimmune thyroiditis			2.0 (1.4–2.9)*	1.6 (1.3–1.9)**	7.7 (4.8–12.3)
Celiac disease				2.3 (1.3–3.8)**	7.2 (4.3–12.1)
Raynaud syndrome					11.9 (5.4–26.3)
Sjogren's syndrome					6.8 (2.8–16.4)
Family History of AIDs					7.1 (3.5–14.5)
Primary biliary cirrhosis		10.7 (4.3–27.3)			
Systemic lupus erythematosus		2.2 (1.3–4.0)			
Autoimmune thyroiditis		5.8 (1.3–26.4)			
Sjogren syndrome					
Other past history					
Urinary tract infection	2.1 (1.1–4.1)	1.5 (1.2–1.9)	2.4 (1.7–3.4)*	1.7 (1.5–2.1)**	2.7 (2.0–3.7)
Shingles			2.5 (1.7–3.5)*		
Tonsillectomy	1.9 (1.0–3.4)			1.6 (1.4–1.9)**	
Appendectomy				1.4 (1.2–1.7)**	
Reproductive history					
Hormone replacement		1.5 (1.3–1.9)			0.6 (0.5–0.8)
Birth control pill					
Never pregnant		0.6 (0.5–0.8)			1.8 (1.5–2.3)
Age at first pregnancy		0.95 (0.93–0.98)			2.0 (1.6–2.5)
Abortion					3.9 (2.8–5.3)
Pruritus during pregnancy				2.1 (1.6–2.7)**	

* epidemiological cases vs. controls; ** foundation cases vs. controls; AIDs: autoimmune diseases; OR: odds ratio; CI: confidential interval.

ic fever [26] and Guillain-Barré syndrome [27], respectively, in patients with autoimmune diseases. A pathogen under such circumstances might express antigens with epitopes that are structurally related to those of autoantigens, resulting in molecular mimicry, which might also play a causative role in the pathogenesis of PBC through human mitochondrial epitopes [28]. The ratio of seropositive AMA is > 90% in patients with PBC and the pyruvate dehydrogenase complex-E2 (PDC)-E2 is a major human anti-mitochondrial autoantigen that is highly conserved among various species and has a high degree of similarity to the PDC sequences of *Escherichia coli*, *Helicobacter pylori*, cytomegalovirus and other microbes [28–30]. Some reports indicate that *Novosphingobium aromaticivorans* is also a potential initiator of PBC [31–33].

Although infectious agents can be associated with PBC, and the degree of similarity between PDC-E2 and the PDC of infectious agents is high, AMA is not considered as a direct cause of biliary damage. Cross-reactivity alone is not necessarily sufficient to evoke PBC. However, it could be a trigger by inducing the initial immune response to PDC-E2.

3.1. *Escherichia coli*

E. coli is a causative agent of PBC and a frequent cause of UTI, particularly among women, and predominantly new infection with recurrent UTI is a feature of PBC [18]. Studies of molecular mimicry and cross-reactivity have shown that the affinity between human and *E. coli* PDC-E2 in patients with PBC is 100-fold higher than in controls [35]. The presence of bacterial products in mononuclear cells damages surrounding bile ducts [36]. AMA from patients with reactive T cells and prokaryotic antigens for several microbes, including *E. coli* and T cell clones derived from human PDC-E2, cross-reacted with peptides of *E. coli* PDC-E2 and *E. coli* OGDC-E2 [37]. These reactions would be caused by the sequences of PDC being highly conserved throughout their phylogeny [38].

3.2. *Novosphingobium aromaticivorans*

N. aromaticivorans is a Gram-negative bacterium of the Sphingomonadaceae family within the class of Alphaproteobacteria that is ubiquitous in the environment and closely associated with the pathogenesis of PBC [39]. Sera from patients with anti-PDC-E2-positive PBC react at 100- to 1,000-fold higher titers against *E. coli* than *N. aromaticivorans* proteins [31],

and such reactivity is evident during the early stages of PBC. The association between PBC and *N. aromaticivorans* infection has been confirmed in a mouse model [40], and it is capable of inducing autoreactive AMA and chronic T-cell-mediated autoimmunity against small bile ducts. Moreover, the specificity of serological findings has been confirmed in an independent cohort of patients and controls [34]. *N. aromaticivorans* could be a candidate for the induction of PBC.

3.3. *Helicobacter pylori*

Helicobacter pylori DNA has been detected in 33% of patients with PBC [41]. Another report has described a positive correlation between the titers of anti-PDC and anti-*H. pylori* antibodies [42]. Molecular mimicry from *H. pylori* infection arising in patients with PBC [28] might induce an autoimmune response due to cross-reactivity with bile ductular antigens. However, a later report did not identify an association between the seroprevalence of *H. pylori* infection and PBC [43]. Two other studies did not identify an increased prevalence of *H. pylori* DNA in liver tissues from patients with PBC [44,45].

3.4. *Chlamydia pneumoniae*

Chlamydia pneumoniae is a cause of community-acquired pneumonia that might play a role in triggering PBC [46]. All of 39 patients with PBC had *C. pneumoniae*, but not *C. trachomatis* antigens. The RNA of *C. pneumoniae* has also been detected in liver tissues from patients with PBC. However, two other reports do not support the notion of an association between *C. pneumoniae* and PBC [47,48]. A Chinese study found that the seroprevalence of *C. pneumoniae* IgG does not differ between patients with PBC and those with post-hepatitis cirrhosis. However, *C. pneumoniae* IgM might contribute to the abnormally high concentrations of total IgM in patients with PBC.

3.5. Other bacteria

Studies of Spanish patients with PBC have revealed extensive, disease-specific cross-reactivity between the 65-kDa heat shock protein of *Mycobacterium goodii* and PDC-E2 [49]. However, others have failed to confirm serum reactivity of *Mycobacterium*, or detect *Mycobacterium* DNA in patients with PBC [50,51].

Mycoplasma antigens have recently been proposed as a trigger of AMA in patients with PBC. Other

cross-reactive responses involving mitochondrial and prokaryotic antigens have been reported, including *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus* and *Salmonella minnesota* [38].

Long-term exposure of mouse models to bacteria triggers non-suppurative, destructive cholangitis associated with multifocal epithelial inflammation, suggesting that persistent and continuous bacterial infection is a trigger in the pathogenesis of PBC [52].

3.6. Viruses

Several viral infections might be associated with the pathogenesis of PBC. A retrovirus resembling the mouse mammary–tumor virus (MMTV) has been identified [53], and although pilot studies of single and combination antiretroviral therapy have indicated an association between MMTV and PBC [54], the findings were not reproducible [55]. The Epstein-Barr (EB) virus, which belongs to the herpes virus family, is a cause of infectious mononucleosis. Increased titers of EB virus antigen in the sera of patients with early PBC suggest that it could trigger PBC [56]. Similar peptide sequences between cytomegalovirus and human PDC indicate that this virus might also trigger PBC [28,56].

4. Xenobiotics and PBC

The ability of infectious agents to induce autoimmune responses has been reported in experimental settings, and molecular mimicry is the most widely studied mechanism explaining these observations. However, titers of bacterial PDC-E2 were lower and appeared later in the disease than self (human) PDC [57]. These observations indicate that another etiological factor is necessary to break tolerance. In this regard, several lines of evidence support a role of xenobiotics in the development of PBC [58].

Xenobiotics are foreign chemicals that may either alter or combine with defined self proteins, inducing a change in the molecular structure of the naïve protein sufficient to induce an immune response in the host. Such an immune response may then result in the recognition of not only the modified or altered protein, but also the unmodified naïve antigen [59]. Chronic presence of both modified or naïve serves to perpetuate the immune response initiated by the xenobiotic-induced adducts and leads to autoimmunity [60]. Many xenobiotics can cause organ-specific autoimmune diseases.

It is well established that AMA and cellular responses in PBC are mainly directed to the lipoyl domain of PDC-E2. Lipoic acid is crucial to PDC-E2 recognition and lies exposed to the exterior part of the complex, thus constituting the ideal target for xenobiotic modification [61,62]. Xenobiotics may modify the lipoyl domain of PDC-E2 by creating neoantigens, possibly in concert with xenobiotic-modifying bacteria such as *Novosphingobium aromaticivorans* [31,32]. This hypothesis was supported by the observation that AMA from patients with PBC recognized a class of xenobiotic-modified PDC-E2 peptides, often with a higher affinity than with their naïve autoantigens [63]. In addition, immunization of animals with xenobiotics conjugated to bovine serum albumin caused serological and histopathological reaction similar to PBC [64–67]. The lipoylated bacterial proteins were further shown to have homology to human 2-oxo acid dehydrogenase enzymes [33]. These findings indicate that bacteria mimics containing lipoic acid residues might be modified by xenobiotics to form immunoreactive products. This modification might trigger a break in tolerance to autoantigens and the development of PBC.

5. Conclusions and directions of future perspectives in the pathogenesis of PBC

Most of the epidemiological, experimental and clinical data concur to indicate the role of environmental factors, such as microorganisms and/or xenobiotics, in the induction of autoimmunity in PBC. Although a cause and effect relationship between infection and PBC has yet to be proven, there is evidence to suggest a role for bacterial agents, especially *E. coli*, in the development of PBC.

However, more data from epidemiological and clinical studies are necessary to directly link microbial infections with PBC. For example, most of the epidemiological studies of PBC have been conducted in the Europe and United States, and few data are available in many parts of the world. In addition, the role of infectious agents in the initiation of PBC has to be further probed, particularly by making a great effort to develop an experimental setting or animal model to ascertain exposure to specific environmental factors [68–70]. Finally, PBC is a multifactor disease in that not only environmental factors are required but also genetic contributions. The informative genome studies, including a genome-wide association study, will help us to understand the pathogenesis of PBC.

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