

Autoimmunity and Environment: Am I at risk?

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Abstract The complex interplay between environmental factors and genetic susceptibility plays an essential role in disease pathogenesis. This is especially true for autoimmunity, where clinical reports, genomic and epidemiological studies, as well as animal models have identified several environmental and genetic risk factors associated with autoimmune disease. The complexity of this relationship is demonstrated by the vast array of environmental factors that have now been implicated in the induction, and possibly the maintenance of autoimmune disease. The multitude of environmental factors implicated includes both infectious and non-infectious agents. Here, we review one specific autoimmune disease, primary biliary cirrhosis (PBC), as a model for environmental risk factors acting in concert with genetic susceptibility in the disease pathogenesis. PBC is an ideal model, as both infectious and non-infectious environmental agents have been identified as risk

factors, and their study provides clues for unravelling the pathogenesis of the disease.

Keywords AMA · Genetics · Estrogen · PBC · Risk factors · Urinary tract infections · Xenobiotics

Abbreviations

AMA	Anti-mitochondrial antibodies
ANA	Anti-nuclear antibodies
<i>E. coli</i>	<i>Escherichia coli</i>
FDR	First-degree relatives
HRT	Hormone replacement therapy
ER	Estrogen receptor
PBC	Primary biliary cirrhosis
PDC	Pyruvate dehydrogenase complex
RA	Rheumatoid arthritis
SLE	Systemic lupus erythematosus

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UC	Ulcerative colitis
UPEC	Uropathogenic <i>E. coli</i>
UTI	Urinary tract infection

Introduction

Observations made at the bedside and in the laboratory have provided evidence for the concurrence of environmental, infectious, genetic, epigenetic, metabolic and immunological factors in the breakdown of immunological tolerance [1–6].

Environmental factors are central to the development of autoimmune diseases, acting in concert with genetic susceptibility, frequently expressed as inability of the host's immune system to suppress harmful autoimmune responses [1, 3, 7, 8]. The list of environmental factors is vast, ranging from microbial agents and xenobiotics to ultraviolet radiation exposure [7–9]. Indeed, individual agents have been associated with specific autoimmune diseases. Support to an environmental influence in autoimmune diseases derives from twin studies where the concordance rate for most autoimmune conditions is less than 50% in monozygotic twins leaving a large scope for non-genetic factors [10–13]. Hints on the identity of these exogenous factors can be obtained from certain characteristics of a given autoimmune disease, such as seasonality in disease onset, geographical and familial clustering [14]. Other clues derive from the observation that genetically similar populations living under different conditions show a different incidence of autoimmune disease occurrence. The mechanisms through which such seemingly unrelated factors concur in the development of an autoimmune response include the alteration of autoantigen structure, stimulatory effects on the immune system such as that exerted by cytokines and chemokines, apoptosis, non-self/self-molecular mimicry and immunological cross-reactivity [15].

Non-infectious agents present a daunting number of possibilities, though pharmacological compounds are often incriminated. This high frequency may be due to two distinct factors, namely the intrinsic ability of a drug to trigger autoimmune reactions and the stringent survey of drug-related side effects. Reported associations of drug with autoimmune disease include that of estrogens with lupus-like syndromes and myositis, of halothane with hepatitis, of iodine with hypothyroidism, and of penicillins with lupus-like syndromes and hepatitis [16].

One of the environmental agents very frequently linked to autoimmune diseases such as rheumatoid arthritis (RA), autoimmune thyroiditis, inflammatory bowel disease and primary biliary cirrhosis (PBC) is cigarette smoking [17–20]. Cigarette smoke accelerates the production of pro-inflammatory cytokines and reduces the levels of anti-

inflammatory cytokines. Free radicals contained in cigarette smoke may also induce cellular necrosis and release of intracellular antigens. In addition, free radicals and other toxins can cause DNA damage and may provoke genetic mutations eliciting gene activation and induction of autoimmune manifestations [19]. Polyprenol-rich glycoprotein present in cigarette smoke can stimulate the proliferation of autoreactive lymphocytes leading to the emergence of self targeting immunopathology. Cigarette smoke exerts anti-estrogenic effects and a modulation of the hormonal balance may favour the induction of autoimmunity [4, 21]. Intriguingly, there are few diseases such as ulcerative colitis (UC), where active smokers seem to incur a reduced risk for UC compared with non-smokers or former smokers and experience a more benign disease course. Symptoms are reportedly exacerbated during periods of smoking abstinence, at least in some individuals.

It is also intriguing that materials developed for cosmetic reasons (such as silicone implants) or to treat diseases (including autoimmune disorders) such as adjuvants, vaccines and nanoparticles, may initiate self-destruction [22–26]. Nanoparticles include molecular entities of various sizes, shapes and physicochemical characteristics used to diagnose or to treat a variety of diseases. Their applications include imaging nanotools, diagnostic nanochips and nanosensors, nanodelivery systems for vaccines and targeted nanotherapeutics based on peptides, proteins or antibodies. Nanoparticles have been shown to interact with the immune system in both inflammatory and immunosuppressive fashion. Concerns have been raised about their toxicity. They have been shown to interact with immune cells, trigger pro-inflammatory cytokine release, and activate the complement cascade. Silica (also known as quartz) is a compound that has long been of interest to those examining environmental materials involved in autoimmune disease [12, 27, 28]. Silica is found in sand, rock and soils and occupational exposure to this particle has been linked with scleroderma, RA, vasculitis and systemic lupus erythematosus (SLE) [12, 27, 28]. Silica acts as an immune stimulant which increases pro-inflammatory cytokine production, induces apoptosis and necrosis and is toxic to macrophages [12, 27, 28]. Silica has also been shown to increase autoantibody production [29] and immune complex formation in animal models. Increases in B cell and CD4 T cell counts, and changes in the T helper and T regulatory cell ratio has been seen in lymph nodes of animals exposed to silica [30]. Apart from silica, exposure to solvents such as trichloroethylene, mineral spirits, and petroleum-based products have been linked to scleroderma and other connective tissue diseases [31].

As mentioned above, infectious agents including bacteria, viruses, and parasites have been implicated in the pathogenesis of autoimmune disease. Their participation in autoimmune disease causation has been suggested to occur through various mechanisms ranging from direct cytotoxicity to microbial/self-

molecular mimics and immunological cross-reactivity at the T and/or B cell level [6, 9, 32, 33]. Few examples of viral, bacterial and parasitic infections linked to autoimmune disease are listed below and include the association of streptococcal infection with rheumatic heart disease, hepatitis C virus with vasculitis, cryoglobulinemia or autoimmune hepatitis, enteroviruses with type 1 diabetes, Epstein–Barr virus with SLE, *Helicobacter pylori* with gastric autoimmunity, *Saccharomyces cerevisiae* with Crohn's disease, measles with multiple sclerosis and cytomegalovirus or Parvovirus B19 with systemic sclerosis [8, 9, 32].

While exposure to environmental agents is linked to autoimmune disease, lack or reduced exposure to certain compounds or dietary components has also been associated to autoimmunity, vitamin D representing a prime example [34, 35]. Vitamin D insufficiency and deficiency are associated with various autoimmune diseases including insulin dependent diabetes, multiple sclerosis, inflammatory bowel disease, SLE, RA and celiac disease [36, 37]. Early studies have indicated that certain autoimmune disorders are more prevalent in regions distant from the equator, or in those with less sun exposure. Decreased levels of vitamin D have been reported in certain autoimmune diseases and an inverse correlation between vitamin D levels and incidence of a variety of autoimmune diseases has been noted [37, 38]. Antibodies against vitamin D have been reported in patients with autoimmune disease and several animal studies described the immunomodulatory role of vitamin D on various cell subsets [39, 40]. Other studies have reported that genetic polymorphisms in vitamin D pathway genes, such as vitamin D receptor, are associated with susceptibility to autoimmune disease [34].

The endless list of environmental and genetic triggers of autoimmunity and the diversity of molecular mechanisms involved adumbrate the complexity of their pathogenic interplay in the induction and/or maintenance of autoimmunity. We will take one specific autoimmune disease, PBC, and use it as a model in delineating a scenario in which environmental and genetic factors have been identified and are likely to interact in the development of the disease. The infectious and non-infectious environmental agents identified as risk factors in PBC are numerous, and recent genome-wide studies have located new genetic elements responsible for the genetic propensity to the development of PBC.

Primary Biliary Cirrhosis: An Overview

PBC is a chronic cholestatic liver disease of autoimmune nature, characterized by inflammatory destruction of the small intrahepatic bile ducts, fibrosis progressing to cirrhosis and subsequent liver failure [41–43]. It is not uncommon for patients to be asymptomatic at the time of diagnosis [44–46]. Autoantibodies specific for the disease,

such as anti-mitochondrial (AMA) or disease-specific antinuclear antibody (ANA) may be present in asymptomatic cases whether biochemical blood tests indicating cholestasis are still normal or already abnormal [41, 45, 47–49]. Symptomatic patients often complain of fatigue, pruritus and arthralgias at the time of presentation [41, 45, 50]. More severe presenting symptoms are related to portal hypertension and hepatic decompensation (jaundice, ascites or variceal bleeding), which may require liver transplantation [41, 45, 50]. PBC progresses slowly, but the course of the disease is often unpredictable [41, 45, 46, 50]. During the last two decades there have been significant advances in the diagnostic workup and management of PBC. Progress has also been made in understanding the pathogenetic mechanisms responsible for the development of the disease. In view of this progress, the number of patients presenting at an earlier disease stage is much higher than that reported in the original 1970s and 1980s studies on the natural history of the disease [41]. Patients at an early stage of the disease tend to respond well to ursodeoxycholic acid (UDCA) with a significant decrease or even normalization of the levels of ALP, gamma GT and other indices of cholestasis [41–43]. Studies reporting findings in large North American and European patient cohorts indicate that the percentage of patients with PBC who require liver transplantation has fallen significantly [43].

The incidence of the disease varies among studies and largely depends on the ethnic background of the affected individuals. Among Caucasians, the incidence of PBC is estimated at 27 per million per year with a prevalence of 402 per million and up to one in 700 women over the age of 40 appear to be affected with PBC in some western European populations [51]. Emerging evidence suggests that the absolute number of patients with PBC has been increasing over the years but it is still unclear whether this is a true increase or is the result of greater awareness of physicians and of the improvement of the diagnostic tools in particular regarding disease-specific serology and imaging analysis [51, 52].

A definite diagnosis is based on the triad of AMA in serum, biochemical markers of cholestasis and liver histological features diagnostic of, or compatible with, PBC. When two of the three criteria are met the diagnosis of PBC is likely [41–43].

Biochemical indices of cholestasis include increased levels of alkaline phosphatase and γ GT [41–43]. AMA are usually present at high titres [41, 47–49]. Less than 3–10% of patients with PBC lack AMA [47, 48, 53–57]. AMA titres do not correlate with the severity of the disease and do not appear to have prognostic significance. When present, disease-specific ANA tend to have elevated titres and identify a subgroup of patients with a more aggressive disease [47–49, 55, 58–69]. There are characteristically

high levels of immunoglobulin M (IgM). Histological features typical for PBC include destruction of biliary epithelial cells and loss of small bile ducts with portal inflammatory cell infiltration or, less frequently, characteristic granulomas [41–43]. These histological findings have led to PBC being described as a non suppurative destructive cholangitis or as granulomatous cholangitis [41–43].

PBC is considered a classic autoimmune disease [70–72]; its pathogenesis is still unfolding but there is evidence suggestive of an immune-mediated pathology targeting the small intrahepatic bile duct cells, as well as high-titre seropositivity for disease-specific autoantibodies [70, 71, 73, 74]. PBC patients often have extra-hepatic autoimmune diseases such as Sicca syndrome, autoimmune thyroiditis and RA [41, 50]. In line with other autoimmune disease, PBC shows a high female preponderance with middle aged women being mainly affected [41]. The female to male ratio ranges from 10:1 to 20:1, which is relatively high compared with other autoimmune diseases [41].

Several features are unique to PBC [75]. Biochemical indices of cholestasis do not respond to immunosuppressive treatment, but do respond to UDCA. Whether the effect of UDCA is solely anti-cholestatic or also immunosuppressive remains to be established [41, 43, 71, 75]. PBC lacks a strong association with genes of the major histocompatibility complex—the HLA in man—which is typical of other autoimmune diseases [71, 76]. Recent large genome-wide association studies have identified as PBC-predisposing elements non-HLA genes closely related to the immune system; however the predisposition imparted by these genes is relatively small [77–80]. A central feature of PBC is the fact that disease defining immune responses, both humoral and cellular, target highly conserved members of the 2-oxo-acid dehydrogenase complexes of enzymes lying within the inner mitochondrial membrane instead of antigens specific to the biliary epithelial cells [47–49, 70, 71, 81].

Risk Factors for PBC

The analysis of risk factors will concentrate on the findings obtained in larger epidemiological studies conducted in the UK, USA, and in continental Europe during the last decade (from 2001 to 2010) [18, 82–84]. The most recent reports have been based on patient questionnaires, which have been formulated to take into account findings from earlier studies. The formulation of these questions has based on findings of both epidemiological and experimental studies suggesting an association between specific environmental triggers and development of PBC. The study of Parikh-Patel et al. [82] was based on a standardized questionnaire posted to 241 PBC patients, 261 siblings of the PBC patients, and 141 friends. As expected, the PBC cohort was overwhelmingly female, with a female to male ratio of 10:1

[82]. The mean age of the PBC patients was 53 years, with 97% being Caucasian [82]. PBC patients had increased odds ratio for having other autoimmune diseases, a history of cigarette smoking, tonsillectomy, as well as recurrent vaginal and urinary tract infections (UTI) [82]. Past medical histories showed an increased incidence of hepatitis A virus infection and shingles [82]. Concurrent autoimmune diseases included Sjögren and Raynaud's syndromes (17.4% and 12.5%, respectively), and autoimmune thyroid disease (11.5%). There was also an increased incidence of first-degree relatives (FDR) with autoimmune diseases [82]: 6% of PBC patients having at least one relative with PBC including two mothers with PBC, two brothers, and four sisters, but no fathers [82]. These findings indicate that a genetic predisposition to autoimmunity is a risk factor in the development of PBC. The findings that recurrent UTI and exposure to cigarette smoke predispose to PBC have been confirmed in subsequent studies [18, 82–84].

One of the most comprehensive and carefully executed studies on the risk factors of PBC was performed by Gershwin and colleagues [18], who enrolled 1,032 PBC patients, and 1,041 controls. PBC patients were referred to the survey from 23 tertiary care centres, where they were being treated for PBC by liver specialists. These patients took part in a telephone interview answering a standardized set of questions [18]. Controls were sex, age, race, and geographically matched and were also interviewed by telephone using the same set of questions [18]. Again, the majority of PBC patients were female (93%) and Caucasian (98%) [18]. Like Parikh-Patel [82], Gershwin et al. found an increased incidence of smoking and UTI among the PBC patients, and an increased risk for PBC among FDR [18]. Interestingly, an association was noted between occurrence of PBC and increased history of hormone replacement therapy (HRT). It should be noted that bone loss, both associated and not associated with cholestasis, is common among females with PBC, and asymptomatic females are often prescribed HRT for this. Therefore, it is unclear whether HRT is a cause or consequence of PBC [18]. Co-existent autoimmune disease—primarily Raynaud's and Sjögren syndromes and to a lesser extent RA—were also found to be more prevalent among PBC patients than in controls (32% vs 13%) [18]. Intriguingly, past usage of nail polish was slightly more frequent among PBC patients [18]. The medical history of PBC patients included a lower incidence of breast cancer as well as a lower amount of alcohol consumption in the 12 months prior to the questionnaire compared to controls [18]. However, lifetime alcohol consumption was similar in patients with PBC and controls. Surgical events that were more frequent in the PBC cohort included tonsillectomy, cholecystectomy, and colonic polypectomy. Akin to Parikh-Patel's study [82], an increased

incidence of PBC and autoimmune diseases was found among FDR, with sisters and mothers being the most commonly affected [18]. The study by Gershwin et al. [18] confirmed findings of earlier studies, but also highlighted important new features that were further examined in later studies. These features include the effects of HRT and exogenous estrogens, as well as exposure to potential toxins found in materials such as nail polish [18].

A more recent study by Prince et al. [83] reports similar findings, focusing on 318 PBC cases from a geographically defined region, 2,258 PBC patients enrolled from a PBC patient support group (known as the PBC Foundation group), and 2,438 controls found through an electoral registry [83]. PBC was associated with smoking, hair dye use, past history of psoriasis, UTI, shingles and autoimmune diseases [83]. Similar to Gershwin's study [18], the PBC groups in the survey by Prince et al., reported a lower level of alcohol consumption compared to controls but this difference was not significant when men from the support group were excluded [83]. A surgical history of tonsillectomy was more frequent in the PBC patients obtained from the Foundation group, but not in those from the geographically defined region [83]. A more frequent history of HRT was found among both PBC groups. Pruritus in pregnancy was reported in patients of the support group but not in the geographically defined group of patients [83]. This was the first study to indicate pruritus in pregnancy as a risk factor for PBC [83].

The largest study of PBC risk factors in continental Europe has been published recently by Corpechot et al. [84], and involved 222 PBC patients and 509 controls. Confirmatory findings were reported, including increased familial incidence of PBC and of autoimmune thyroid disease [84]. Patient histories comprised recurrent UTI, cigarette smoke exposure, co-morbid autoimmune disease (which preceded the diagnosis of PBC by 4 years on average). Pruritus during pregnancy was again noted [84]. An increased incidence of autoimmune hepatitis was also noted [84]. Incidences of missed abortions and extrauterine pregnancies were more frequent in patients than in controls. The authors indicate that the increased rate of abortion and extrauterine pregnancy may indicate a possible exposure to fetal alloantigens, leading to a breakdown in immunological tolerance in young women evolving to a full-blown disease later in life [84]. At variance with other studies, no association was found with hair dye use or exposure to specific toxins, and no association with history of previous surgical events [84]. No increased incidence of cancers (including breast cancer) was reported among the PBC group [84]. Interestingly, no link was found with HRT, but there was a less frequent use of oral contraceptives among female PBC patients [84]. This report was the first to show high rates of gastroesophageal reflux in PBC patients with the suggestion that this may be related to subclinical

scleroderma, UDCA treatment or increased use of bisphosphonates [84].

The studies reviewed reveal common features in PBC patients (Table 1) such as histories of recurrent UTI, co-morbid autoimmune disease and autoimmune disease among FDR. There are conflicting results in regards to toxins and HRT exposure. The potential role for these latter factors has been investigated in greater depth in other studies, and will be discussed individually (Table 1).

Urinary Tract Infection

A common finding of the larger epidemiological studies is a more frequent history of recurrent UTI in female PBC patients compared to controls [18, 82–84]. UTI was included in epidemiological surveys, firstly, in view of the original findings from the Royal Free Hospital in London of an increased rate of bacteriuria in female PBC patients compared to demographically matched controls [85–88] and secondly, as a consequence of experimental studies by Shimoda et al. and Bogdanos et al. who showed T and B cell cross-reactivity involving *Escherichia coli* and mitochondrial antigens [89–96]. As mentioned previously, the study by Gershwin et al. [18] showed that 60% of patients with PBC have a history of UTI. The propensity of PBC to affect middle aged women has been linked with recurrent UTI. This hypothesis suggests that recurrent infections with *E. coli* trigger the processes leading to the disease's development.

A mechanism through which *E. coli* may induce PBC is based on the homology between *E. coli* and the pyruvate dehydrogenase complex (PDC)-E2 epitopes which become targets of cross-reactive immune responses [89–91, 97]. In experimental UTI models, uropathogenic *E. coli* (UPEC) invades the bladder epithelial cells predisposing to recurrent episodes of bacteriuria. Mice inoculated with UPEC can develop AMA and histological features of PBC in liver tissue [98]. An early report by Burroughs and Baum [99] proposed that infection with *E. coli* induces an anti-microbial immune response directed against *E. coli* as well as its homologous human PDC-E2 sequences, in a cross-reactive immune response. Testing this hypothesis at the B and T cell levels have shown that patients with PBC do indeed mount such cross-reactive immune responses [89–91, 93, 94, 100, 101]. Experimental studies have also documented cross-reactive immune responses between human PDC-E2 and sequences of *E. coli* and other bacteria that are unrelated to bacterial PDC-E2 [93, 94, 101–104].

Xenobiotics

Although the French epidemiological study by Corpechot et al. [84] did not find a significant association of PBC with hair dye or toxins, other large studies did [18, 83].

Table 1 Major epidemiological studies cite common and differing risk factors for the development of primary biliary cirrhosis (PBC), which are compared here

Risk factor	Parikh-Patel et al.	Gershwin et al.	Prince et al.	Corpechot et al.
UTI	Yes	Yes	Yes	Yes
History of HRT	–	Yes	Yes	No relation found
Exposure to cigarette smoke	Yes	Yes	Yes	Yes
Toxin or Xenobiotic exposure	–	Yes (nail polish)	Yes (hair dye)	No association found
Oral contraceptive use	–	–	–	Yes (decreased uptake in PBC patients)
Surgical history	Tonsillectomy	Tonsillectomy, cholecystectomy, colonic polypectomy	Tonsillectomy (but only in a subgroup of the PBC cohort)	No association found
Medical history	Other autoimmune disease; increased HepA vaccine uptake; shingles	Other autoimmune disease; decreased ethanol intake; decreased breast cancer incidence	Other autoimmune disease; psoriasis; decreased ethanol intake; obstetric pruritis	Other autoimmune disease; obstetric pruritis; autoimmune hepatitis
Family history of PBC	Yes (6% of first-degree relatives)	Yes (5.9% of first-degree relatives)	Yes	Yes (4% of first-degree relatives)
Reproductive history	–	No association but younger age of first pregnancy	No association found	Increased incidence of abortion and extrauterine pregnancy; younger age of first pregnancy

Risk factors indicated in all studies include history of urinary tract infection (UTI) in females, exposure to cigarette smoke, history of a co-morbid autoimmune disease, and family history of PBC. AMA seropositivity is so common among PBC patients, and those who will develop PBC, and is not included in the Table

AMA anti-mitochondrial antibodies, HRT hormone replacement therapy

Individual elements of these chemical components, known as xenobiotics, have been linked to autoimmune disease [105]. Xenobiotics have been shown to alter self and form complexes with these proteins which may elicit a cross-reactive immune response, targeting, in the case of PBC, mitochondrial autoepitopes [105, 106]. This process is similar to molecular mimicry, where a microbial epitope mimics a self-epitope, promoting an autoreactive immune response [23–25, 32, 33, 72, 95, 99, 105–120]. According to this mechanism, infectious agents initiate an immune response directed against specific microbial sequences and their self-mimics leading to the development of cross-reactive immunity against autoantigens and ultimately to autoimmune disease [23–25, 32, 33, 49, 72, 93–96, 99, 102–122]. In vitro studies using specific PDC-E2 T-cell lines obtained from the peripheral blood or from the liver of PBC patients, together with studies in animal models, suggest that xenobiotics can trigger PBC [41, 71, 123–126]. Gershwin's group has provided evidence that modifying the lipoylated major mitochondrial antigens could elicit the production of autoantibodies [41, 81, 105, 106, 115, 126, 127]. One xenobiotic found in cosmetics and nail polish is 2-nonynoic acid, which has been implicated in PBC [124, 125]. The presence of this compound in cosmetics is interesting given the high female preponderance of PBC. Sera from PBC patients

show a high affinity reactivity for 2-nonynoic acid, implicating this chemical in the induction of cholangiopathy in immunised mice [41, 81, 105, 106, 115, 126, 127].

In addition to chemicals found in cosmetics, environmental toxins present in water and contaminated soils have been suggested as potential xenobiotic triggers of PBC. A 1980 study by Triger [128] found that a cluster of patients in Sheffield received their water from a common source, known as the Rivelin reservoir. Testing both the reservoir and delivery system only revealed an increased level of copper, as well as lower concentrations of fluoride but the relevance of this finding to the development of the disease remains elusive [128]. No further studies were done to determine if other chemicals were present in this water supply, and the link between these clusters and the water supply remains unexplained [129]. Ala et al. [130] has reported a cluster of PBC patients living near a superfund toxic waste site contaminated with volatile aromatic hydrocarbons and trichloroethylene in the New York State [130]. As groundwater is not a source of water in these areas, Ala and co-workers suggested that air contamination by particle bound chlorinated hydrocarbons (such as benzene) would be a more likely method of exposure [130]. This suggestion finds support in early and more recent epidemiological studies reporting an association between benzene-containing cigarette smoke, and PBC [18, 82, 84].

Estrogens and PBC

Reproductive histories in the larger epidemiological studies have indicated a potential association between the disease and estrogen deficiency, with both Gershwin and Prince citing an increased uptake of HRT among PBC patients [18, 83]. These results were not reproduced in the study by Corpechot et al. [84], though that group noted a decreased uptake of oral contraceptives among PBC patients. Both Prince and Corpechot found no association between PBC and number of pregnancies [83, 84], while Corpechot and Gershwin noted a younger age of first pregnancy [18, 84]. Menstrual abnormalities as well as abortions and extrauterine pregnancies have also been noted to occur at higher rates in PBC patients [84, 131]. These findings, together with the overwhelming female predominance of the disease, implicate estrogens—or lack thereof—as a potential risk factor. Alvaro et al. [123, 132, 133] investigated the intracellular pathways involved in the stimulatory effects of estrogens on cholangiocyte proliferation, finding that they induce cholangiocyte proliferation in an animal model by activating the Src/Shc/ERK molecular signalling cascade, which is usually activated by growth factors acting through tyrosine kinase receptors [133]. According to Alvaro and colleagues this suggests a synergism between estrogens and growth factors in cholangiocyte proliferation [133]. Estrogen receptor antagonists inhibited cholangiocyte proliferation, and ERK/Shc protein expression was reduced [133]. Administration of 17- β -estradiol was associated with increased Src/Shc/ERK protein expression and cholangiocyte proliferation in vitro [133]. These results demonstrate that estrogens are most likely to play a role in cholangiocyte proliferation [123, 132, 133].

Alvaro and colleagues also investigated the role of estrogens in the progression of PBC [123]. Liver biopsies from PBC patients and controls were evaluated for estrogen receptor alpha (ER)- α , estrogen receptor beta (ER)- β , cytokeratin 19, proliferating cellular nuclear antigen (PCNA) and Fas using terminal deoxynucleotide transferase end labelling and immunohistochemical techniques [123]. Control liver samples were obtained from those with normal liver, primary sclerosing cholangitis (PSC), and alcoholic cirrhosis [123]. ER- α and ER- β were found in PBC samples but not in normal liver [123]. ER- β was expressed at all histological stages in 50–65% of PBC patients. ER- α increased from 1% at stage 1, to 12% at stage 3, while it was completely absent at stage 4 in association with low PCNA and maximal ductopaenia [123]. ER- α was significantly decreased in PBC compared to PSC and alcoholic cirrhosis [123]. These findings suggest that ER- α is reduced in advanced PBC, and that this reduction favours the progression towards ductopenia, which is a common histological feature in PBC [41, 123].

Familial PBC

Having a relative with PBC has been noted to increase the probability of developing PBC [41]. The large epidemiological studies quoted above all noted a higher incidence of PBC in families, in addition to a higher incidence of autoimmune disease in general [18, 82–84]. As expected, family members affected by PBC are mostly female. The most common profile of affected individuals is mother–daughter, followed by sister–sister, and mother–sister–daughter [134, 135]. These findings emphasize a genetic component to the pathogenesis of PBC. Lazaridis et al. [136] reports the prevalence of PBC among sisters to be 20.7%, 7.8% in brother–sister pairs, and 3.7% in father–daughter pairs. It should be noted that the diagnosis of one family member may prompt relatives to undergo screening for PBC. This may reveal laboratory features of the disease in the absence of clinical signs or symptoms and may explain the short interval between the diagnoses in members of the same family.

A retrospective review to determine the familial prevalence of PBC was conducted by Bach and Schaffner at the Mount Sinai Hospital in New York [137]. A total of 405 patients with PBC were enrolled, 26 of whom had at least one FDR with PBC or ‘liver disease’. Those with liver disease did not have a pathology specified, but their history and disease features were suggestive of PBC. The familial PBC prevalence was calculated to be 4.3% (when only diagnosed PBC cases were included), or 14.9% when taking into account those with undiagnosed liver disease [137]. A higher incidence of autoimmune disease among FDR’s of PBC patients (approximately, 71%) was also recorded, as well as seropositivity for AMA [137]. It is important to note that most family members included in the study by Bach and Schaffner did not share a common environment [137]. Similar familial prevalence rates have been reported by Floreani, who found the prevalence to be 3.8% in an Italian cohort [138] and by Tsuji et al. who found a prevalence of 5.1% in a cohort from Hiroshima [139]. Studies by Gershwin and colleagues indicate a familial prevalence of 5.9% [18], and Corpechot et al. report 4% [84].

Finally, monozygotic twins share a high prevalence of PBC. A twin study by Selmi et al. [11] involved 16 twin pairs, who were selected from a database of 1,400 PBC patients. There were eight pairs of monozygotic and eight pairs of dizygotic twins (verified through DNA typing), four pairs being female–female, and the remaining four being male–female [11]. A concordance rate for PBC was 0.63 among monozygotic twin pairs, which is one of the highest among autoimmune diseases [11]. It is possible that non-concordant pairs will eventually become concordant taking into account the latent period before presentation. Whether discordant twins have become concordant for

PBC cannot be determined from the work of Selmi and co-workers since no follow-up has been reported.

HLA and Non-HLA Genes

Specific genetic factors predisposing to PBC are now emerging, with genes both in HLA and non-HLA regions being implicated. At variance with most autoimmune conditions, no strong HLA link has been established for PBC. Several common HLA haplotypes have been identified in pairs or small clusters of PBC patients, but only HLA-DR8 has been reported consistently [41, 71, 76, 140, 141]. A series by Tsuji et al. [139] identified HLA-DR8 in 29.4% of PBC patients, and HLA DR8 is deemed a risk factor for the development of PBC also in Caucasian populations [76]. Hirschfield et al. [77] has carried out a genome-wide association analysis of 536 PBC patients and 1,536 controls from Canada and USA, and associations were made with 13 loci across the HLA class II region, with HLA DQB1 showing the strongest disease association [77]. Associated non-HLA loci included two single-nucleotide polymorphisms (SNPs) at the interleukin 12 (IL12) A locus, and one SNP at the IL12RB2 locus [77]. Weaker associations were found with STAT4 and CTLA4 loci, regions previously associated by Hemminki and colleagues to RA and SLE in the case of STAT4, and to RA and other autoimmune disease in the case of CTLA4 [142]. Liu et al. [78] made similar observations with IL12A and IL12RB in an Italian cohort. Additional associations were disclosed with HLA regions DRB1, DQA1, DQB1, and DQA2 and with non-HLA regions such as IRF5, SPIB, and the IKZF3-ORMDL3 on chromosome 17q12–21. Liu et al. [78] emphasizes potential relevance of the link with STAT4, since this transcription factor is involved in IL12 signalling, and has been linked to other autoimmune diseases (although none of them is commonly associated with PBC). Finally, Hirschfield et al. have also identified variants at IRF5-TNPO3, 17q12–21 and MMEL1 as being associated with PBC [79]. Collectively, these studies confirm a genetic component in the pathogenesis of PBC and concur with the findings of a high familial and monozygotic twin prevalence.

AMA Preceding Disease Onset

AMA are the diagnostic hallmark for PBC, being present in 95% of PBC patients and individuals who are asymptomatic but who are destined to develop PBC [41, 44, 49, 71, 84, 143]. A study by Mitchison and colleagues indicates that despite the lack of symptoms and in presence of normal liver biochemistry, individuals with AMA positivity are likely to have histological evidence of PBC [44]. Twenty-nine asymptomatic patients with normal liver biochemistry

were incidentally found to be positive for AMA [44]. Biopsies from 12 of them fulfilled criteria for the diagnosis of PBC, with a further 12 patients having PBC-like features, and only two having a normal histology [44]. Follow-up studies were done in 16 of these patients, five of whom had become symptomatic for PBC, and 11 eventually showing raised alkaline phosphatase levels [44]. The same cohort was re-investigated 10 years later, giving a median follow-up of 17.8 years in these. Twenty-two (76%) patients had developed symptoms of PBC, and 24 (83%) had persistent laboratory signs of cholestasis. The studies from Newcastle show the predictive power of AMA.

PBC-specific AMA target components of the 2-oxo-acid dehydrogenase complexes (also known as M2 antigen previously), primarily the E2 subunit of the PDC [41, 47, 49, 70, 144]. More than 90% of the AMA positive PBC cases have detectable anti-PDC-E2 antibodies, which cross-react with PDC-E3 binding protein (E3BP) [41, 47, 49, 70, 144, 145]. The E2 subunits of branched-chain 2-oxo-acid dehydrogenase complex and 2-oxoglutarate dehydrogenase complex are targeted in 20–70% of the cases, and the PDC E1 α and E1 β subunits have been identified as subdominant targets [41, 47, 49, 70, 144, 145]. It can therefore be concluded that individuals who are positive for PBC-specific AMA, mainly those directed against PDC-E2, branched-chain 2-oxo-acid dehydrogenase complex, and/or 2-oxoglutarate dehydrogenase complex epitopes are at risk of developing PBC, even in the absence of symptoms or abnormal liver biochemistry [41, 44, 47, 49, 70, 144, 145].

Individuals at High Risk of Developing PBC

In an hierarchy of risks predisposing to the development of PBC the most important is familiarity, especially in female members of a PBC patient: sisters and daughters of PBC patients are at the greatest risk. This risk is likely to be higher in subjects with a history of cigarette smoke exposure or with a history of recurrent UTI. These individuals should be encouraged to be screened for AMA in view of its predicting value. An obvious question is at what age, and how often one should screen these individuals. Current North American and European guidelines, issued for the management of patients with PBC, do not provide a clear course of action for this group of predisposed individuals [43, 146]. In familial PBC, subsequent generations tend to develop the disease at a younger age, and in some cases, PBC progression is more aggressive. More work needs to be done to establish criteria indicating who needs to be screened, when to start screening, and how often screening should be performed. What is clear is that there is a sub-group of PBC patients who will never proceed to end-stage liver disease, but will

be affected by the symptoms of PBC, such as pruritus and fatigue. Identification of these individuals may allow an early tailored treatment. Although some risk has also been attributed to the use of hair dyes, nail polish and other cosmetics, it is uncertain whether these truly pose a significant risk: it may be premature and counterproductive to advise against their use. More research is also needed on the role of estrogens. Genome-wide studies have provided new tools to identify individuals at risk of PBC. Identification of disease associated genes is not only diagnostically and prognostically relevant but provides clues for unravelling the pathogenesis of PBC.

Finally we note that this paper is part of a special issue highlighting the 7th Congress of Autoimmunity. This Congress focussed on environment and geoepidemiology as risk factors for the development of autoimmunity [1, 7, 14, 16, 19, 147–190]. It was the first Congress of Autoimmunity that focused on this issue and, much as we have emphasized herein, it is a critical issue not only for physicians/scientists but also for patients/families. We expect with the era of genomics, epigenetics and next generation sequencing that we are just at the cusp of this important subject.

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