Primary biliary cirrhosis: Environmental risk factors

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Abstract. Primary biliary cirrhosis (PBC) is an autoimmune disease of unclear etiology. It is a chronic, progressive condition that causes intrahepatic ductal destruction ultimately leading to symptoms of cholestasis, cirrhosis and liver failure. The disease predominantly affects middle aged Caucasian women. It has a predilection to certain regions and is found in higher incidences in North America and Northern Europe. It also has a genetic predisposition with a concordance rate of 60% among monozygotic twins. Combinations of genetic and environmental factors are proposed in the pathogenesis of this disease with a compelling body of evidence that suggests a role for both these factors. This review will elucidate data on the proposed environmental agents involved in the disease’s pathogenesis including xenobiotic and microbial exposure and present some of the supporting epidemiologic data.

1. Introduction

Primary biliary cirrhosis (PBC) is a disease of presumed autoimmune etiology that causes inflammation within the portal tracts. This leads to destruction of small and medium sized intrahepatic biliary ducts. Ultimately, this may result in symptoms of cholestasis, cirrhosis and liver failure [1]. The serologic hallmark of the disease is the production of anti-mitochondrial antibodies (AMA), known to react most frequently with the E2 subunit of private dehydrogenase complex among other autoantigens [2]. The disease predominates among women with a 10:1 female to male ratio and is usually diagnosed in middle aged women though it has been diagnosed in patients as young as 15 and as old as 93 [1,3].

The pathogenesis of the disease remains enigmatic since the cause is likely complex and multi-factorial. The current view is that PBC occurs in a genetically susceptible population with external triggers precipitating its development. Considerable evidence points to environmental triggers involved in the development of this disease. In this review, we will focus on observed and circumstantial data pertaining to possible environmental exposures including xenobiotics, and microbes.

2. Genetic predisposition

The prevalence of PBC amongst families with an affected member is estimated to be 1000 times greater than the general population [4,5]. PBC has been weakly associated with the HLA-DRB1*0801 haplotype and a recent study by Hirschfield et al suggests a significant association between HLA class II, IL12A and IL12RB2 loci as well [6]. This is especially interesting as these haplotypes are associated with IL12 immunoregulatory signaling. These findings further affirm the genetic predisposition to this disease.

In 2004, Selmi et al identified 8 pairs of monozygotic twins and 8 pairs of dizygotic twins from a large cohort of patients with PBC. 60% of the monozygotic twins had PBC affecting both siblings while none of the dizygotic twins were dually affected. This concordance rate for the monozygotic twins is one of the highest of all diseases. This study confirms the strong role genetics plays in the pathogenesis of this disease [4]. However, genetics alone are not sufficient to explain the pathogenesis as 3 of the 8 monozygotic twins were not concordant for the disease. There is likely a second trigger.
3. Disease triggers

PBC demonstrates a large geographic variation in its incidence and prevalence. The incidence of the disease is significantly higher in Northern Europe and North America when compared to Mediterranean and African countries [1]. In addition, disease clustering with and without environmental associations has been reported by several groups (Table 1). Abu-Mouch et al described 4 unique clusters including: a family of 10 siblings with half having PBC, a husband and wife with PBC, a family of two genetically unrelated individuals with PBC, and a cluster of PBC in Alaska [7]. Their data adds to the considerable body of clinical and molecular data suggesting clustering of this disease is associated both with genetic and likely environmental triggers. The epidemiologic and molecular data presented below help support this hypothesis.

4. Epidemiology

The first real suggestion of disease clustering was reported by Triger in 1980 when he found clustering of PBC cases in Sheffield, England. He proposed groundwater contamination as a possible source for this clustering. However, analysis of the suspected water reservoir, showed no significant differences from the other reservoirs in the region [8]. James et al. also proposed water contamination as a possible mechanism of the PBC clustering that his group noted in urban areas of Northeast England [9].

In 2006, we investigated whether our perception of a higher prevalence of PBC from an area known to be developed on landfill sites was accurate. Superfund sites (SFS) are hazardous waste sites designated for clean-up by the Department of Environment and Conservation (DEC). We looked at prevalence rates of PBC near designated SFS in New York City. Data were collected from PBC and PSC patients in the Mount Sinai School of Medicine PBC database (MSSM PBC database). To avoid referral bias, we also collected data from all PBC and PSC patients listed for liver transplantation by the Organ Procurement Transfer Network (OPTN) in the five boroughs of New York City from 1995–2003. In the first part of our study we examined zip codes for all PBC (n = 99) and PSC (n = 73) patients listed for liver transplant in New York City. Expected prevalence ratios, adjusted for age, gender and race were calculated. Median standardized prevalence ratios in zip codes containing/adjacent to a SFS (n = 89) were compared to the zip codes without SFS (n = 85). Median prevalence ratios for PBC were significantly higher in clusters associated with SFS compared with PSC (Table 2).

In the second part of our study, we applied SaTScan technology (a validated statistical analysis software program looking for disease clusters) to detect specific clusters of PBC near SFS using our OPTN and MSSM PBC databases [10]. Again, an increased prevalence of PBC patients was identified in clusters around SFS. Furthermore, the clusters of PBC in our cohort (MSSM PBC database) correlated with the OPTN data [11]. The major contaminants in these superfund sites were halogenated aromatic hydrocarbons including polychloroethylene and benzene.
Since then many studies continue to show geographic clustering of PBC [8,9,11–18]. McNally et al showed space-time clustering in a population from Northeast England [19]. This phenomenon is interesting as it supports the role for a transient environmental agent in the disease pathogenesis. Space-time clustering is an epidemiologic term used to describe a large number of cases presenting in a small geographic area within a limited time period. When space time clustering is identified, a transient, possibly environmental agent becomes suspect. Using population based data from the northeast regions of England over a 16 year period (1987–2003), space time clustering was noted for PBC. The Knox test and K-function methods used in statistical determination of spatiotemporal patterns, were used in this study to analyze space-time clustering. Variations in population density were adjusted based on nearest neighbor thresholds. Individual space-time clusters were identified using Kulldorff’s scan statistic. Using these methods, 1015 cases of highly significant space-time clustering were identified using Kulldorff’s scan statistic. Using population based data from the northeast regions of England over a 16 year period (1987–2003), space time clustering was noted for PBC. The Knox test and K-function methods used in statistical determination of spatiotemporal patterns, were used in this study to analyze space-time clustering. Variations in population density were adjusted based on nearest neighbor thresholds. Individual space-time clusters were identified using Kulldorff’s scan statistic. Using these methods, 1015 cases of highly significant space-time clustering were identified (P < 0.001). Cases diagnosed within 1–4 months of each other showed the highest degree of clustering. McNally’s identification of space-time clustering using rigorous statistical tools in a stable population without migration is highly suggestive of transient environmental agent involvement in disease pathogenesis.

In the largest epidemiologic study involving PBC, Gershwin et al interviewed 1032 individuals with PBC and a randomly picked control group [20]. Using multivariate analysis, they were able to show multiple risk factors for developing PBC. These included: having a first degree relative with PBC, recurrent urinary tract infections, past smoking history, use of hormone replacement therapy, and the frequent use of nail polish. Of note, an increased frequency of urinary tract infections has previously been reported in PBC [21]. As will be described in the next section, this association may be one related to molecular mimicry.

5. Molecular data

5.1. Xenobiotics

Xenobiotics are small molecular weight foreign chemicals that can complex with the body’s own proteins to alter structure and induce an immunogenic response. These small compounds are found ubiquitously in our environment in household detergents, cleaners, food preservatives, pesticides and pollutants among other things. They have been implicated in the pathogenesis of other autoimmune disease. For example, systemic lupus erythematosis can be induced by the drug hydralazine and scleroderma has been linked to silica exposure [22]. In a similar manner, the involvement of xenobiotics in the pathogenesis of PBC has also been studied. Most xenobiotics are processed by the liver.

The liver is the major detoxification organ of the body and the biliary tree is thereby exposed to high levels of xenobiotics during their clearance. Gershwin et al have extensively studied the immunoenicity of modified forms of PDC-E2, the major autoantigen recognized by anti-mitochondrial antibody. They propose loss of tolerance to PDC-E2, secondary to modification of the autoantigen by xenobiotic conjugation, as a mechanism for triggering autoantibody production (AMA) in PBC [23]. Several studies have implicated halogenated compounds as the mimotope that triggers this cascade of events [24].

In one study, rabbits immunized with 6-bromohexionate, a halogenated xenobiotic complex, all developed AMA that reacted with PDC-E2 and inhibited its enzymatic function [25]. Similar results were seen in guinea pig models [26]. Interestingly, in rabbit models, tolerance was recovered and a gradual reduction in autoantibody production was documented following repeated immunization with the xenobiotic [27]. Other examples of possible xenobiotics include halothane. Halothane is an antiquated anesthetic which oxidizes to a reactive intermediate that binds to cellular proteins leading to tri fluoro acetyls (TFAs). These TFAs not only cause an immunogenic response but also cross react with PDC-E2 [28].

2-octynoic acid has also been identified as having possible in vivo activity in modifying PDC-E2. This xenobiotic is widely used in perfumes, makeup and nail polishes possibly suggesting a linkage for the female predominance of the disease [29]. Additionally as mentioned earlier, an epidemiologic study suggested nail polish use as a risk factor for PBC. Despite the many identified immunogenic xenobiotics, identifying potential causative agents is not an easy task as exposures and molecular modifications likely take place long before a diagnosis of PBC is made. The triggering agent may no longer be present at the time of disease manifestation.

5.2. Microbial exposure

A number of microbes have been implicated in the pathogenesis of PBC. DNA of gram positive bacteria
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and LTA, an antigenic bacterial cell wall component, have been detected in the bile of PBC patients. It is postulated that the peri-portal inflammation seen in PBC patients may cause increased ductal permeability. This may lead to leakage of bacterial antigens into the ductal system thereby provoking an immunogenic response [30]. The suggested mechanism of action for microbial exposure causing an immunogenic response is molecular mimicry. This refers to the hypothesis that antigens on bacteria and viruses may be similar enough to host antigens to elicit an autoimmune response by host T cells.

Escherichia coli have been suggested as a possible culprit. This is interesting because epidemiologic studies have shown an increased incidence of urinary tract infections in the PBC population [20,21,31]. As E. coli is the most common cause for these infections; this theory is an attractive one. The PDC-E2 auto-antigen is a highly preserved protein sequence in phylogeny and is found in many prokaryotes. Shimoda et al had shown that a peptide derived from E. coli was able to activate a host response against PDC-E2. These observations further suggest the possibility of molecular mimicry in the initiation of this disease [31]. Those that refute the theory have suggested that titers of antibodies against E. coli are often much less than titers against human complex. They are also often not seen in patients with early PBC but rather in those with advanced disease. Another gram negative bacterium that has been implicated in PBC is the aerobic Novosphingobium aromaticivorans. This microbe is found in soil and water and is non-pathogenic in humans. Studies have shown a high degree of homology between the sequence of human PDC-E2 and lipoylated proteins for N. aromaticivorans. The reactivity against PDC-E2 is also 100 to 1000 fold greater than that seen with E. coli antigens. One study showed 100% reactivity with the bacterium in PBC patients compared to none in the control group [32]. Another study demonstrated that autoreactive AMA and T-cell mediated autoimmunity can be induced in the biliary ducts after immunization with this bacterium [33]. However, this was done in murine models. Interestingly, the bacteria also have a role in activating estrogens suggesting a role in the female predominance of PBC [34]. Furthermore, this bacterium is commonly found in soil and water, again linking with the aforementioned epidemiologic studies of PBC.

A role for viruses in the pathogenesis of PBC has also been explored. One group of investigators was able to isolate human beta retrovirus in the peri-hepatic lymph nodes of PBC patients. The retrovirus bore similarities to a murine mammary tumor and human retrovirus cloned from breast cancer tissue [35]. However this study could not be replicated by other groups. There have also been studies of Propionibacterium, Chlamydia and a case report of lactobacillus as causative factors of PBC; these findings have yet to be reproduced [36–38]. Identifying a single causative microbe in the pathogenesis of PBC is unlikely given the natural course of the disease. It is also probable that there are multiple agents capable of inciting the immune mediated response (Table 3).

5.3. Current perspective and future direction

Rather than solving the enigma of PBC, we have presented many of the individual pieces of this still unsolved puzzle. Much of our knowledge is based on observational and circumstantial evidence. While some of the pieces seem to form a cohesive theory others may be a “red herring”. The current thought remains that the pathogenesis of PBC is both complex and multi-factorial in nature.

Like other autoimmune conditions, the two hit hypothesis has been suggested in which an already genetically susceptible individual faces a secondary insult triggering the cascade of autoimmune events. However, neither the genetic inheritance pattern nor the inciting triggers are yet clear. Though numerous studies have supported a role for environmental agents such as xenobiotics and microbes, further work is needed to elucidate this disease’s development. The development of animal models will likely help us move towards putting the pieces of the puzzle together. Clearly, great strides remain to be made in the study of PBC and other autoimmune diseases.

Table 3  Proposed antigens in pathogenesis of PBC

| Xenobiotics:          |
|-----------------------|
| Benzene               |
| Polychlorethylene     |
| 6-bromohexonate       |
| 2-octynoic acid       |
| Halothane             |
| Other halogenated hydrocarbons |

| Microbes:            |
|----------------------|
| E. coli              |
| N. Aromaticivorans   |
| Human β retrovirus   |
| Chlamydia            |
| P. acnes             |
| Lactobacillus        |
| Mycobacterium        |

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