Infection and Primary Biliary Cirrhosis

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ABSTRACT: Primary biliary cirrhosis is an autoimmune cholestatic liver disease characterized by humoral and cellular response directed at mitochondrial autoantigens, mainly the E2 component of the pyruvate dehydrogenase complex. The etiology of PBC, like most polygenic autoimmune diseases, belongs to the "complex" category, including genetic elements and environmental factors. Many environmental factors, such as xenobiotics, smoking, hormonal therapy, toxins, oxidative stress and recurrent urinary tract infections, are associated with PBC. Infectious agents can trigger autoimmunity via several mechanisms and are associated with various autoimmune diseases. A relationship between PBC and several infectious agents, and a possible role for *Escherichia coli* in the pathogenesis of PBC, have been suggested. The identification of a culprit agent that induces or exacerbates PBC might have diagnostic and therapeutic implications. This review evaluates the evidence for an infectious agent role in the pathogenesis of PBC.

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Primary biliary cirrhosis is a chronic, slowly progressive cholestatic liver disease that affects mostly women over the age of 40 years and may progress to cirrhosis and liver failure [1]. PBC prevalence differs considerably between different geographic areas, and has increased in the last decade to 1 in 700 people in some western populations [2]. PBC is characterized biochemically by cholestasis, serologically by antimitochondrial antibodies, and histopathologically by inflammation and destruction of intrahepatic bile ducts [1,3]. The etiology of PBC is unknown, but considerable evidence points to a multi-hit process involving an autoimmune activation triggered by environmental factors in genetically susceptible individuals [4]. Although no major histocompatibility complex alleles are clearly associated with PBC, genetic predisposition is strongly supported by the 50–100 fold higher relative risk of first-degree family members, and the concordance rate of PBC in identical twins which is among the highest reported for any autoimmune disease [5,6].

PBC = primary biliary cirrhosis

PBC predominates in women with a ratio of 10 to 1, and X-chromosome monosomy in lymphoid cells was proposed as an explanation for this predilection [1].

Epidemiological studies suggest environmental factors that can trigger or exacerbate PBC by molecular mimicry, hapten modifications, xenobiotic modification, direct toxic effect and other mechanisms that break self-tolerance and induce autoimmunity [7]. In a large-scale study that evaluated 1032 PBC patients, the risk factors for developing the disease apart from familial predisposition were past smoking, the use of hormonal replacement therapy, history of urinary tract infections, and frequent use of nail polish [4]. Different toxins and oxidative stress were also associated with PBC [8]. Today a large body of evidence supports a role for xenobiotics in the pathogenesis of PBC. Xenobiotics are chemicals such as drugs, pesticides and organic molecules, foreign to an organism, that are removed by metabolism, deactivation and secretion and occur mostly in the liver. Xenobiotics may modify the host protein to create a neo-antigen and thereby induce the breakdown of self-tolerance; a number of those chemicals are associated with autoimmune diseases [5]. Xenobiotic modifications of self-proteins were strongly reactive with sera of PBC patients. Immunization of animals with xenobiotics conjugated to bovine serum albumin in complete Freund's adjuvant caused serological and histopathological reactions similar to PBC, providing the first inducible animal model of PBC [7].

Infectious agents have also been suggested as causative agents of PBC [1]. The possibility of an autoimmune disease being invoked by an infectious agent has been researched and different mechanisms have been proposed [9,10]. The aim of this review is to evaluate the evidence for an infectious agent role in the pathogenesis of PBC.

PBC AND THE IMMUNE SYSTEM

The immune system's ability to distinguish self from non-self is essential for the protection of self-antigen from autoimmune destruction as well as for host defense against microbial antigens [11]. From an immune system perspective PBC is characterized by the breakdown of self-tolerance to highly conserved mitochondrial and nuclear antigens, and by profound changes in the adaptive and innate immunity [12]. The autoimmune nature of PBC is further supported by

geographic variation with a 'north-south' gradient, familial occurrence, and associations with other autoimmune diseases [13].

The diagnostic hallmark of PBC is the presence of antimitochondrial antibodies in 95% of the patients. Although AMA are not the direct cause of biliary damage, they can predict the disease and are detectable long before the clinical and histological features of PBC appear [14]. Twenty years ago Gershwin et al. [1,7] reported the cloning and identification of the immunodominant autoantigen in PBC. AMA are directed at the members of the 2-oxo-acid dehydrogenase complex family, which includes four autoantigens with substantial homology and are located in the inner mitochondrial matrix [1,3]. The dominant antibody response in PBC is directed at the pyruvate dehydrogenase complex. Within the PDC the reactivity in more than 90% is to a highly conserved motif present within the dihydrolipoamide acetyltransferase (PDC-E2). B cells, which are responsible for AMA release, respond to the PDC-E2 and may have an additional role as antigen-presenting cells that prime a specific T cell response. T cells in PBC, both CD4 and CD8 cells infiltrating the liver, are directed at self-PDC-E2. These specific T cells are present in portal tracts of PBC patients and entirely absent from portal tracts of normal and chronic liver disease controls [15,16]. Human PDC-E2 is the dominant CD4 T cell epitope restricted with HLA-DR53, and CD4 T cells are 100–150 times more frequent in the liver and regional lymph node of patients with PBC than in the circulation [1,17]. CD8 T cell response targeted at PCD-E2 is highest in the early stages of the disease and correlates with the highest bile duct loss [15]. Autoreactive PDC-E2-specific CD8 T cells are tenfold more common in the liver of PBC patients compared to peripheral blood [15,18]. Activated T cells transferred into naïve animals induce bile duct lesions resembling PBC [19]. Furthermore, CD4 autoreactive T cells were identified even in AMA-negative PBC patients [7]. These data suggest that although AMA have a limited role in disease development, the antimitochondrial multi-lineage immune response of autoantibodies, B cells, CD4 and CD8 T cells directed at PDC-E2 is directly related to the initial immunological insult in PBC [7,20].

However, there is a conceptual problem associated with an apparently highly tissue-specific pathological process (biliary epithelium) mediated by an immune response directed at a ubiquitous self-antigen. Antigenic mimicry and bile ducts biology may account for this tissue-specific pathology. PDC-E2 are normally located in the inner surface of the mitochondrial membranes; however, molecules that cross-react with PDC-E2 are expressed on the apical surface of biliary epithelial cells, selectively in patients with PBC [7]. Primed T cells may recognize the conserved sequence in association with class 2 human leukocyte antigen, which are inappropriately expressed on bile duct epithelia [18]. Aberrant apoptosis of biliary epithelial cells with less glutatiolation of PDC-E2 enables the immune system to recognize the autoantigen specifically on biliary epithelial cells [7].

The innate immune system has been less well studied in PBC, but augmented mononuclear response to Toll-like receptor ligands and increased level of cytokine transcription within the liver of PBC support chronic activation of the innate immune system [21,22].

PBC AND INFECTIOUS DISEASES

The relationship between infections and autoimmune diseases has been extensively studied [23]. In genetically susceptible individuals, infectious agents can initiate or enhance an autoimmune disease via several mechanisms [24]. Molecular mimicry is a direct mechanism, where the infection agent presents an epitope structurally similar to a self-antigen [11]. Epitope spreading is a different mechanism, where infection accelerates an ongoing autoimmune process by local activation of antigen-presenting cells and over-processing of antigens [25]. Viral agents may enhance polyclonal activation of B lymphocytes, which will further enhance the production of antibodies and immune complexes that may damage self-tissues [11]. Viral and bacterial superantigens can bind to a wide variety of MHC class 2 molecules, and to the T cell receptor beta chain variable domain. Super-antigens may accelerate T cell-mediated autoimmunity via priming of large numbers of T cells irrespective of their specificity [11]. Furthermore, infection itself may induce autoimmunity via bystander activation, meaning an enhanced cytokine production that induces the expansion of autoreactive T cells [11].

Several infectious candidates have been suggested as causative agents of PBC. Molecular mimicry of human mitochondrial epitopes to microbial antigens is the most widely proposed mechanism [26]. PDC-E2, the major human antimitochondrial autoantigen, is a well-conserved sequence among various species, with a high degree of similarity to microbial PDC sequences of *E. coli*, *Helicobacter pylori*, cytomegalovirus and others [26]. In addition, AMA from PBC patients cross-react with bacterial PDC-E2, and immunoglobulin G anti-PDC can be identified in humans during infectious diseases in the absence of liver damage [27,28].

Even though infectious agents have been associated with PBC, it is important to note that molecular similarity and cross-reactivity alone do not necessarily imply that the infectious agent is the cause of PBC, or that the initial immune response was to the microbial PDC-E2.

AMA = antimitochondrial antibodies
PDC = pyruvate dehydrogenase complex
MHC = major histocompatibility complex

ESCHERICHIA COLI

E. coli is a common cause of urinary tract infection, which has attracted attention as a causative agent of PBC. A high annual incidence of UTI (35%) in women with PBC, and predominantly new infection in PBC patients with recurrent UTI were reported [29]. Intestinal colonization by R(rough)-forms *E. coli* was found in the stool of 22 PBC patients (100%) vs. 1 of 20 healthy controls and 25% of patients with other liver diseases [30]. Molecular mimicry and cross-reactivity of human PDC-E2 and *E. coli* PDC-E2 were the main mechanisms evaluated in several studies. A low titer of AMA was measured in the sera of healthy women with a history of recurrent urinary tract infection regardless of any evidence for liver disease [31]. A high affinity of anti-human PDC-E2 to *E. coli* PDC-E2 was 100-fold higher in PBC patients [32]. T cell clones selected by human PDC-E2 were reactive with peptides of *E. coli* PDC-E2 and *E. coli* OGDC-E2 [33], while T cell clones specific for *E. coli* OGDC-E2 were reactive with human mitochondrial autoantigens [20].

However, antibodies to *E. coli* PDC are more frequent in the later stages of the disease and in low titers, whereas antibodies to another xenobiotic metabolizing gram-negative bacterium, *Novosphigobium aromaticivorans*, are 1000 times higher than the titers to *E. coli* and can be found in the early stages of the disease [34].

CHLAMYDIA PNEUMONIAE

Chlamydia pneumoniae is a common cause of community-acquired pneumonia, which may have a role in chronic inflammatory processes such as atherosclerosis, multiple sclerosis and sarcoidosis [35]. A potential role for *C. pneumoniae* as a triggering or even a causative agent of PBC was suggested in one study by demonstrating CP-DNA in 100% of 39 liver biopsies of PBC patients, as compared to 8.5% of 105 controls biopsies [35]. By contrast, two other studies failed to demonstrate correlation between *C. pneumoniae* and PBC; in one of them chlamydial rRNA gene or antigens were not increased in liver specimens from PBC patients, and in the other, CP-IgG levels were not increased in Chinese patients with PBC compared to patients with post-hepatitis cirrhosis [36].

HELICOBACTER PYLORI

Helicobacter pylori infection is acquired in most cases during childhood and induces autoantibodies to the H+K+ATPase located in the gastric parietal cell. *H. pylori* can cause chronic gastric inflammation, peptic ulcer disease, gastric carcinoma and gastric MALT lymphoma and has been associated with numerous extragastric diseases [37]. Microbial mimicry of *H. pylori* peptides and human pyruvate dehydrogenase has been observed [26]. However, clinical or serological association could not be demonstrated, and seroprevalence of *H. pylori* infection in 149 PBC patients was not increased compared to 619 healthy volunteers [37]. Two other studies failed to identify increased prevalence of *H. pylori* DNA in liver tissues from PBC patients [10,38].

MYCOBACTERIUM

Mycobacterium infections, especially *Mycobacterium gordonae*, have been suggested to have a role in the pathogenesis of PBC. Cross-reactivity of sera from PBC patients with an extract of *M. gordonae*, as well as cross-reactivity of antibodies to *M. gordonae* heat-shock protein with human mitochondrial antibodies were demonstrated by the same group [39]. Reactivity of sera from tuberculosis patients with the PDC-E2 subunit was reported by another group [27]. By contrast, others failed to confirm serum reactivity of PBC patients with mycobacteria, or to detect Mycobacterium DNA in the liver of PBC patients [10,40].

VIRAL INFECTIONS

Few viral infections have been associated with PBC. An association between a retrovirus resembling mouse mammary-tumor virus and PBC was reported, however this association was not reproducible [1]. Epstein-Barr virus is a virus of the herpes family and the cause of infectious mononucleosis. Recently, increased titers of EBV early antigen were observed in sera of patients with PBC [9]. Cytomegalovirus is another virus of the herpes family, with peptide sequences similar to the human PDC sequence [26].

DISCUSSION

PBC is an autoimmune cholestatic liver disease characterized by a multi-lineage immune response directed at the mitochondrial epitope PDC-E2. The etiology of the autoimmune activation in PBC is multifactorial, including genetic susceptibility and different environmental factors such as xenobiotics, smoking, toxins, and probably infectious agents. Infections may lead to autoimmunity and are associated with various autoimmune diseases. Although a cause and effect relationship between an infectious agent and PBC has yet to be proven, there is evidence to suggest a role for *E. coli* in the development or aggravation of PBC. The presence of AMA in women with recurrent UTI regardless of liver disease, the

UTI = urinary tract infection
CP = *C. pneumoniae*
Ig = immunoglobulin

EBV = Epstein-Barr virus

increased prevalence of recurrent UTI in women with PBC, and the high molecular mimicry and cross-reactivity between *E. coli* and human PDC-E2 are in favor of this thesis. Data regarding the role of other agents in PBC as *Helicobacter pylori, Chlamydia pneumonia*, Mycobacterium, and viral infections are yet inconclusive, and require further studies.

In the present era, early diagnosis and treatment of PBC might delay or abort disease progression [1]. The identification of a culprit agent or agents that induce or exacerbate PBC might have therapeutic implications, since early identification of high risk patients and specific therapy may help delay or prevent the evolution of autoimmunity.

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