

# Infectious agents in the pathogenesis of primary biliary cirrhosis

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**Abstract.** Primary biliary cirrhosis (PBC) is a chronic progressive cholestatic liver disease which is characterized by the breakdown of self-tolerance to the highly conserved pyruvate dehydrogenase complex, specially the pyruvate dehydrogenase E2 complex (PDC-E2). The breakdown of the tolerance to such antigens leads to an autoimmune process characterized by portal inflammation and immune-mediated destruction of the intrahepatic bile ducts. Epidemiological studies have suggested that infectious agents can trigger or even exacerbate the disease. Among other gram negative bacteria, *Escherichia Coli*, and *Nosphiogobium aromaticivorans* are the most associated agents reported hitherto. Epidemiological and molecular evidence points towards molecular mimicry between some components of these microorganisms and specific amino-acid sequences that are present in proteins on normal cells of the biliary tract. In this review, we revisit all reports suggesting that infectious agents might be associated with the autoimmune pathogenesis of PBC. We also retrieve the immune molecular mimicry mechanisms that are likely involved with the autoimmune process in PBC.

**Keywords:** Primary biliary cirrhosis, infectious agents, molecular mimicry, tolerance, autoimmunity

## 1. Introduction

Primary biliary cirrhosis (PBC) is a chronic progressive cholestatic liver disease that affects mostly women over the age of 40 years and may progress to cirrhosis and liver failure [1]. The etiology of PBC is unknown, but considerable evidence points to a multi-causal process, involving an autoimmune activation triggered by environmental factors on the basis of a genetic background. Limited studies have indicated the association of HLA-DR8 with PBC [2], but in general, no particular HLA haplotype is considered to be associated with the occurrence of PBC. However genetic predisposition is strongly supported by almost 50 to 100 fold higher relative risk in first-degree relatives as well as the highest concordance rate between identical twins when com-

pared to other autoimmune diseases (ADs) [3]. There are also some differences regarding gender, PBC predominates mainly in women with a ratio of 10 to 1, suggesting some hormonal influence [1]. From a molecular perspective, PBC is characterized by the breakdown of self-tolerance to the highly conserved pyruvate dehydrogenase complex, specially the pyruvate dehydrogenase E2 complex (PDC-E2) [4]. Although, there is no general consensus, ten long amino acid row residues 163 through 176 carrying a lipoyl domain site in human PDC-E2 (163–176) are thought to be the prominent immunodominant epitope. Extensive studies have shown that that amino acid residues E, D, and K at positions 170, 172, and 173, respectively, are essential for recognition by the T-cell clones [5,6]. Comparison in homology of some bacterial antigens and normal human PDC-E2 has shown high degree of identity in such critical amino-acid sequence and also antibodies against PDC-E2 (163–173) are able to cross-react with specific bacteria components. Likewise, epidemiological studies have suggested that infections can trigger or even exacerbate the disease [7,8]. In this regard,

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several infectious candidates have been suggested as potential causative agents of PBC [9–11], *Escherichia Coli*, *Chlamydia Pneumoniae*, *Mycobacterium gordonae*, and *Nosphiogobium aromaticivorans* among others have been reported [7,12–15]. The evidence points towards molecular mimicry between some components of microorganisms and specific amino-acid sequences that are present in proteins on normal cells of the biliary tract [4,7]. Herein, we review all reports suggesting that infectious agents might be associated with the autoimmune pathogenesis of PBC.

## 2. Autoimmune mechanisms in PBC and cross-reactivity

PBC is the result of the breakdown of self tolerance to the mitochondrial and nuclear antigens as well as profound changes in the adaptive immunity [4,16]. The dominant antibody response in PBC is directed to the evolutionarily pyruvate 2-oxo-acid dehydrogenase complexes (2-OADC) located in the inner mitochondrial membrane. In this regard, the diagnostic hallmark of PBC is the presence of antimitochondrial antibodies (AMA) in 95% of the patients. The targets of the AMA includes the E2 subunits of the pyruvate dehydrogenase complex, the branched-chain 2-oxo-acid dehydrogenase complex, the ketoglutaric acid dehydrogenase complex, and the dihydrolipoamide dehydrogenase binding protein [17]. Although AMA are not the direct cause of biliary damage, they can predict disease onset and are detectable long before the clinical and histological features of PBC develop [18].

Most commonly, antibodies react against PDC-E2 [1,17]. The E2 enzymes have a common structure consisting of the N-terminal domain containing the lipoyl group (Fig. 1). A number of studies using oligopeptides or recombinant proteins have demonstrated that the dominant epitope recognized by AMA is located within the lipoyl domain. Gershwin et al. [19], suggested the important role of lipoic acid in determining the immunogenicity of mitochondrial antigens in PBC, and also hypothesized that modifications of this domain are involved in the breakdown of tolerance in animal models [20]. Several studies have suggested that microbial infection has a role in the induction of AMA, through a mechanism of molecular mimicry [9, 14,21]. Protein data based studies have shown that more than 10 microbial sequences have high degree of similarity with human PDC-E2 [14]. Reactivity and competitive cross-reactivity sera studies between

human mitochondrial and microbial components have shown competitive inhibition, suggesting a positive association [9,14,21]. The large number of immunogenic mimics may account for the dominance of the PDC-E2 in the pathogenesis of the disease.

Regarding T Cell response against mitochondrial antigens, the frequencies of CD8+ T cells, natural killer T-cells, and B-cells that are reactive with the PDC-E2 are also higher in number in the liver than in peripheral blood [22]. Activated T-cells transferred into naive animals induce bile duct lesions resembling human PBC [23]. Furthermore, CD4+ autoreactive T cells have been identified even in AMA-negative PBC patients [19]. These data suggest that although AMA have a limited role in disease development, the whole antimitochondrial multi-lineage immune response addressed by autoantibodies, B cells, CD4+ and CD8+ T cells mainly directed to PDC-E2 might constitute the initial immunological insult in PBC [19,24].

## 3. Infectious agents in PBC

Several infectious candidates have been suggested as causative agents of PBC and molecular mimicry of human mitochondrial epitopes to microbial antigens is the most widely proposed mechanism [14]. Molecular mimicry is a direct mechanism, where the infection agents present an epitope structurally similar to a self-antigen [25]. As described above PDC-E2, the major antimitochondrial autoantigen, is a well-conserved sequence among various species, with a high degree of similarity to microbial PDC sequences of some microorganisms such as *E. coli*, *Helicobacter pylori*, *Cytomegalovirus* among others [14]. In one study, AMA from patients with PBC were able to cross-react with PDC-E2 components derived from such microorganisms [26]. Although this theory is the most widely accepted, there is another potential mechanism known as epitope spreading, in which, infection accelerates an ongoing autoimmune process by the enhancement of local activation of antigen-presenting cells and over-processing of antigens in individuals prone to develop PBC [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 agents can cause PBC by themselves, or that the initial immune response is directed to the microbial PDC-E2. It rather implies that this is a multi-causal process occurring in genetic prone patients [29].

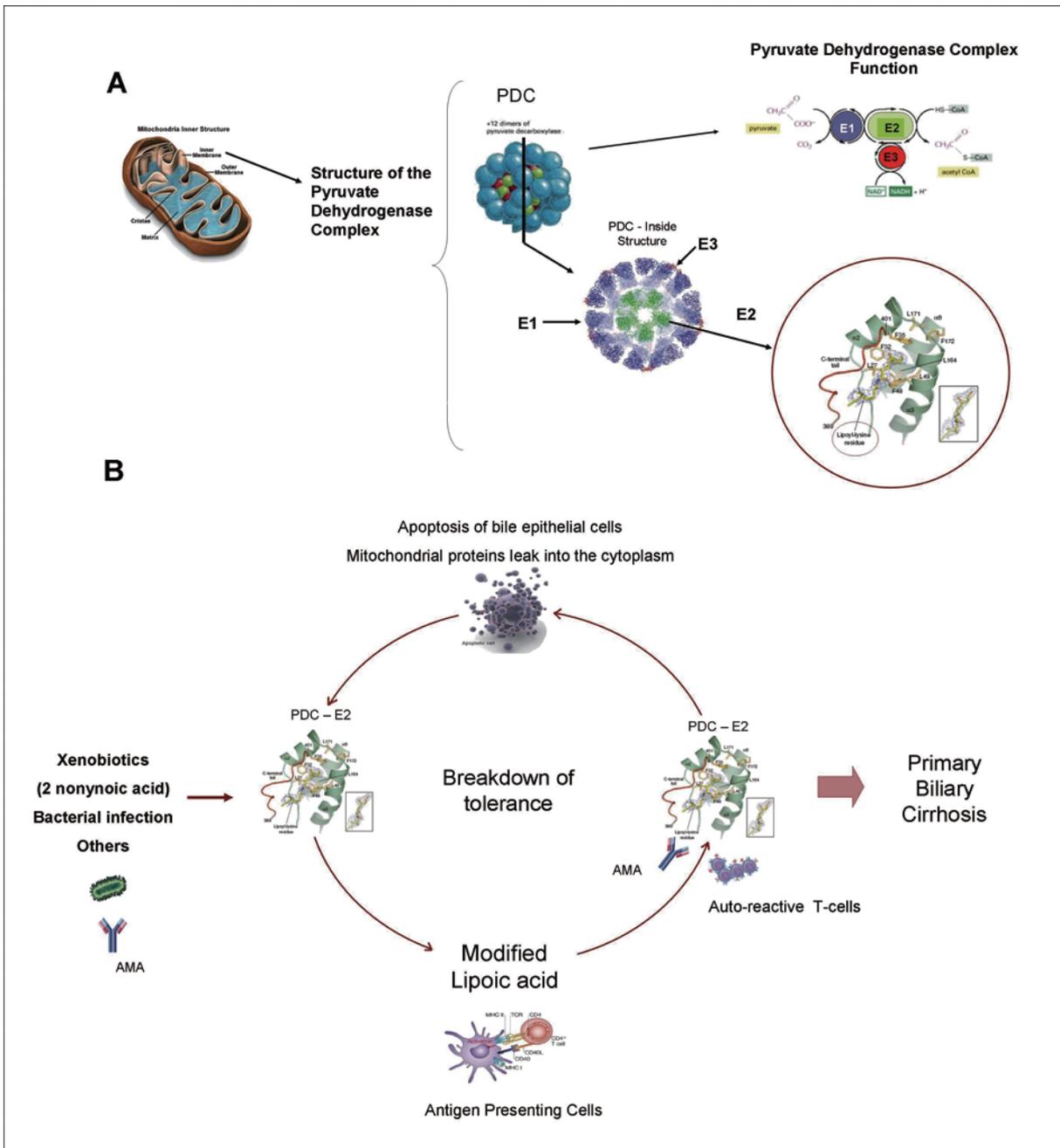


Fig. 1. (A) The structure of the pyruvate dehydrogenase a multienzyme complex (PDC), which is located on the inner membrane of mitochondria. It contains 60 polypeptide chains organized in three main complexes 2-oxo acid dehydrogenase (E1 subunit), dihydrolipoamide acyltransferase (E2 subunit), and dihydrolipoamide dehydrogenase (E3 subunit). The three enzymes are coupled and reaction intermediates are passed directly from one enzyme to another. PDC converts pyruvate to acetyl CoA in the mitochondrial matrix; NADH is also produced in this reaction. The E2 subunits carry lipoic acid, an essential cofactor, which is covalently bound to the lysine residue within the lipoylated domain of the protein. (B) Apoptosis of bile epithelial cells might expose critical epitopes with high degree of homology in specific aminoacid sequences with antigens from several infectious agents. In addition to such process, environmental risk factors (xenobiotics), and other unknown factors may cause modification in the lipoic acid causing the break of tolerance and the onset of an autoimmune destructive process against mitochondrial normal components. Antimitochondrial antibodies (AMA) are mainly directed against the lipoyl domain site inside PDC-E2. AMA are able to cross react with both human and infectious agents antigens.

### 3.1. *Nosphingobium aromaticivorans*

*N. aromaticivorans* is a gram negative strictly aerobic bacteria which is found worldwide in soil, water, and coastal plain sediments that metabolizes xenobiotics and activates environmental estrogens. *N. aromaticivorans* has been suggested as a pathogenic cofactor in the development of PBC [30]. In one study, it was reported that antibodies against *N. aromaticivorans* were found in 100% of 77 PBC Italian patients and cross-reacted with human PDC-E2 [31]. An important question was whether this finding was generalizable or only limited to PBC patients from Italy. To address this Olafsson et al. studied 14 Iceland patients with PBC, and 85 first-degree relatives [32]. The majority of Icelanders are of Scandinavian and Celtic origin and genetically distinct from Italian population. Thirteen sera samples of 14 Icelandic PBC patients who were positive for antibodies against *N. aromaticivorans* reacted against at least one of the 2 oxo-acid dehydrogenase-E2 complexes [32]. It is of interest that one healthy first-degree relative had antibodies to *N. aromaticivorans* and developed PBC two years later. Approximately 25% of PBC patients and controls had *N. aromaticivorans* in their stools but only those with PBC had antibodies to *N. aromaticivorans*. Earlier reports described an association between PBC and drinking water from one reservoir in Northeast England, raising the possibility of a common exposure to *N. aromaticivorans* from the water supply [33,34].

In support of this notion, studies in mice have shown that infection with *N. aromaticivorans* induced the appearance of antibodies against microbial PDC-E2 and its mitochondrial counterpart. In addition to the cell injury, concomitant production of anti-PDC-E2 antibodies that cross-react with conserved PDC-E2 epitopes display by *N. aromaticivorans* was also confirmed [35]. It also triggered chronic T cell-mediated autoimmunity against small bile ducts [35]. Furthermore, the infection leads to the development of liver lesions resembling human PBC. Of 13 contiguous amino acids comprising the core sequence of human PDC-E2 (GDL-LAEIETDKAT), 12 are identical and only 1 is different in the corresponding sequence of *N. aromaticivorans* (Table 1). It has been shown that *N. aromaticivorans* exhibits PDC-E2-like proteins in their membranes with higher degree of homology to the immunodominant region of human PDC-E2 than any microorganism thus far studied (100–1,000 times greater than that of *Escherichia coli*) [36].

Furthermore, *N. aromaticivorans* can metabolize xenobiotics that are similar to the chemical compounds that react with sera from PBC patients [37]. Some of these xenobiotics are immunologically related to lipoic acid, the cofactor that is at the active center of PDC-E2. Even more, Xenobiotic incorporation into PDC can occur [38]. Thus, *N. aromaticivorans* can theoretically break down self-tolerance in two ways: by molecular mimicry due to subclinical infection and by the metabolism of xenobiotics that are present in the environment [37,38].

### 3.2. *Escherichia coli*

Molecular mimicry between *E. coli* and human PDC-E2 was first described by Burroughs et al. [39]. Intestinal colonization by R(rough)-forms *E. coli* was found in the stool of 22 PBC patients (100%) vs. 1 of 20 healthy controls as well as in 25% of patients with other liver diseases [40]. A high affinity of antibodies to PDC-E2 of *E. coli* was reported to be 100-fold higher in PBC patients [41]. Several studies have found an epidemiological association between *E. coli* and PBC [3,42]. The main finding comes from a controlled epidemiological analysis showing a positive association of PBC with recurrent urinary tract infection (UTI) [42]. Another epidemiological study performed on 1,032 PBC patients followed-up in 20 tertiary referral centers, and including 1,041 demographically-matched controls also confirmed the connection of UTI with PBC [3]. Reactivity to PDC-E2 up to 52% of sera from patients with chronic UTI was reported [43]. Other studies found that at least 33% of patients with PBC have antibodies that cross react to the ATP-dependent Clp protease of *E. coli*, especially to one specific aminoacid sequence (177–194) located inside the protein [44,45]. Moreover, the epitope of *E. coli* also maps to similar lipoyl domains which are present in human PDC-E2 [14] (Table 1). Such domain has been shown to be essential for T cell epitope recognition of both human PDC-E2 and *E. coli* PDC-E2 proteins [14,46]. This reactivity is strongly associated with a history of recurrent UTI [14]. Although there is a large amount of information supporting such an association, it is important to remark that antibodies to *E. coli* PDC-E2 are more frequent in the later stages of the disease and in low titers, whereas antibodies to *N. aromaticivorans* are found 1000 times higher in early stages of the disease [31].

Table 1  
Comparison in homology between normal human mitochondrial antigens and antigens of infectious agents presumably involved in the pathogenesis of primary biliary cirrhosis

Infectious Agents	Microorganism protein	ID protein	Critical aminoacid sequence position	Similarity to PDC-E2 a.a Sequence at positions <sup>a</sup> : 159K L S E G D L L A E I E T D K 173	Number of identity aminoacids at positions 150-173 on the PDC-E2	Similarity to the critical PDC-E2 a.a sequence (%)	Serological association with PBC in Clinical studies	Ref.
<i>N. aromaticivorans</i> <sup>b</sup>	Novo 1	Novo 1	100-115	100K V S S G D I M A E I E T D K 115	11	11/15 (73)	Positive	(30)
	Novo 2	Novo 2	21-36	21E V R S G D I L A E I E T D K 36	10	10/15 (67)		(31)
	Novo 3	Novo 3	28-43	28A Y E P G Q V L Y E I E T D K 43	8	8/15 (53)		(32)
	Novo 4	Novo 4	30-45	30T V E E D G R L A D M M T D K 45	6	6/15 (40)		(33)
<i>E. Coli</i>	ATP-dependent clp X	ClpX	280-294	280K A S E G E L L A Q V E P E D 294	8	14/15 (93)		(3)
	ATP-dependent Helicase hrpA	HRPA	153-167	153L M T D G I L L A E I Q Q D R 167	7	12/15 (80)	Positive	(14)
	Pyruvate dehydrogenase complex- E2	PDC-E2	231-245	231K V A E Q S L I T V E G D K 245	5	11/15 (73)		(40)
<i>P.auriginosa</i>	Diaminopimelate decarboxylase	DCDA	663-677	663V T P N V D L L A E L M A R S 677	5	10/15 (67)	N/A	(14)
	Heat Shock Protein 65 Kda	Hsp-65 Kda	90-104	90D Q S I G D L I A E A M D K V 104	6	7/15 (47)	Negative	(7)
<i>H. influenzae</i>	tRNA (uracil-5)-methyltransferase	TRMA	205-219	205Q N S E G D L L E L Y C G N G 219	6	8/15 (53)	N/A	(39)
	Beta galactosidase	BGAL	265-280	266R D S E G D L V A E K L G P I 280	7	10/15 (67)	N/A	(49)
<i>Y. Enterocolitica</i>	Caseinolytic protease	ClpX_E	280-294	280K A T E G E L L R Q A E P E D 294	6	13/15 (87)	N/A	(50)
	Caseinolytic protease	ClpX_S	280-294	280K A S E G E L L S Q V E P E D 294	7	14/15 (93)	N/A	(14)
<i>S. Typhimurium CMV</i>	Capsid assembly protein UL47	V120	663-677	663V T P N V D L L A E L M A R S 677	5	10/15 (67)	N/A	(64)
								(57)

Variations in the amino acids of bacterial proteins are presented in italics; variants are likely to match the corresponding amino acids position on human PDC-E2. NA: non-available.

<sup>a</sup>The amino acid sequence highlighted on normal human PDC-E2 mitochondrial enzyme constitutes the amino acid residues 163 through 173, which has been identified as the minimal T-cell epitope.

<sup>b</sup>Amino acids variants for Novo 1, 2, 3 and 4 from *N. aromaticivorans* are not available.

### 3.3. *Chlamydia pneumoniae*

A potential role for *C. pneumoniae* as a triggering agent of PBC was suggested in one study performed by Abdulkarim et al. [47]. From 39 patients with PBC who were enrolled, *C. pneumoniae* antigens were present in the 100% as compared to 8.5% of 105 controls biopsies including patients with primary sclerosing cholangitis and chronic hepatitis C virus infection. This association detected by immunohistochemistry and confirmed by *in situ* hybridization was specific for *C. pneumoniae* whereas any association was found with *Chlamydia trachomatis* which was also consistently negative in controls [47]. In another study, reactivity to Chlamydia antigens was found in the serum of 91% AMA-positive and 21% AMA-negative PBC patients but was practically absent in pathological and normal controls [48]. The presence of -16s-RNA from *C. pneumoniae* was demonstrated by transcription-polymerase chain reaction (PCR) in liver samples of those patients [48]. However, other authors have suggested that Chlamydia infection is not directly involved in PBC, rather, those findings might represent an epiphenomenon [13].

### 3.4. *Mycobacterium spp*

An association between infection with Mycobacteria and PBC has been seen since a long time ago. One early study reported that serum from patients with PBC was able to react to Mycobacterium spp [49]. Another study determined that binding can be done to one specific antigen, the head shock protein –65 kDa (hsp65) which is especially present in *Mycobacterium gordonae*. The sera of such patients did not react to other Mycobacterium species [7,50]. Burroughs et al. [39]. demonstrated a high degree of homology between hsp65 of *Mycobacterium gordonae* with human PDC-E2 (Table 1) [39]. In the same study, patients from two different geographical regions were included but surprisingly the sera of patients from one of the regions did not react to the hsp65 of *Mycobacterium gordonae*. The authors remark that atypical bacteria (*Mycobacterium gordonae*) are less prevalent in that region which can be responsible of the absence of serological reaction [39]. Such findings are also in agreement with disparities found regarding the incidence of PBC among different populations [51,52]. In general, differences in infectious diseases and the incidence of ADs are found from one country to another [53, 54]. ADs are not evenly distributed among continents, well-circumscribed regions within a given country, or

ethnic groups. An examination of the distribution reveals several important and probably interrelated phenomena. One is the north–south gradient: the incidence of disease decreases from north to south in the Northern Hemisphere (and reciprocally from south to north in the Southern Hemisphere). Epidemiologic data provide strong evidence of a steady rise in the incidence of ADs in developed countries over the past three decades. Concomitantly, there has been an obvious decrease in the incidence of many infectious diseases in developed countries as a result of antibiotics, vaccination, or more simply, improved hygiene and better socioeconomic conditions [55]. A plausible explanation for such findings might be the limited exposure to a significant infectious agent's burden in the North-South gradient. Another example of this gradient regarding PBC is the presence of AMA in patients with active pulmonary tuberculosis and active leprosy in almost 43% of patients. However none of them developed PBC, suggesting that at least the exposure to a single agent *per se* is not critical in the development of the disease [26,56]. Furthermore, Mycobacterium species have not been isolated from any histological samples of patients with PBC. Attempts to detect mycobacterial DNA in the histological samples has also failed [49]. Therefore, the role of *Mycobacterium gordonae* in the pathogenesis of PBC remains uncertain.

### 3.5. *Lactobacillus spp*

The group of Bogdanos et al. [57]. described the presence of autoantibodies to human PDC-E2 able to cross-react with a Lactobacillus-mimic antigen. The authors reported that the enzyme beta galactosidase (lactase) of *L. delbrueckii*, sub-specie *bulgaricus* shares eight aminoacid sequences with human PDC-E2 (213–226) (Table 1). Such association seems to be the highest when compared to other Lactobacillus species [57]. Although Lactobacillus does not cause infection, the enzyme lactase of *Lactobacillus* is broadly used in the production of lacteous for human consumption. It was suggested that fermented products might constitute another environmental risk factor for PBC. Regarding cross-reactivity, the same group also showed that the serum of more than 50% of PBC patients with positive AMA -subclass IgG3 are able to react with lactase of *L. delbrueckii*, with high specificity [57]. The affinity of the antibody for *L. delbrueckii* mimic was greater than for human PDC-E2.

### 3.6. Other gram negative bacteria and PBC

Apart from *E. Coli*, other gram negative bacteria have been reported to be epidemiologically associated with PBC prevalence. The common antigenic denominator for such gram negative bacteria is the presence of the ATP-dependent caseinolytic protease (Clp). ClpP is a serine protease complex that together with the regulatory subunit ClpX plays an important physiological role in protein degradation [58]. The Clp protease complex is broadly distributed among gram negative bacteria, and its structure is highly conserved. ClpP of *E. coli* is identical to the corresponding ClpP of *Salmonella typhimurium* and also has a high degree of homology with ClpP of *Yersinia enterocolitica* [in particular to one amino acid sequence of Clp(177–194)], and to a lesser extent to ClpP of *Haemophilus influenza* [14,58] (Table 1). Mayo et al. [45]. enrolled 45 patients with PBC, 44 patients with Hepatitis C and autoimmune thyroiditis (AT) and 32 healthy controls. Reactivity to at least one of the ClpP peptides was observed in 21 (47%) of PBC patients, 5.8% of patients with Hepatitis C and AT and 3.1% of healthy controls. Among these 21 seropositive PBC patients, 71% reacted at least to the amino acid sequence 177–194, located within the proteolytic subunit of the Clp *E. Coli*, alone or in association with Clp of *Y. Enterocolitica* and/or Clp of *H. Influenzae* peptides [45]. Simultaneous reactivity to homologous sequences was also observed. By competition assays, the authors confirmed that it was due to cross-reactivity [45]. In a large follow-up study of patients with infection by *Y. Enterocolitica*, the induction of chronic liver diseases and stimulation of various non-organ specific autoantibodies was observed in some patients [59]. Although previous reports suggest cross reactivity, the overall frequency of infection by *Y. Enterocolitica* has been described to be quite similar between PBC and non-PBC controls [60]. No additional epidemiological studies searching for such association have been performed.

### 3.7. Viral infectious agents

Although the association between virus infection and PBC is less clear compared with bacteria, there are some remarkable studies supporting such association. Two herpes virus have been described to share sequence homology with human PDC–E2. The first one is Epstein-Barr virus (EBV), a virus belonging to the herpes family, which infection has been largely associated with multiple ADs [61,62]. Recently, we have found in-

creased titers of the early antigen of EBV in sera of patients with different ADs [63]. Morshed et al. [64]. had reported long time ago, that peripheral blood mononuclear cells of patients with PBC showed increased levels of EBV-DNA (61%) in contrast to chronic active hepatitis patients (19%), liver cirrhosis patients (14%) and healthy individuals (11%) [64]. They also showed that formalin-fixed paraffin-embedded liver tissues, and saliva from the same PBC patients, also demonstrated increased levels of EBV-DNA when compared to healthy individuals [64]. The second herpes virus is the cytomegalovirus (CMV) and even though no clinical studies have reported a direct association with PBC, one peptide sequence shows sharing similarity to the human PDC [14] (Table 1). Regarding other viruses, a plausible role for retroviruses was suggested by Mason et al. [65]. They reported a long time ago that the sera from patients with PBC react with proteins from the human immunodeficiency virus 1 (HIV-1) and human intracisternal A-type particle (HIAP). The same group identified a human betaretrovirus that shares high homology with the mouse mammary tumor virus (MMTV) [66]. Then, MMTV was identified and cloned in the liver and lymph nodes of patients with PBC by the same group [66]. By means of PCR, it was only found in 20% of the serum samples that contained antiretroviral reactivity. This retrovirus appears to be more lymphotropic than hepatotropic [65,66]. It was also reported that co-culture of healthy biliary epithelial cells together with lymph node tissue from patients with PBC, lead to the expression of an AMA – specific auto-antigen on the apical surface of such epithelial cells [67,68]. Ultrastructure studies also suggested the presence of a viral-like antigen in PBC samples [69]. Otherwise, the group of Gershwin did not detect reactivity of the sera of patients with PBC against any MMTV encoded protein [70]. In the same study, two proteins of different molecular weight present in the MMTV extracts were recognized by a small proportion of PBC-sera with positive AMA. They demonstrated that such proteins were lipoylated and not recognized by any of the anti-MMTV polyclonal sera tested [70]. They also did not find either evidence of MMTV in liver samples obtained from patients with PBC, nor specific sequences of MMTV in peripheral blood mononuclear cells of the same patients [70]. Therefore whether or not retroviruses are associated with the pathogenesis of PBC remains controversial.

#### 4. Concluding remarks

There is a large amount of evidence suggesting an association between infectious agents and PBC. Most of the evidence points to molecular mimicry between specific bacteria components and human mitochondrial antigens [4,14,26]. Although *in vitro* studies and some animal models support the concept of molecular mimicry in PBC [20,35], only some clinical studies support such association [3,32,42]. Some other studies have failed to find a direct relation [70–72]. Direct epidemiological association between exposure to a determined infectious agent and PBC onset remains unclear. In this regard, a plausible explanation for epidemiological differences in the incidence of PBC might be the variation in the exposure to several infectious agents burden rather than to a single agent exposure [54,55]. Clearly, whatever is the reason for this marked epidemiology discrepancy; we must admit that genetics and environment are both also involved. Therefore, it is worthy to remark that the association of PBC with infectious agents constitutes only one piece belonging to the “big puzzle”, but the exact mechanism of disease’s origin remains unknown. Other components such as genetic, hormonal, environmental, and xenobiotic also make PBC etiology enigmatic [73–76]. Moreover, the exact mechanism implicated in the induction of PBC-specific anti-mitochondrial responses is not clearly understood and since PBC is an autoimmune disease in which several aspects contribute to its pathogenesis, the interaction among them is also unknown [77]. Such interactions are highly amenable to additional exploration through new clinical and experimental studies.

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