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REVIEW

Role for mycobacterial infection in pathogenesis of primary biliary cirrhosis?

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Abstract

Primary biliary cirrhosis (PBC) is a progressive cholestatic liver disease characterized by the immune-mediated destruction of biliary epithelial cells in small intrahepatic bile ducts. The disease is characterized by circulating antimitochondrial antibodies (AMAs) as well as disease-specific antinuclear antibodies, cholestatic liver function tests, and characteristic histological features, including granulomas. A variety of organisms are involved in granuloma formation, of which mycobacteria are the most commonly associated. This has led to the hypothesis that mycobacteria may be involved in the pathogenesis of PBC, along with other infectious agents. Additionally, AMAs are found in a subgroup of patients with mycobacterial infections, such as leprosy and pulmonary tuberculosis. Antibodies against species-specific mycobacterial proteins have been reported in patients with PBC, but it is not clear whether these antibodies are specific for the disease. In addition, data in support of the involvement of the role of molecular mimicry between mycobacterial and human mitochondrial antigens as triggers of cross-reactive immune responses leading to the loss of immunological tolerance, and the induction of pathological features have been published. Thus, antibodies against mycobacterial heat shock protein appear to cross-recognize AMA-specific autoantigens, but it is not clear whether these autoantibodies are mycobacterium-species-specific, and whether they are pathogenic or incidental. The view that mycobacteria are infectious triggers of PBC is intriguing, but the data provided so far are not conclusive.

Key words: Antimitochondrial antibodies; Autoantibody; Autoimmunity; Cholestasis; Heat shock; Infection; Liver disease; Liver failure; *Mycobacterium*; Tuberculosis

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INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic, autoimmune liver disease characterized by inflammatory destruction of the small intrahepatic bile ducts, fibrosis progressing to cirrhosis[1-5], and subsequent liver failure[6,7]. PBC primarily affects middle-aged women, with an increased incidence within families[2,8,9]. The prevalence of the disease is estimated to be less than 1/2000[10]. Despite the variance noted amongst countries/ethnic groups, there is a notion that the incidence of the disease is increasing[10-13].

Several autoantibody profiles have been found to be specific for the disease[14], and aid in the diagnostic workup of PBC. These include antimitochondrial antibodies (AMAs)[15-19], and/or disease-specific antinuclear antibodies (ANAs)[20-22], which are found in both symptomatic and asymptomatic patients[23]. Most common symptoms at presentation are nonspecific and include fatigue, pruritus, Sicca symptomatology and arthralgia. In more severe cases, symptoms relate to portal hypertension and hepatic decompensation (jaundice, ascites or variceal bleeding), which may indicate the need for liver transplantation[2,5,24]. The progression of PBC is generally slow and unpredictable[6,24].

The diagnosis of PBC is based on the presence of serum AMA, biochemical markers of cholestasis and histological features on liver biopsy[2]. The diagnosis of PBC is likely when at least two of these criteria are met[2,4]. Biochemical indices of cholestasis include increased levels of alkaline phosphatase and γ glutamyltransferase (GT). The diagnostic hallmark of PBC is the presence of AMA in high titers, with less than 3%-10% of patients with PBC being negative for AMA[23]. Positivity for AMA appears to be predictive of future PBC development in asymptomatic individuals[17,25]. The prevailing notion is that the presence of AMAs and their titers/concentrations do not correlate with the severity of the disease[23]. AMA seropositivity appears unable to identify patients at risk for faster progression of their liver disease compared to the seronegative cases[19,26]. Disease-specific ANAs[27-30] are also found in PBC, to a lesser degree than AMAs (20%-50% *vs* 90%-97%)[31-34], but tend to have elevated titers, and more importantly, appear to characterize patients with a more aggressive form of the disease[35-38]. Levels of IgM are also raised in most cases[2,4]. Histologically, PBC features include destruction of biliary epithelial cells and loss of small bile ducts with portal inflammatory cell infiltration, and granuloma formation (see below)[2,4,5].

The antigenic specificity of AMA[15-19] and ANA[20-22] responses have been extensively studied. PBC-specific ANAs recognize either nuclear body proteins such as the speckled protein 100 and the promyelocytic leukemia protein, or the gp210 and nucleoporin 62 nuclear membrane proteins[28,29,39-41].

AMAs in PBC are directed against the 2-oxo-acid dehydrogenase complex family of enzymes, and in particular the E2 subunits of pyruvate dehydrogenase complex (PDC-E2), branched-chain 2-oxo acid dehydrogenase complex (BCOADC) and 2-oxoglutarate dehydrogenase complex (OGDC)[2,42-44]. PDC-E2 reactivity is found in > 95% of patients with PBC, and 70% recognize BCOADC-E2 and/or OGDC-E2[2]. Reactivity to all three antigens occurs in < 50% of patients[19]. The immunodominant antigenic regions recognized by (CD4 and CD8) T lymphocytes[45-47] on PDC-E2[48-50] comprise a region within the inner lipoyl-binding domain of the subunit, spanning amino acids 212-226 (PDC-E2$_{212-226}$)[51-53]. This region is also the core target of B cell receptors, which are antibodies in their soluble form[54-56].

Medical treatment of PBC includes ursodeoxycholic acid, with the best response seen in patients who initiate treatment early in the disease[2]. These patients often show decreased or even normalized levels of alkaline phosphatase (ALP), γGT and other markers of cholestasis[2,4,6]. 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[2,57].

The cause of PBC remains undetermined[58-61], but it is believed to be the result of a genetic predisposition compounded with several lifetime exposures[62,63], similar to a "multi-hit" model[61,64-67] of disease pathogenesis[50,68,69]. Recent genome-wide association studies[70,71] have identified several HLA[72] and non-HLA[73-76] genes to be associated with PBC. Environmental factors implicated are numerous[77-81], and range from cosmetic products and xenobiotics[82], to estrogen deficiency and infectious organisms[50,58,83-85] including bacteria and viruses[50,54,58,59,63,65,66,68,69]. Mycobacteria have been included in the list of infectious organisms, partially due to the presence of granulomas in the histopathology of PBC, and the association of granulomas with mycobacteria[2,86]. In addition, AMA is found in some patients with mycobacterium-related infections[87,88]. This review will critically analyze the evidence surrounding the role of mycobacteria in the pathogenesis of PBC.

GRANULOMAS IN PBC

Granulomas consist of focal collections of inflammatory cells and cellular debris[89-92]. Their formation occurs when nondegradable products persist, as well as in hypersensitivity reactions or a combination of the two[91]. They form in a complex process involving the interaction of the infectious organism, antigen, macrophages, T cell responses, B cell hyper-reactivity, and circulating media-

Table 1 Prevalence of granulomas in liver disease *n* (%)

Ref.	Origin	Total number liver biopsies	Total number liver biopsies with granulomas	Diagnosis of PBC in granuloma group
Drebber *et al*[100]	Germany	12 161	442 (3.6)	215 (48.6)
Dourakis *et al*[101]	Greece	1768	66 (3.7)	41 (62)
Gaya *et al*[95]	United Kingdom	1662	63 (3.8)	15 (23.8)
McCluggage *et al*[96]	Northern Ireland	4075	163 (4)	90 (55)
Satti *et al*[99]	Saudi Arabia	404	59 (14.6)	Unknown

PBC: Primary biliary cirrhosis.

tors[91]. T cells involved in granuloma formation may be of the T helper (Th)1 or Th2 type[91]. Several organisms may initiate granuloma formation, including *Mycobacterium*, *Yersinia*, *Toxoplasma gondii* and *Bartonella henselae*[93,94]. Zumla *et al*[91] have noted three categories of granulomatous infections: those due to well recognized organisms; those due to organisms that are detected by molecular techniques but not by conventional microbiological techniques; and those due to organisms that have not been identified, but which are suspected. *Mycobacterium tuberculosis* (*M. tuberculosis*) is the most common organism associated with granulomas, and is histologically characterized by epithelioid cells, lymphocytes, histiocytes, Langerhans giant cells and fibroblasts surrounding a core of necrotic debris[91]. The necrotic core is usually caseating, although non-caseating granulomas may also occur[91,92].

The presence of granulomas in liver biopsies has been well documented, with a prevalence ranging from 2% to 15% in some studies, with geographical differences in prevalence rates (Table 1)[95-99]. A German study conducted by Drebber *et al*[100] examined 12 161 liver biopsies for the presence of granulomas, in addition to determining the etiology of the granuloma through histology, clinicopathological data, and polymerase chain reaction (PCR) for the detection of infectious organisms. Granulomas were found in 442 (3.6%) of the liver biopsies and interestingly, 215 were from PBC patients (1.8% of all biopsies, and 48.7% of all biopsies with granulomas)[100]. PCR demonstrated the presence of infectious organisms in 15 samples (3.4%), with *M. tuberculosis* being detected in three of the 15 (20%). It was not indicated whether any of the samples with positive PCR results came from PBC patients[100]. Although only 1.8% of liver biopsies contained granulomas, nearly half of these were obtained from patients with PBC. However, if mycobacterial infection with associated granuloma formation is a feature of PBC, a much higher percentage of granulomas would be expected. The preponderance of granulomas in liver biopsies from PBC patients has been demonstrated in other studies as well, which show geographical differences in prevalence rates. In a Northern Irish study, 55% of liver biopsies with granulomas were from PBC patients[96], compared to 23.8% in a United Kingdom study[95]. A Greek study indicates an underlying diagnosis of PBC or overlap syndrome in 62% of liver biopsies with granulomas, followed by viral hepatitis in 7.5%, and autoimmune hepatitis (AIH) in 6%[101]. Despite these findings, it should be noted that only a small percentage of granulomas can be attributed to mycobacteria, with the liver disease in these patients being due to infection and not to PBC. Figure 1 illustrates PBC-related liver granulomas contrasted with liver granulomas seen in conditions not directly related to PBC such as sarcoidosis and schistosomiasis.

The predominance of PBC as the underling liver disease in most cases of hepatic granulomas and the association of granulomas with mycobacteria, raise the question as to whether mycobacteria play a role in the pathogenesis of PBC[58,86,102]. If mycobacteria play a role in the pathogenesis of PBC (or at least in some cases of PBC), it would be expected that certain PBC-specific features, such as disease-specific AMA positivity, would also be present in patients with mycobacterial infections. Likewise, evidence of mycobacterial infection would be expected in PBC patients. This evidence may comprise more prevalent immune responses against mycobacterial antigens, or detectable mycobacterial DNA in liver tissues of PBC patients, compared to healthy and pathological controls. If mycobacterial infection plays a role in the pathogenesis of PBC, it is likely that it is limited to a triggering event, because PBC has been known to recur following transplantation[103-106] in up to 35% of patients[107]. The recurrence rate ranges from 21% to 37% at 10 years, to 43% at 15 years[108-111]. Immunosuppression with tacrolimus, as well as warm ischemic time, is associated with an increased risk of recurrence[108-112]. The more aggressive course of recurrent disease suggests that the pathological process involved in the development of PBC persists after transplantation[113]. This does not, however, suggest that the triggers of PBC also persist, and may indicate their transient involvement in the pathogenesis of PBC[113]. Neuberger highlights that hepatitis A virus can trigger autoimmune hepatitis after viral clearance, and also indicates there are several similarities between the recurrence of hepatitis C virus and PBC after transplantation, suggesting a possible infectious trigger of PBC in some individuals[113]. Although these theories are plausible, no conclusive evidence exists to demonstrate an infectious agent (including mycobacteria) as the trigger of PBC.

PBC-specific features in patients with mycobacterial infection

In an analysis of sera from 28 patients with active pul-

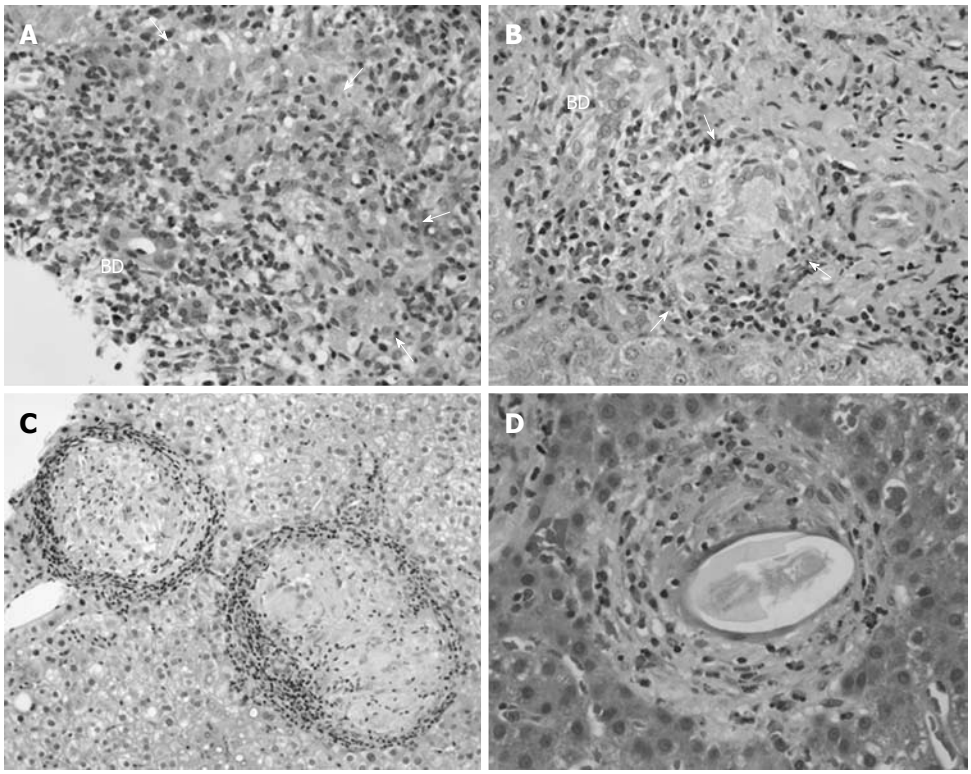

Figure 1 Epithelioid granuloma in liver biopsy. A, B: Liver specimens from patients with primary biliary cirrhosis show granuloma (arrows) close to bile ducts (BD) in portal tracts; C: Granuloma in sarcoidosis is more discrete and large in size; D: Granuloma associated with schistosomiasis contains a large number of eosinophils.

monary tuberculosis (*M. tuberculosis* infection), Klein and colleagues[88] found that 43% of these sera recognized PDC-E2 by immunoblotting based on purified mitochondrial fraction derived from beef heart mitochondria as an antigenic source. Only 2% of sera from individuals with other viral and bacterial infections [including 25 individuals with *Escherichia coli* (*E. coli*)] reacted with PDC-E2, and there was no reaction with sera from healthy controls[88]. The titers of anti-PDC-E2 antibodies were low, with no seropositive cases showing immunofixation of the 70-kDa band corresponding to PDC-E2, at a dilution of < 1/500[88]. Of relevance, none of the anti-PDC-E2 antibody-positive sera (1/10 dilution) gave an immunofluorescent pattern typical of PBC when tested by indirect immunofluorescence based on rat kidney and stomach tissue as substrates[14,16,17,88]. Among the 12 AMA-positive cases with active tuberculosis, six had abnormal levels of γGT and four had slightly elevated levels of alkaline phosphatase; only two of the cases had increased IgM levels[88]. Two of the 12 cases had alcoholic disease and of the remaining 10, none had clinical evidence of chronic liver disease[88].

A variety of autoantibodies have also been reported in the sera of patients with leprosy [caused by *Mycobacterium leprae* (*M. leprae*)], including antibodies against mitochondrial antigens[87,114,115]. In one study, the most common autoantibodies found in leprosy patients were antibodies to SS-B and mitochondria, including cardiolipin[115]. Despite the presence of AMA in the sera of leprosy patients, very few develop liver disease. Gilburd and colleagues[87] screened 69 leprosy patients with no clinical or biochemical evidence of liver disease, for the presence of anti-PDC antibodies. Positive controls consisted of three PBC patients and 18 healthy individuals served as negative controls[87]. Twenty-seven (39%) patients were found to have elevated anti-PDC antibodies by enzyme linked immunosorbent assay, but the absorbance values were relatively low; none of the normal controls had detectable AMA reactivity[87]. By immunoblotting, AMA reactivity was directed against the 54-, 41- and 35-kDa PDC subunits, and only two leprosy patients reacted with PDC-E2[87]. Inhibition studies showed that in contrast to PBC sera, none of the AMA-positive sera gave significant PDC enzyme inhibition; the rate of inhibition being similar to that noted in normal controls. Immunofluorescence testing was not performed[87].

Although AMAs are found in patients infected with mycobacteria, the reactivity patterns observed differ, with *M. tuberculosis*-infected patients showing reactivity to PDC-E2, which has not been observed in those infected with *M. leprae*[87,88]. The source of the AMAs in either case remains unknown. Gilburd *et al*[87] have suggested that the AMAs seen in leprosy patients may arise from the presence of bacterial antigens (such as *M. leprae*), which share sequence homology with the 35-, 41- and 54-kDa subunits. It is not clear whether all bands correspond to PDC subunits (such as PDC-E1 α and β) but may indeed be contaminants of other 2-oxo-acid dehydroge-

nase complexes (formerly known as M2 antigen) such as BCOADC-E2 and OGDC-E2, but reactivity to these antigens has not been tested[87].

Evidence of mycobacterial infection in PBC

In the search for evidence of antimycobacterial antibody reactivity in patients with PBC, Vilagut *et al*[86] investigated a cohort of 19 PBC patients from Spain, using membrane extracts from 10 atypical mycobacteria and found that all cases specifically reacted with *Mycobacterium gordonae* (*M. gordonae*) antigens of approximately 65 kDa and 55 kDa. It remained elusive why the other nine atypical mycobacteria were not targets of antibody responses. The unreactive mycobacterial membranes were those prepared from *Mycobacterium chelonei*, *Mycobacterium flavescens*, *Mycobacterium fortuitum* (*M. fortuitum*), *Mycobacterium intracellulare*, *Mycobacterium kansasii* (*M. kansasii*), *Mycobacterium malmoense* (*M. malmoense*), *Mycobacterium scrofulaceum*, *Mycobacterium xenopi* (*M. xenopi*), and *Mycobacterium terrae*[86]. The extraction of the 10 atypical mycobacterial membranes was performed using the same protocol, therefore, Vilagut *et al*[86] considered that their findings were not a consequence of methodological problems and suggested a biological significance for the role of *M. gordonae* in the pathogenesis of PBC.

This intriguing finding has initiated a series of subsequent studies from the same investigators as well as from independent groups[116-120]. Following the original Spanish report, a study was conducted at King's College Hospital in London investigating reactivity to *M. gordonae* and seven other atypical mycobacteria[119]. O'Donohue *et al*[119] found that 23 of 26 (88%) PBC sera reacted with a 65-kDa protein in extracts of six of the mycobacterial species tested (*M. gordonae*, *M. kansasii*, *M. fortuitum*, *Mycobacterium chelonae*, *Mycobacterium szulgai* and *M. malmoense*). Also, 15 and nine of these samples reacted with the 65-kDa band in membrane extracts of *Mycobacterium avium-intracellulare* and *M. xenopi*, respectively. However, the antibody reactivity to *M. gordonae* or other atypical mycobacteria was not restricted to PBC, but was also present in a similar prevalence in patients with other chronic liver diseases as well as in normal controls[119].

Several studies have attempted to provide evidence of mycobacterial infection in liver tissues from PBC patients. Broomé and colleagues[121] conducted an immunohistochemical study investigating liver biopsy specimens from 10 PBC cases, 13 from primary sclerosing cholangitis, five chronic hepatitis C, four alcoholic liver disease, and six healthy controls. Samples were studied using a monoclonal antibody specific for mycobacterial heat shock protein (hsp) 65. Positive staining was observed in nine of the 10 PBC cases, all primary sclerosing cholangitis, three chronic hepatitis C, and three alcoholic liver disease cases, but in none of the healthy controls[121]. Both interlobular and septal bile duct staining was observed in the PBC cases, with perinuclear staining in all positive interlobular ductal cells, and perinuclear as well as apical staining in the positive septal cells[121].

Direct evidence of microbial products in an affected tissue is best studied at the molecular level using PCR techniques. Vilagut *et al*[116] assessed the presence of *M. gordonae* DNA in liver tissue from PBC patients and controls using PCR based on amplification of a 565-bp fragment of mycobacterial gene coding for 16S rRNA on M. *gordonae*. They detected *M. gordonae* in nine of 11 (82%) PBC liver tissues but in none of the six control livers from patients with other liver diseases[116]. Contradictory results were obtained by O'Donohue *et al*[118] who investigated whether mycobacterial DNA could be detected in archival liver biopsy material from PBC and AIH. Archival material was obtained from 11 PBC and 11 AIH cases, with five lymph nodes from patients with tuberculous lymphadenopathy acting as positive controls[118]. Three of the positive controls also had liver biopsies taken for concurrent tuberculous hepatitis. No mycobacterial DNA was detected in PBC or AIH cases, while four of the five positive controls had detectable mycobacterial DNA[118]. A similar study also utilized a PCR approach for the detection of mycobacterial and other organisms in archived (paraffin embedded) liver tissues from 29 cases of PBC, as well as pathological and healthy controls. Again, no mycobacterial DNA was detected in PBC samples, and only *Helicobacter pylori* DNA was found in one case of PBC[122].

Mycobacteria and PBC: The role of molecular mimicry

The above studies do raise doubt as to whether mycobacteria play a role in PBC, although it is unclear whether a negative detection test only implies that there is no current active infection or can rule out previous infection as well. However, it has been suggested that mycobacteria and other infectious agents may not be actively present in PBC patients, but rather involved in the initiation of autoimmunity by microbial/self immunological cross-reactivity[55,56,123,124]. We and others have studied the role of molecular mimicry[68,69,123,125-128] and immunological cross-reactivity[129-133] as a mechanism responsible for the induction of autoantigen-specific immune responses in viral-hepatitis-triggered autoimmunity and several autoimmune diseases (including those affecting the liver)[134-138] in susceptible individuals[139,140]. Impairments in T-regulatory functions also appear to be a feature[141,142]. Cross-reactivity between PBC-specific mitochondrial antigens and mycobacterial proteins has been investigated by two groups[86,117]. Vilagut *et al*[86] have found that antibody responses to the 65-kDa *M. gordonae* antigen cross-reacted with anti-PDC-E2 antibodies. The same group of investigators went on to demonstrate that the 65-kDa protein was the mycobacterial hsp65, and that preincubation with PBC sera prevented binding of antibodies against hsp65[117]. The apparent cause of this cross-reactivity remained elusive until another group noted a sequence similarity between human PDC-E2 and *M. gordonae* hsp65[120]. These authors have found that the hexameric motif [GDL(IL)AE)] is shared by *M. gordonae* hsp65$_{94\text{-}99}$ and the major PBC-specific mitochondrial autoepitopes,

Table 2 Summary of studies in support or against a role of mycobacteria as triggers of primary biliary cirrhosis

Ref.	Year	Mycobacterium	M2 antigen	In support	Against
Klein *et al*[88]	1993	*M. tuberculosis*	Bovine heart	Anti-M2 AMAs (70 kDa) found in 12/28 (43%) tuberculosis patients	None of the patients had PBC AMA by IIFL were negative Anti-PDC-E2 titers were low
Gilburd *et al*[87]	1994	*M. leprae*	Bovine heart	Anti-M2 against 54-, 41- and 35-kDa bands found in 27/69 (39.1%) leprosy patients	None of the patients had PBC Only 2/27 (1%) sera reacted with the PDC-E2 band Anti-M2 inhibited anti-PDC activity in 19.1%
Vilagut *et al*[86]	1994	*M. gordonae*	Porcine heart	Anti-hsp65 *M. gordonae* antibodies found in 19/19 (100%) of PBC patients. No reactivity to other atypical mycobacteria was found Anti-porcine AMAs (anti-PDC-E2 and BCOADC-E2) cross-reacted with anti-hsp65 *M. gordonae* antibodies and *vice versa*	
O' Donohue *et al*[119]	1994	*M. gordonae*, *M. kansasii*, *M. fortuitum*, *M. chelonae*, *M. szulgai and M. malmoense*, *M. avium-intracellulare and M. xenopi*		23 of 26 (88%) PBC sera reacted with a 65-kDa protein in extracts of six of the mycobacterial species tested Sera from 15 PBC patients reacted with *M. avium-intracellulare* Sera from 9 PBC patients reacted with *M. xenopi*	Reactivity to the atypical mycobacterial mimics was found in non-PBC liver disease patients and controls
Bogdanos *et al*[54]	2004	*M. gordonae*	Primate liver	The motif GDL(IL)AE is shared by *M. gordonae* hsp65$_{94-99}$ and PDC-E2$_{216-221}$	Reactivity to *M. gordonae* hsp65$_{90-104}$ /human PDC-E2$_{212-226}$ seen in Spanish (47.5%), but rarely British patients (4%)
Broome *et al*[121]	1993	-	-	Immunohistochemistry demonstrated positive mycobacterial hsp65 staining in PBC liver tissue	Positive staining was also observed in pathological controls
Vilagut *et al*[116]	1996	*M. gordonae*	-	*M. gordonae* genetic material detected in 9 of 11 (82%) PBC liver tissues	
O'Donohue *et al*[118]	1998	*M. gordonae*	-		Mycobacterial DNA not detected in PBC liver tissues, but was detected in positive controls
Tanaka *et al*[122]	1999	Mycobacterial genus specific primers	-		Mycobacterial DNA not detected in PBC liver tissues

M. tuberculosis: *Mycobacterium tuberculosis*; *M. leprae*: *Mycobacterium leprae*; *M. gordonae*: *Mycobacterium gordonae*; *M. kansasii*: *Mycobacterium kansasii*; *M. fortuitum*: *Mycobacterium fortuitum*; *M. chelonae*: *Mycobacterium chelonae*; *M. szulgai*: *Mycobacterium szulgai*; *M. malmoense*: *Mycobacterium malmoense*; *M. avium-intracellulare*: *Mycobacterium avium-intracellulare*; *M. xenopi*: *Mycobacterium xenopi*; IIFL: Indirect immunofluorescence; PDC: Pyruvate dehydrogenase complex; PBC: Primary biliary cirrhosis; AMAs: Antimitochondrial antibodies; hsp: Heat shock protein.

namely, the inner lipoyl PDC-E2$_{216-221}$ and the outer lipoyl domain human PDC-E2$_{102-107}$[120]. No other sequences were found to be in common with *M. gordonae* hsp65 and human PDC-E2[120]. A database search analysis has found that, among bacteria, the motif SxGDL[IL]AE is virtually unique to mycobacterial hsp, and the only human sequence containing that motif was the inner lipoyl domain of PDC-E2[120]. This excellent and almost unique match between sequences of the dominant epitope of human PDC-E2 and of mycobacterial hsp65 has led the authors to investigate whether the corresponding sequences were targets of immunological cross-reactivity specifically present in patients with PBC. That study involved testing sera from 40 Spanish and 50 British PBC patients, with antibody reactivity to *M. gordonae* hsp 65$_{90-104}$/human PDC-E2$_{212-226}$ being detected in 47.5% of Spanish PBC patients and in only 4% of British PBC patients[120]. No reactivity was observed in controls. Inhibition studies confirmed that the reactivity to the mimics was due to cross-reactivity. In addition, the affinity of anti-*M. gordonae* hsp 65$_{90-104}$ antibodies was higher than that against human PDC-E2$_{212-226}$, raising the possibility that antibody reactivity to the microbe long precedes that against the human homolog[120]. Table 2 provides a summary of the above evidence for and against the role of mycobacteria in PBC.

It remains unclear whether the differing reactivity patterns noted among PBC patients from differing geographical locations (highly prevalent in Spanish PBC cases, practically absent in British PBC cases), could be explained by differing prevalence exposure rates of certain atypical mycobacteria in geographical locations. It should also be noted that multinational epidemiological studies on PBC have not indicated an association with mycobacterial infection, in contrast to what has been reported for *E. coli* or other microbes[77,78,80,81].

CONCLUSION

The presence of granulomas in liver biopsies of PBC patients raises the suspicion that bacterial infections are involved in the pathogenesis of PBC. Although infection with several bacterial species is associated with granulomatous disease, mycobacteria are the most common culprits. It is therefore not surprising that mycobacteria have been added to the list of infectious agents implicated in the pathogenesis of PBC. Furthermore, it has been observed that AMAs, which are characteristic of PBC, are also found in many patients with mycobacterial infections but their titers are low, their epitope specificity differs from that seen in PBC, and they do not appear to be detectable by conventional immunofluorescence. Additionally, mycobacteria have not been found in any significant proportion of hepatic granulomas associated with PBC. When found, the liver disease is often attributed to infection and not PBC.

It is unlikely that ongoing mycobacterial infection is a characteristic of PBC, but an early, transient infection with immunological clearance may be capable of inducing cross-reactivity. Genetic studies on PBC are now demonstrating a genetic susceptibility to the disease, and it is likely that a variety of other factors act in an additive fashion towards PBC development. These factors probably not only vary from patient to patient, but also from one geographical location to the next. A correlation between PBC and certain organisms in the context of their geographical prevalence is warranted. In addition, sequence homology between PDC-E2 and mycobacterial epitopes at the T-cell level has not been fully explored, which is of interest given that differing reactivity patterns have been observed between a variety of mycobacteria and the major mitochondrial epitope of PBC. Experimental studies in animal models of PBC involving mycobacteria may provide useful hints as to whether mycobacteria play a role in the induction of PBC. They may also distinguish between mycobacteria being a trigger of the disease, or being epiphenomena secondary to an increased susceptibility to infection in PBC patients.

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