

PATHOGENESIS OF PRIMARY BILIARY CIRRHOSIS

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Primarily biliary cirrhosis (PBC) is a chronic cholestatic liver disease that affects up to one in 700 women in western populations (female to male ratio is approximately 10 to 1, with the disease typically presenting over the age of 40 years).^{1–2} PBC is characterised histologically by damage to, and eventual loss of, the biliary epithelial cells (BEC) lining small intrahepatic bile ducts. BEC loss is typically accompanied by a significant portal tract inflammatory infiltrate that is mixed in phenotype (T cells (CD4 and CD8, with the latter predominating in the periductal areas), B cells, macrophages, eosinophils and natural killer cells).^{3–4} Early descriptions of PBC emphasised the predominant role played by the progression of bile duct loss, accompanied by increasing portal tract and linking fibrosis leading to biliary cirrhosis, in the clinical expression of PBC, and described an aggressive and uniformly fatal condition.⁵ Increased awareness of the condition and, in particular, the availability of diagnostic tools such as serology has led to broader and earlier diagnosis.⁶ This has had the effect that we now recognise PBC far more frequently than was previously the case, and typically do so at an earlier stage in the disease process. Broadening of the diagnostic base has, in addition, led us to appreciate that there is a significant subgroup within the PBC population who have a low risk of disease progression and who are unlikely to develop end-stage liver disease during a normal lifetime (but who remain at risk of developing the often life-altering symptoms of the disease such as fatigue, which are seemingly unrelated to the severity of the underlying liver disease).^{2–7–8} The factors that determine individual risk of disease progression remain unclear, precluding us, at present, from targeting disease-modifying therapies at “high-risk” patients.

Study of the pathogenesis of PBC has focused, until relatively recently, almost entirely on immunological aspects of the disease.⁹ PBC was one of the first conditions in which the presence of autoantibodies in the serum was identified, and was one of the first conditions in which the antigen specificity of this autoreactive response was characterised.³ This led, quite naturally, to the view that PBC is an autoimmune disease. This led, again not unreasonably (but largely unsuccessfully), to a focus on developing and applying therapies aimed at modifying this increasingly well-characterised autoreactive immune response. More recently, however, the true complexities of the processes seemingly contributing to PBC pathogenesis have begun to be appreciated and the exclusively autoimmune disease model has been challenged.

PATHOLOGICAL FACTORS IN PBC

PBC pathogenesis from an immune system perspective

PBC is characterised immunologically by the breakdown of immune self-tolerance to highly conserved mitochondrial and nuclear antigens (table 1).^{3–10–19} The first autoreactive responses to be characterised in PBC were the serum antibody responses directed at antigens present on the inner mitochondrial membrane (antimitochondrial antibodies; AMA). AMA, which are present, often at very high titre, in over 95% of patients, are directed at the members of the 2-oxoacid dehydrogenase complex family of multi-enzyme complexes. These complexes, which all play important roles in mitochondrial energetics, share a common multicomponent, multidomain structure. The dominant autoantibody response in PBC is directed at pyruvate dehydrogenase complex (PDC), although reactivity is seen to a lesser extent to all other 2-oxoacid dehydrogenase complex family members (table 1). Within PDC, reactivity is seen, in particular, to the dihydrolipoamide acetyltransferase (E2) and enzyme 3 binding protein (E3BP) components. Reactivity is seen to these two antigens alone in over 90% of PBC patients. It was appreciated from relatively early on that the antibodies reactive with these two enzymes are fully crossreactive and that they can block the function of both enzymes. The mechanistic explanation for these two, in retrospect key, observations is the reactivity seen to a highly conserved structural motif present within both PDC-E2 and E3BP,¹¹ which consists of the binding domain for a lipoic acid (LA) co-factor that plays an essential role in enzyme function. The

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Table 1 Characteristic autoantibodies seen in primary biliary cirrhosis and their reported frequencies in populations

Antibody group	Antigen	Patient frequency (%)
Mitochondrial	PDC-E2 ¹⁰	95*
	PDC-E3BP ^{10, 11}	95*
	PDC-E1 α ¹²	40–60 [†]
	PDC-E1 β ¹²	10
	OGDC-E2 ¹³	40–90 ^{†,‡}
Nuclear	BCOADC-E2 ¹⁴	10 [‡]
	gp210 ¹⁵	10–40 [†]
	Sp100 ¹⁶	10–30 [†]
	p62 ¹⁷	20–30 [†]
	Centromere ¹⁸	10
	Lamin B receptor ¹⁹	2

*Autoantibodies to pyruvate dehydrogenase complex (PDC)-acetyltransferase (E2) and PDC-enzyme 3 binding protein (E3BP) are fully crossreactive (reactivity is to the shared, highly conserved lipoic acid binding domain). The identical population frequency reflects the fact that these are, *de facto*, the same antibodies.

[†]Primary biliary cirrhosis population frequencies to these antibodies appear to show significant geographical variation and this is reflected in the varying reported frequencies.

[‡]2-Oxoglutarate dehydrogenase (OGDC)-E2, branched chain 2-oxoacid dehydrogenase (BCOADC)-E2 and PDC-E3BP share an apparent molecular weight on sodium dodecylsulphate-polyacrylamide gel electrophoresis in the range 50–52 kDa (with subtle intermammalian species differences that can be important depending on the source of the PDC used in immunoblots). This molecular weight similarity has led, in the past, to errors in the identification of antibodies by immunoblot.

importance of LA in the autoantibody response to PDC is confirmed by the finding that LA itself forms part of the PDC-E2 and PDC-E3BP autoepitope.^{11, 20, 21}

Despite the importance of serum AMA in the history of PBC (and its ongoing importance in the serological diagnosis of the disease),²² the actual role it plays in disease pathogenesis appears to be limited (although a number of potential mechanisms, such as antibody-directed cell cytotoxicity (itself relevant because of the upregulation of cell surface PDC seen on BEC in PBC)²³ remain to be fully explored (fig 1A)).⁹ Observations in human infectious disease settings, in which serum IgG anti-PDC can be identified in the apparent absence of liver damage,²⁴ have suggested that the presence of AMA does not, in isolation, result in BEC damage (although the potential significance of differences in the IgG isotype of the resulting antibody response (PBC is characterised by a specific increase in responses of the the IgG3 isotype)²⁵ again remains to be explored). More recently, it has been suggested that it may be non-IgG isotypes of AMA (in particular IgA, which can undergo transcytosis in cells such as BEC, theoretically causing cytopathic effects through its intracellular interaction with PDC) that are pathogenetic. These models remain unconfirmed despite the demonstration that such transcytosis can indeed occur (fig 1A).^{26, 27}

Following the original identification and characterisation of AMA in PBC more recent studies have characterised apparently PBC-specific antibodies reactive with highly conserved nuclear structures such as the gp210 and Sp100 antigens (giving rise to characteristic nuclear dot and perinuclear staining patterns on immunofluorescence).³⁵ In a scenario redolent of AMA it has, again, been difficult to show any direct pathogenetic role for antinuclear antibodies in PBC (although there are emerging data to suggest that their presence is associated with a poorer

clinical outcome).³⁶ More recently it has been postulated that although AMA do not play a direct role *per se* in causing BEC damage in PBC, the B cells that are responsible for their release may have an additional important “upstream” pathogenetic role in priming the breakdown of T-cell tolerance described below, which has a more plausible effector role.³⁷ This observation has echoes in an emerging body of literature suggesting that B cells can, contrary to earlier beliefs, function as “professional” antigen presenting cells able to prime naive T-cell responses.³⁸ It is certainly the case that B cells in PBC have the surface activation phenotype required if they are indeed to function as antigen presenting cells.³⁹

The potential for T-cell autoreactive immune responses to contribute to bile duct injury in PBC (fig 1A) is suggested by a number of observations, not least the almost universal presence of activated T cells in the portal tract infiltrates surrounding affected bile ducts.³ Both CD4 and CD8 autoreactive T-cell responses directed at self-PDC-E2 have now been extensively characterised in PBC patients (and have been found to be, importantly, entirely absent from normal and chronic liver disease controls).^{30, 40–42} The key class I and class II restricted autoepitopes appear to be, once again, located within the LA binding domain of PDC-E2 spanning the LA binding residue itself. Both CD4 and CD8 PDC-reactive T-cell responses are enriched within the liver and the CD8 response, which appears to be at its highest level in the early stages of the disease when bile duct loss appears to be at its height, is effective at lysing PDC-E2 peptide pulsed target cells.³⁰ These observations would all be compatible with a cytotoxic T-cell-mediated response as the final pathway for BEC loss in PBC (fig 1A).

In a separate strand of work, animal modelling studies have suggested that the breakdown of tolerance to PDC at the T-cell level is associated with the development of portal tract inflammation and bile duct changes with features redolent of early human PBC.⁴³ Transfer studies from primed into naive animals suggest that the transfer of AMA alone is insufficient to induce bile duct lesions, whereas the transfer of activated splenocytes is able to achieve this effect.⁴⁴ These preliminary transfer observations, together with the observation that the timing of bile duct lesion development mirrors the timing of the breakdown of T-cell tolerance to self-PDC,⁴⁵ would all support a T-cell pathogenesis model in PBC. Furthermore, the murine studies identified conservation of the LA binding domain structure across evolution and, in particular, immune crossreactivity at the level of LA itself, as a potential mechanism for the breakdown of immune self-tolerance to PDC.⁴⁶

Recent studies in autoimmune disease have highlighted a potentially significant role in the orchestration of target cell damage for a CD4 T-cell subset characterised by release of the cytokine IL-17.⁴⁷ Development of the “Th17” cell subset (well characterised in the mouse and postulated to be present in the human) is induced in the joint presence of TGF β and IL-6, is suppressed by the actions of IL-2, and is associated with the reciprocal regulation of IFN- γ .^{47, 48} Although “Th-17” type CD4 T-cell function remains to be studied in PBC there is indirect evidence to implicate cells of this phenotype. Evidence of upregulated TGF- β signalling is present in the liver in PBC, and IL-6 release is augmented (partly by the actions of hydrophobic bile salts).^{28, 49} Studies in this area are clearly warranted.

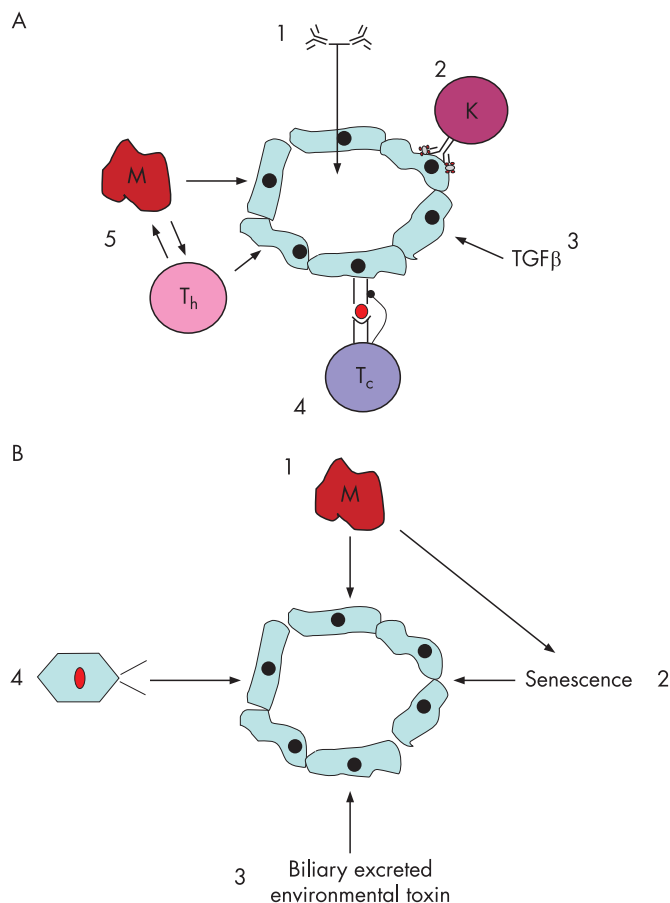


Figure 1 (A) Potential immune effector mechanisms for biliary epithelial cell (BEC) damage in primary biliary cirrhosis (PBC). 1. IgA (and, potentially, IgM) transcytosing across BEC inducing cytopathy as a consequence of interactions with nascent pyruvate dehydrogenase complex (PDC) component peptides.²⁷ 2. Antimitochondrial antibody (AMA)-mediated antibody-directed cell cytotoxicity against BEC by killer cells. 3. BEC epithelial to mesenchymal transition potentially induced by transforming growth factor beta (TGF β). In this model TGF β could be released by cells of either immune or non-immune system origin^{28, 29} (the true role, if any of this pathway in BEC “loss” in PBC remains to be determined). 4. PDC-acetyltransferase (E2)-derived epitope directed cytotoxic T-cell response.³⁰ 5. Actions of pro-inflammatory cytokines released by CD4 (and CD8) T cells interacting with macrophages. (B) Potential non-immune effector mechanisms for BEC damage in PBC. 1. Oxidant stress resulting from macrophage activation. 2. BEC senescence (which may, in turn, be augmented by oxidant stress).³¹ 3. Cytotoxic effect of hypothetical environmental toxic trigger excreted into the bile.^{32, 33} 4. Cytopathic effect of postulated (but not confirmed) PBC-associated beta-retrovirus.³⁴

Despite the evidence, however, some investigators in the field retain doubts about the precise extent of the role played by autoimmunity in the pathogenesis of PBC. Several observations have been used to argue against the autoimmune model. First, several of the “classic” features defining autoimmune diseases are absent in the case of PBC (table 2). Second, treatments that might be expected to modify autoreactive T-cell responses significantly have proved disappointing in clinical trials in PBC. Third, there is difficulty in explaining the now widely recognised recurrence of PBC seen post-transplantation, which can clearly occur across human leukocyte antigen (HLA) boundaries, by an HLA-restricted process (table 3). Finally,

there is the conceptual problem associated with an apparently highly tissue-specific pathological process (BEC loss) being mediated by a T-cell response directed at a ubiquitous self-antigen.

The innate immune system has been less well studied in PBC. There are, however, emerging data suggesting that chronic activation of the innate immune system may occur. Peripheral blood mononuclear cells from PBC patients show an augmented response to Toll-like receptor ligands, suggesting the potential for chronic high-level inflammatory cytokine release.⁴⁹ This observation mirrors findings suggesting increased levels of cytokine transcription within the liver in PBC. The presence of chronic activation of the innate immune system would fit with characteristic clinical features of PBC, such as fatigue and chronic sleep disturbance,^{50, 51} which can, in other circumstances, occur as a consequence of chronic exposure to inflammatory cytokines.^{52, 53} The mechanisms underpinning chronic inflammatory cytokine release are at present unclear, although observations concerning the release of high levels of such cytokines by cells, including BEC themselves,^{54, 55} also after exposure to hydrophobic bile salts, suggest that this may be a consequence of cholestasis rather than a disease-initiating event.

PBC pathogenesis from a target cell perspective

Until relatively recently BEC were regarded as passive “victims” in the PBC pathogenetic pathway (a conventional effector cell/target cell immunological paradigm). It is becoming increasingly clear that the nature of the BEC response to harmful processes and, in particular, the balance between damage and proliferative repair is both critical in the development of bile duct lesions and may provide important opportunities for the development of new approaches to treatment (fig 2).

The earliest observations identified (perhaps unsurprisingly in light of the then prevailing immune model for disease pathogenesis) apoptosis as a key mechanism for BEC loss.^{56–61} The identification of markers suggestive of T-cell cytotoxicity, such as perforin and granzyme in PBC liver,⁶² together with the data suggesting PDC-E2-derived epitope restricted CD8 T-cell killing by PBC patient-derived clones, supported this model of immune effector cell-mediated BEC apoptosis as a key pathogenetic step. Although BEC apoptosis undoubtedly occurs the temporal association between BEC apoptosis and cytotoxic T-cell responses appears more complex than has previously been appreciated. Studies of apoptotic markers in PBC suggest that apoptosis is actually at its peak in the middle stages of the disease (stage II–III) rather than in the very earliest stages when, intuitively, the BEC-directed cytotoxic T-cell response might be predicted to be at its peak.^{63, 64} Moreover, hydrophobic bile salts of the type known to be progressively retained in the liver in cholestatic liver disease are, themselves, pro-apoptotic.^{65, 66} These observations raise the possibility that, rather than being a feature of the cardinal anti-BEC process, apoptosis may represent, in part at least, a secondary process occurring as a consequence of cholestatic bile salt retention and/or failed replicative homeostasis. This model gives rise, of course, to the key question as to the cause of the primary BEC loss if apoptosis is a secondary phenomenon. The concept of apoptosis as a secondary process in PBC also gives rise to an interesting potential implication for the anti-PDC response. Data from our

Table 2 Classic characteristics of autoimmune disease aetiology and the extent to which primary biliary cirrhosis demonstrates those characteristics

Characteristic	Characteristic seen in PBC?	Comment
Autoreactive immune responses to defined autoantigens	Yes	PDC association is among the strongest seen in any autoimmune disease
Female predisposition	Yes	10 : 1 Female to male ratio although classical disease occurs in men ruling out an obligate female association
HLA association	Yes	Significant associations but with atypical alleles and weaker than in other autoimmune diseases
Induction of disease by sensitisation with autoantigen	Possibly	Observation awaits replication
Response to immunosuppressive therapy	Possibly	Comprehensive trials equivocal. Probably effective in selected individuals with significant inflammatory component
Production of disease by adoptive transfer of autoreactive cells and/or antibody	Possibly	Preliminary data suggestive. As yet unconfirmed
Spontaneous animal model with identical antigen specificity	No	No confirmed spontaneous model
Both children and adults affected	No	Disease in children is anecdotal
Autoantigen distribution matches disease distribution	No	PDC is universal but disease is highly restricted in its distribution

HLA, human leukocyte antigen; PBC, primary biliary cirrhosis; PDC, pyruvate dehydrogenase complex.

group have suggested that apoptosis may be a cause of the aberrant cell-surface expression of PDC seen on PBC BEC.^{23 67} If, as has previously been postulated, aberrant PDC processing and expression contribute to its immunogenicity, and this altered processing occurs as a consequence of apoptosis,⁶⁸ it raises the possibility that the anti-PDC immune response seen in PBC is actually also a secondary phenomenon, which, although potentially contributing to the expression of the disease, is not its initiating feature.

Emerging data suggest intriguing alternative potential mechanisms for BEC loss in PBC. Observations in both animal models of biliary obstruction, and to a more limited extent in PBC itself, have suggested that one such mechanism is the process of EMT in which epithelial cells undergo phenotypic "reprogramming" (fig 1A).^{28 29 69-71} Phenotypic studies demonstrate an intermediate stage in which both epithelial (CK19-9, E-cadherin) and mesenchymal (S100A4, Vimentin, α SMA) markers are expressed, with the eventual loss of epithelial features and acquisition of a full mesenchymal functional and surface expression phenotype. This model is attractive as an explanation for the "loss" of BEC and their "replacement" by fibroblasts. Studies using cultured BEC have demonstrated that EMT can be driven by TGF β , an observation that is supported by the presence of markers of TGF β signalling such as nuclear

pSMAD 2/3 in BEC in PBC liver.²⁹ Critically, the induction of EMT in cultured BEC can be reversed by hepatocyte growth factor (HGF; which is, in turn, able to reverse liver fibrosis secondary to bile duct ligation and ameliorate PBC-like bile duct lesions in chronic graft versus host disease).^{71 72} The observation that HGF is itself expressed by BEC in affected bile ducts in PBC⁷³ is in keeping with a dynamic balance model in which the net extent to which ductopenia develops reflects the degree to which homeostatic BEC protective/repairative processes such as EMT reversal by physiological HGF are able to counterbalance ductopenic processes such as EMT and/or apoptosis (fig 2).⁷⁰ Studies in tubular renal disease, in which EMT plays a prominent role, have identified other potential therapeutic approaches that may be of relevance for use in PBC. These include the TGF β antagonising cytokine bone morphogenetic protein 7^{74 75} and rapamycin,⁷⁶ an agent already demonstrated to attenuate liver fibrosis in the bile duct ligated rat model.⁷⁷

A further potential mechanism for BEC loss in PBC has been suggested with the demonstration that senescence of BEC is both a feature of PBC lesions in liver biopsy samples and can be induced in cultured BEC by oxidative stress (a phenomenon previously demonstrated to be present *in situ* in PBC patients; fig 1B).^{31 78} Senescence-associated markers such as β -galactosidase and

Table 3 Summary of the postulated upstream pathogenetic processes in primary biliary cirrhosis and their characteristics

Category	Mechanism/process/factor	Observation confirmed in repeat studies	Mechanism observed in majority of PBC patients	Mechanism able to explain post-transplant disease recurrence across HLA boundaries	Mechanism able to explain tissue tropism of PBC
Immunological	AMA	Yes	Yes	Yes (ADCC)	No
	ANA	Yes	No	? (BEC surface expression not reported)	No
	AMA transcytosis	Yes	?	Yes	Yes
	CD4 T-cell response to PDC	Yes	Yes	No	?
	CD8 T-cell response to PDC	Yes	Yes	No	?
	T-cell response to nuclear antigen	No data yet	?	No	?
	? TGF β -driven EMT	No	?	Yes	?
Non-immunological	Retrovirus	No	?	Yes	Yes
	Environmental toxin effect	Yes	?	Yes	Yes
	Accelerated BEC senescence	No	?	Yes	Yes
	Oxidative stress	Yes	?	Yes	Yes

ADCC, antibody-directed cell cytotoxicity; AMA, antimicrobial antibody; ANA, antinuclear antibody; BEC, biliary epithelial cell; EMT, epithelial to mesenchymal transition; HLA, human leukocyte antigen; PBC, primary biliary cirrhosis; PDC, pyruvate dehydrogenase complex; TGF β , transforming growth factor beta. ? Denotes the fact that this aspect of the process has not been studied in large enough study cohorts and/or that the appropriate mechanistic studies have not been performed.

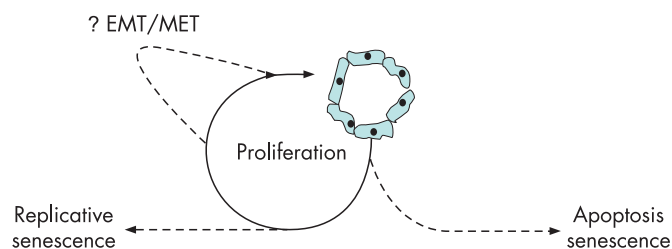


Figure 2 The balance between damage and proliferative repair in the expression of ductopenia in primary biliary cirrhosis. Irreversible biliary epithelial cell (BEC) “loss” through, for example, apoptosis or oxidant-stress-induced senescence is counterbalanced by a homeostatic proliferative response. Ultimately, ongoing proliferation will result in replicative senescence and failure of homeostasis (cellular “exhaustion”). The recently identified process of epithelial to mesenchymal transition (EMT), in which BEC appear to undergo transition to a fibroblast phenotype could, theoretically, result in both further ductopenia and paradoxically in proliferative homeostasis if the resulting mesenchymal cells themselves undergo proliferation and subsequent mesenchymal to epithelial transition (MET; reversion to the epithelial phenotype possibly driven by hepatocyte growth factor function). Studies in this area are required.

p16^{INK4} and p21^{WAF1/CIP} were found to be present in the majority of a representative sample of PBC patient livers and can be induced in culture by the actions of hydrogen peroxide.³¹ The in-situ changes were closely associated with the presence of myeloperoxidase-positive inflammatory cells suggesting the potential for senescence to occur as a consequence of chronic inflammation. As yet the full role played by cellular senescence in disease pathogenesis has not been explored, but the apparent role of inflammation oxidative stress in driving the process gives rise to at least the theoretical potential for therapeutic intervention using anti-inflammatory and anti-oxidant medication approaches. It should be noted, however, that neither of these approaches has previously been shown to be of particular benefit in empirical trials of therapy in PBC.

POTENTIAL PATHOGENETIC FACTORS IN PBC

There is now a broad consensus in the field that, regardless of the precise nature of the pathways responsible for BEC damage in PBC, susceptibility to the disease in individual patients results from a combination of environmental trigger(s) acting on a genetically susceptible individual.

Environmental factors

A significant role for environmental factors in the triggering of PBC is suggested by the demonstration, using formal cluster analysis, of disease “hot spots” in the north-east of England.³² Geographical approaches, and studies of individual potential risk factors, have highlighted diverse and potentially interesting putative PBC triggers. The original UK cluster analysis suggested that PBC was most frequently seen in former industrial and/or coal mining areas. Furthermore, a recent study from New York has suggested high levels of disease in the environs of highly toxic federal waste disposal sites.³³ Taken together, these observations raise the possibility of a chemical environmental factor, potentially associated with contaminated land, which could either trigger disease (fig 1A) or cause disease through a direct toxic effect (potentially explaining the tissue tropism of PBC if the toxin or toxins are excreted into the

bile (and therefore concentrated in the biliary tree); fig 1B). A potential mechanism for the triggering of disease by environmental factors is suggested by the key role played by LA in the immunogenicity of PBC. Homologues of LA, which could be encountered in the environmental setting and which are crossreactive with AMA in terms of in-vitro reactivity with AMA (such as 6-bromohexanoic acid), appear to be able to induce AMA in experimental animals.^{79–80} Unpublished work from our group has suggested that xenobiotic agents such as 6-bromohexanoic acid can enter the physiological post-translational lipoylation enzyme pathway in the mitochondria and be incorporated into PDC components in place of LA, thereby generating potentially immunogenic “altered-self” (one of the postulated mechanisms for the breakdown of immune self-tolerance to PDC in PBC; fig 3). Although these models are potentially very exciting in terms of the opportunity they offer for the identification of the first true environmental chemical trigger for a human autoimmune disease, they have, as yet, not translated into clinical practice and there is currently no advice that should be given to people concerned about the development of PBC (such as family members of affected patients) regarding potentially hazardous environmental exposure.

The second form of environmental trigger that has been proposed in PBC is exposure to infectious organisms. From the earliest observations that bacteria contain PDC components fully crossreactive with the mammalian form it has been proposed that exposure to these homologues could trigger crossreactive immunity (fig 3). Furthermore, more recent data suggesting that Toll-like receptor ligands induce an augmented inflammatory response in PBC would fit with bacteria both providing crossreactive antigen and a pro-inflammatory environment theoretically able to break tolerance.^{49–51} Although attractive, this model has little direct evidence to support it and there remain to be any objective data, obtained either from prospective follow-up cohorts or through case-control epidemiological approaches, confirming a role for bacteria in triggering PBC. An alternative infectious aetiology has recently been proposed with the identification of a human retrovirus in both liver tissue and draining lymph nodes from PBC patients that has the capacity to infect cultures inducing marked phenotypic change.³⁴ Retroviral infection could cause BEC damage either through a direct viral cytopathic effect or through crossreactivity between viral protein and self-PDC (a “molecular mimicry” model; fig 1 and fig 3). In the latter scenario the surface upregulation of “PDC” on BEC would reflect expression of the viral crossreactive protein (although virus-induced apoptosis would represent another explanation for the phenomenon). Although theoretically attractive in terms of its capacity to explain key phenomena in PBC (such as disease recurrence that can occur extremely rapidly after transplantation; table 3),²⁸ well performed follow-up studies have failed to replicate the key findings of that study and it is too early, in the absence of such confirmation, to think in terms of antiviral therapy.⁸²

Genetic factors

A high concordance rate is seen for PBC in monozygotic twins. This observation, together with a significantly increased incidence of PBC seen in the first-degree relatives of probands, would argue for a genetic component to PBC susceptibility.^{83–84}

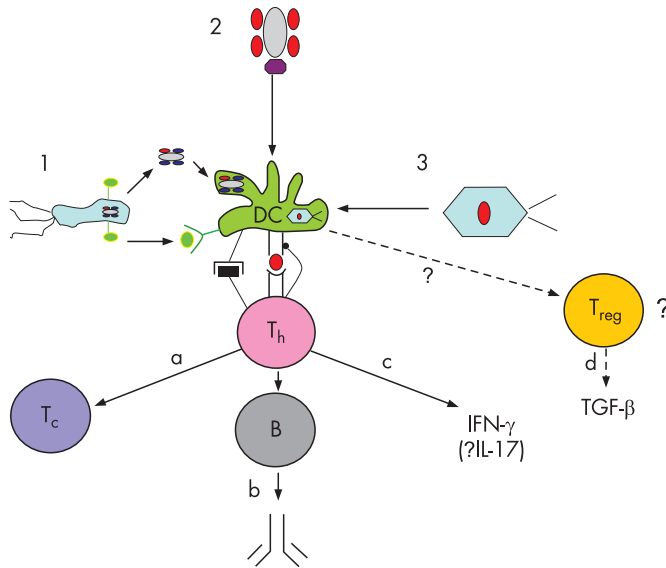


Figure 3 Potential mechanisms for, and sequelae of, the breakdown of immune self-tolerance to pyruvate dehydrogenase complex (PDC) in the pathogenesis of primary biliary cirrhosis (PBC). 1. Induction of a response to bacterial PDC, which is cross-reactive with self-epitopes (denoted in red). In this postulated model the antigen-presenting cell (represented here as a dendritic cell; DC) is activated by bacterial Toll-like receptor ligand (e.g. lipopolysaccharide, denoted in green) through cell surface Toll-like receptors). 2. Response to self-PDC modified by environmental xenobiotic (purple lozenge). This is an “altered-self” model. 3. Response to a virus containing a protein that shows molecular mimicry with self-PDC. In each of these models (most particularly models 1 and 2) activated B cells could replace dendritic cells as antigen presenting cells. The potential effector mechanisms that could result from this breakdown of tolerance are: (a) cytotoxic T cells; (b) anti-PDC antibody (potentially able to mediate biliary epithelial cell (BEC) damage through antibody-directed cell cytotoxicity or BEC transcytosis); (c) inflammatory cytokines (IFN- γ and potentially IL-17, although data are at present lacking); (d) release (potentially) of TGF β by FOXP3+ CD103+ regulatory phenotype T cells arising in response to the primary autoreactive T-cell response. There are data to suggest the presence of transforming growth factor beta (TGF- β) signalled effects in the liver in PBC, but the identity of the cells responsible for TGF- β release/activation remains unclear.

Moreover, the increased incidence of autoimmune disease in both PBC patients and their families would support this susceptibility being expressed at least partly via immunoregulatory genes.⁸⁵ Despite the strong supportive evidence for a genetic contribution to PBC pathogenesis, relatively limited progress has been made in identifying susceptibility loci. The genetic associations for which there is the strongest supporting evidence in PBC are with major histocompatibility complex-encoded genes.^{86–98} There is now a broad consensus that PBC is associated with the *DRB1*08* family of alleles. Significant variation is seen, however, between different ethnic groups. Association is seen with the *DRB1*0801*-containing haplotype in populations of European origin (*DRB1*0801-DQA1*0401-DQB1*0402*). In contrast, in populations of Asian origin an association is seen with the *DRB1*0803* allele (table 3). A protective association has been described with *DRB1*11* and *DRB1*13* but, once again, significant population differences are seen. Although associations with other loci have been described in individual populations, the vast majority have not been replicated (an example being the CTLA-4 association originally

described by our group). A discussion of these associations and the potential factors underpinning the failure to replicate associations is beyond the scope of this review and the issue has been addressed in detail elsewhere.^{99–100} To date, none of the genetic associations identified in PBC have proved sufficiently strong to be useful clinically in the prediction of disease risk.

The strongest inherited trait associated with PBC is, of course, with female sex. This gives rise to obvious and intriguing questions as to which potential mechanisms might explain a strong, but not obligate (approximately 10% of PBC patients are men who have clinical features seemingly identical to those seen in female patients) female preponderance. A role for fetal microchimerism has been proposed but not proved. Moreover, the clinical observation of disease in men and nulliparous women, and a lack of association between parity and disease frequency would argue against such a mechanism. An alternative mechanistic explanation for the female predominance of PBC has been proposed following the observation of an increased frequency of X chromosome monosomy in PBC (and other female autoimmune disease patients).¹⁰¹ It has been suggested that this increase in X chromosome monosomy may lead to haploinsufficiency for specific X-linked genes thereby increasing disease predisposition. At the tissue level cholangiocytes from PBC patients in the earliest disease stages (but not normal controls) express oestrogen receptors¹⁰² and polymorphisms in oestrogen receptor genes have been shown to be associated with the disease in some populations.¹⁰³ Oestrogen signalling has been proposed to play a role in the homeostatic proliferative response of cholangiocytes outlined in fig 2, and agents able to modulate oestrogen receptor-mediated responses (such as tamoxifen) have been proposed as novel, BEC homeostasis targeting, therapies.¹⁰⁴ As yet, this potentially interesting therapeutic approach has not undergone formal clinical trial assessment.

MODELS FOR THE PATHOGENESIS OF PBC

In what way do these exciting emerging data lead us to modify our existing views regarding disease pathogenesis?

The concept of “upstream” and “downstream” pathogenetic processes in PBC

One important implication of the emerging data regarding the cycle of augmented BEC damage innate immune system activation, and, ultimately, fibrosis that appears to occur as a consequence of cholestasis, is that we should consider the disease as occurring in sequential phases (fig 4). In this model “upstream” refers to the originating pathological process responsible for early BEC loss and the resulting initiation of ductopenia and cholestasis. “Downstream” processes are the further cytopathic, pro-inflammatory and fibrotic mechanisms that occur as a consequence of cholestasis and the sequelae of the homeostatic proliferative BEC response (such as replicative senescence). One important implication of this model is that there are elements to the pathogenesis of PBC that are likely to be unique to PBC (the upstream elements) and elements that are likely to be common to many ductopenic cholestatic diseases (the downstream elements; fig 5). It may also help us to rationalise our approach to therapy by suggesting the potential value of combination approaches, which address both downstream processes (probably the principal site of action of ursodeoxycholic acid) and upstream mechanisms. The dual

Table 4 Human leukocyte antigen associations in primary biliary cirrhosis

Allele	Nature of association	Reproducible	Population group	Frequency range in PBC
DRB1*0801 [†]	Susceptibility	Yes	Northern & southern European, USA Caucasian, Canadian ⁸⁶⁻⁹⁰	12-18%
DRB1*0803	Susceptibility	Yes	Japanese ⁹¹⁻⁹³	30-35%
DQA1*0401 [†]	Susceptibility	Yes	Northern & southern European, USA Caucasian ⁸⁷⁻⁹⁰	14-18%
DQB1*0402 [†]	Susceptibility	Yes	Northern & southern European, USA Caucasian ⁸⁶⁻⁸⁹	11-21%
DRB1*0701	Susceptibility	No	Chinese ⁹⁴	29%
DRB1*13	Protection	Yes	Northern & southern European ⁸⁷	10-14%
DRB1*1101	Protection	Yes	Southern European & USA ^{87 95 96}	7-28%
DPB1*0301	Susceptibility	No	Northern European ⁹⁷	50%
DPB1*0501	Susceptibility	No	Japanese ⁹⁸	85%

PBC, primary biliary cirrhosis.
[†]Alleles within disease associated-haplotypes.

process model may also explain the apparent lack of efficacy of immunomodulatory drugs in PBC. Agents such as prednisolone would be expected, in this model, to have their principal effects on upstream mechanisms. They have, however, largely been used in clinical trials that were restricted to relatively advanced, symptomatic, disease patients in whom downstream processes might be expected to have come to predominate.

Improvement in treatment of both upstream and downstream processes would be of real value in both PBC and other ductopenic cholestatic liver disease. Improved treatment of upstream processes is an obvious goal as it offers the hope of curative treatment. There are, however, two major problems inherent in targeting upstream mechanisms in PBC. The first is our ongoing lack of understanding of the precise mechanism(s) responsible for BEC damage initiation, which means we remain unsure as to the process we are actually targeting (fig 1). The second is the relatively late stage in the disease process (in cell biology terms) at which the disease is diagnosed (existing concepts of disease stage and progression largely relate to the degree of fibrosis development (an exclusively downstream

process) rather than the degree of BEC loss and balancing regeneration). Effective upstream process-directed treatment is likely to require very early disease diagnosis if it is to have any chance of success. In light of the potential limits of upstream process-directed treatment, there is real value in our continuing to optimise treatment aimed at ameliorating downstream damage. Given the important role played by bile salt effects (in particular those mediated by retained hydrophobic bile salts) in progressive ductopenic and fibrosis development, an improvement in bile salt-directed therapy represents a major opportunity in PBC treatment.

“Upstream” pathogenetic processes in PBC

Although improved treatment of downstream mechanisms in PBC will bring undoubted clinical benefit in PBC, we must find ways to arrest and reverse the upstream processes if we are ever to approach the long-term goal of curing the disease. We can now integrate recent findings regarding pathogenetic processes in PBC into three theoretical pathogenetic models, the last of which represents a unifying model.

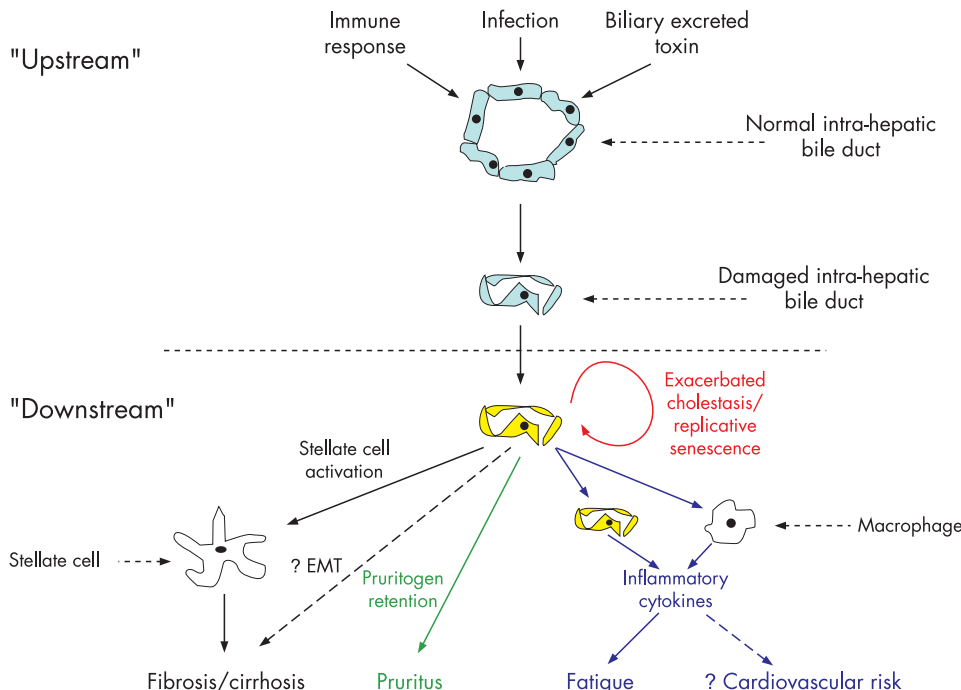


Figure 4 Sequential model for primary biliary cirrhosis pathogenesis. In this model one (or more) processes causes biliary epithelial cell damage, which in turn leads to ductopenia (“upstream” phase). The cholestasis that results from this effect induces a subsequent series of secondary effects, the nature of which is independent of the cause of the upstream process (“downstream” phase). The sequelae include biliary fibrosis and the characteristic clinical features of cholestasis. There is growing interest in a more generic effect of cholestasis on clinical outcomes (including cardiovascular morbidity and mortality) that may result from pro-inflammatory effects. EMT, Epithelial to mesenchymal transition.

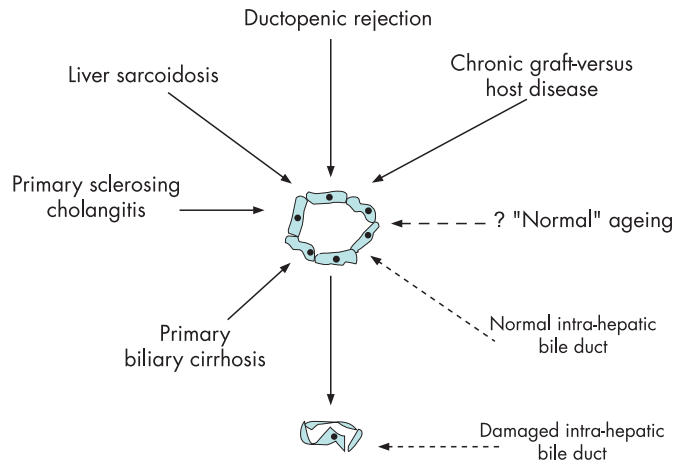


Figure 5 Application of the proposed unique upstream/common downstream model for the pathogenesis of cholestatic liver diseases to the broader issue of the pathogenesis of the vanishing bile duct syndromes.

The immune model for BEC damage

The balance of evidence in PBC remains strongly in favour of an autoimmune process in which autoreactive effector mechanisms are directed at epitopes within self-PDC-E2 expressed normally or aberrantly by BEC (fig 1A, fig 3). There is a strong evidence base to support both "altered-self" and molecular mimicry mechanisms for the breakdown of tolerance to self-PDC, with crossreactivity between the lipoic acid co-factor and environmental xenobiotics, between bacterial and self-PDC and between self-PDC and viral protein all potentially playing important roles. Animal modelling data would argue that a crossreactive B-cell response induced to xenobiotic modified self-PDC can, through a process of epitope spreading driven by antigen-specific crossreactive B cells, translate into the breakdown of T-cell tolerance responsible for the effector T-cell mechanisms thought to be directly responsible for BEC loss. The clinical implication of this observation is that immunomodulatory approaches to therapy should play a role in the very earliest stages of PBC. The key outstanding paradoxes inherent in the immune-mediated model of PBC pathogenesis are the tissue tropism of the disease (why is an immune response mounted to a ubiquitous antigen such as PDC in a tissue-specific manner?), the previously highlighted lack of a response seen to immunomodulatory therapies, and the mechanism whereby disease recurrence can occur post-transplant. The potential role played by liver-excreted xenobiotics in the earliest stages of the immune pathogenesis model would potentially provide an explanation for the first of these issues.

The cytopathic model for BEC damage

An alternative pathogenetic model is that non-immune-mediated cytopathic processes are responsible for BEC damage and eventual ductopenia. Potential mechanisms for this would include the direct cytopathic effects of viruses that are trophic to BEC, a process of BEC apoptosis secondary to environmental factors excreted in the bile, and BEC senescence (fig 1B). The potentially directly cytopathic models would certainly be compatible with the clinical observation of recurrent PBC occurring in occasional cases very rapidly after transplant and across HLA boundaries (table 2). In the direct cytopathy model,

the characteristic autoreactive immune responses seen in PBC would be regarded as epiphenomena arising as a consequence of the BEC cytopathic process and as playing no role in mediating or accelerating BEC damage. Major progress is likely to be made in the next few years in exploring directly cytopathic processes that may affect BEC in PBC and which may be amenable to therapeutic intervention.

A consensus multistage model for PBC pathogenesis

The intriguing final possibility is that all the highlighted processes play a role in the mechanism of expression of PBC, and that the apparent involvement of different processes in different patients simply reflects different timepoints in the disease development pathway. In this consensus model the earliest effect is a directly cytopathic process that induces BEC morphological change either as an associated process or part of the homeostatic mechanism designed to retain BEC function. Failure of homeostasis in the earliest stages (which might typically be expected to occur before clinical presentation) is followed by the progressive development of ductopenia. BEC damage through apoptosis and/or related processes then alters PBC-E2 expression patterns (as exemplified by the upregulation of PBC-E2 seen in apoptotic cells), which, through exposure to the immune system of an altered form of self-PBC-E2, promotes a secondary immune phase with a BEC tropism. This leads to a second stage of immune-mediated ductopenia in which immune-mediated apoptosis becomes prominent. In the tertiary, downstream, phase of the disease progressive ductopenia and resulting cholestasis cause hydrophobic bile salt retention and BEC replicative senescence, with direct further consequential cytopathic effects. Such a model would have major implications for our approach to therapy in PBC, not least because it would imply that the appropriate therapeutic mechanism would depend entirely on the stage of the disease process in which patients are treated. Therapeutic intervention opportunities are likely to be most marked in the very earliest stages of the disease, which will raise important practical questions regarding disease identification and early diagnosis, particularly as serological disease markers, by this model a mid-stage phenomenon, could lose their value.

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