

# Addison's other disease: primary biliary cirrhosis as a model autoimmune disease

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**ABSTRACT** – Thomas Addison described three of the classical autoimmune diseases, so can justifiably be regarded as one of the fathers of the study of autoimmunity. The least well recognised of these conditions, the autoimmune liver disease primary biliary cirrhosis (PBC), is in some ways the most interesting. As a result of the relatively advanced state of knowledge of its immunopathogenesis, it represents a good model for the study of autoimmune disease. The classical pathological lesion of PBC is apoptotic damage to the biliary epithelial cells lining the small intrahepatic bile ducts. The disease is typified by two symptom sets:

- fatigue and pruritus, which can occur at any stage of the disease process, and
- the features of advanced liver disease, which occur when secondary liver damage results from bile retention.

Although autoantibodies directed at, in particular, pyruvate dehydrogenase complex (PDC) are almost universally present in PBC (and represent an important diagnostic tool), it appears likely that CD8+ cytotoxic T cells reactive with self-PDC derived epitopes are directly responsible for target cell damage. Recent studies in humans and a novel murine disease model have shed light on the mechanism of breakdown of immune tolerance to self-PDC; they provide important insights into the pathogenesis of PBC in particular and of autoimmunity in general.

**KEY WORDS:** animal models of disease, apoptosis, autoimmune disease, autoimmunity, biliary, immune tolerance, liver cirrhosis, pyruvate dehydrogenase complex, xenobiotic

Thomas Addison (1785–1860) can be described as the father of the study of autoimmune disease, having described three of what are now widely accepted to be classical autoimmune diseases. In addition to the eponymous disease of the adrenal gland and pernicious anaemia (also known as Addisonian anaemia), he and William Gull described in 1857 the first cases of what is now recognised as

the autoimmune liver disease primary biliary cirrhosis (PBC).<sup>1</sup> He was born in an area with one of the highest prevalences of PBC yet described,<sup>2</sup> and there is some suggestion from contemporary sources that he himself succumbed to liver disease.

Of the three autoimmune diseases originally described by Addison, PBC is perhaps the least familiar to physicians and the one least likely to be used as an example of autoimmunity. This is surprising, given the extent to which the autoimmune responses characteristic of PBC have been dissected (in contrast to many of the other more 'typical' autoimmune diseases) and the progress made in recent years in understanding its pathogenesis.

## Clinical features

PBC typically affects women over the age of 40. The female predominance mimics that seen in other classical autoimmune diseases but, given the typical 9:1 female bias, is more marked than in most other equivalent conditions. The mechanism underlying the female predominance remains unclear. Several hypotheses, including fetal microchimaerism, have not been supported by definitive studies. Early descriptions of the disease following its 're-discovery' by Ahrens in the 1940s emphasised the development in affected patients of end-stage liver disease, characterised by hepatocellular failure and the complications of portal hypertension. Survival following the diagnosis of disease in this era was typically, at most, one or two years. The advent and widespread availability of non-invasive diagnostic testing (in particular, the characteristic serological tests described below) have changed appreciation of the prevalence of the disease; it is now widely accepted to be significantly more prevalent than previously thought. Most patients, however, have a slowly progressive, relatively indolent form of the disease.<sup>3</sup>

The modern appreciation of the wide spectrum of PBC severity helps to understand the range of clinical problems encountered. One highly characteristic symptom set (typified by fatigue and pruritus) affects patients at all stages of disease, probably due to a failure of biliary excretory function resulting

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from bile duct loss (Fig 1). The second symptom set, resulting from hepatocellular failure and the complications of portal hypertension, is restricted to the subset of patients developing end-stage disease.

A detailed description of modern treatment approaches is outside the scope of this review and has been well covered recently elsewhere,<sup>4</sup> but it is important to emphasise that the dichotomy in clinical problems encountered by patients should receive targeted treatment approaches. Traditional treatment dogma has been to slow or prevent disease progression, thereby treating a 'problem' (advanced disease risk) which in practice affects only a minority of patients. It is now appreciated that, although important, the issue of disease progression is not the only one facing patients, and at least equal effort should be put into treating the symptoms (such as fatigue and pruritus) which can dramatically affect patient quality of life. Indeed, for the majority of, usually elderly, patients at only very low risk of developing end-stage disease, treatments directed at reduction of symptom burden and improving quality of life should take precedence.

### Pathological features

Pathological change in PBC is restricted to the liver and, to a lesser extent, the salivary glands. The classical histological motif is damage to and destruction of the biliary epithelial cells (BEC) lining the small intrahepatic bile ducts (Fig 2).<sup>5</sup> As the disease progresses, bile duct radicles can be lost from the majority of portal tracts. Bile duct lesions are accompanied by a CD4+ and CD8+ T cell rich mononuclear cell infiltrate; CD4+ cells typi-

cally predominate, and the richest CD8+ infiltrates surround affected bile ducts.<sup>6</sup> There is now a significant body of evidence to suggest that the final effector mechanism leading to BEC loss is apoptosis.<sup>7</sup> The associated presence of CD8+ cytotoxic T cells has led to the suggestion that BEC apoptosis is mediated by cytotoxic effector cells.

### Primary biliary cirrhosis as an autoimmune disease

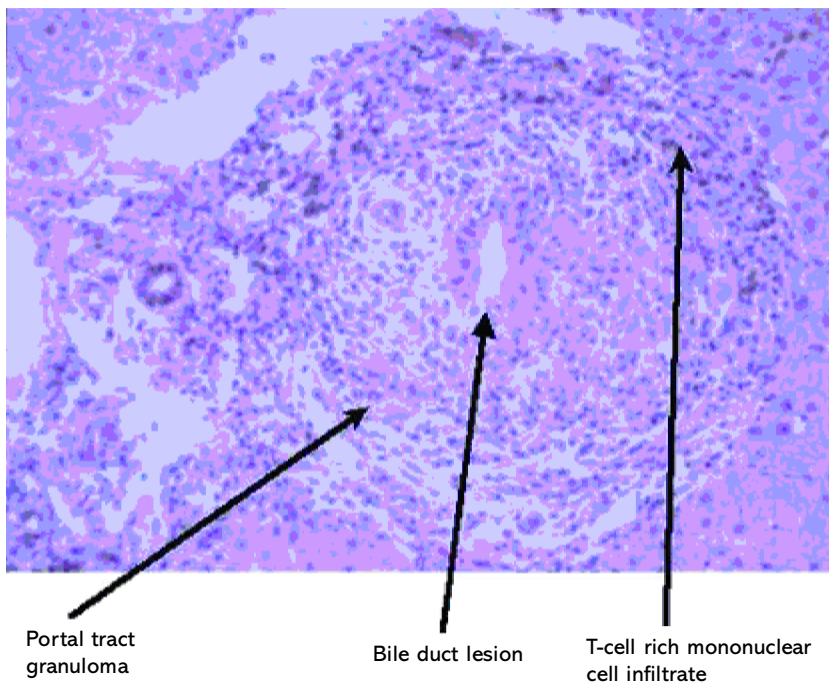
The key observation that led to the suggestion of an autoimmune aetiology for PBC was that almost all patients have high titre serum autoantibodies. The most typical (present in over 95% of patients) are specific for non-organ, non-species specific antigens localised to the inner mitochondrial membrane in normal cells. Detection of these 'anti-mitochondrial'(AMA) antibodies using an immunofluorescence technique remains a cornerstone of the diagnosis of PBC.<sup>8</sup> The characteristic mitochondrial antibodies have been identified as members of the 2-oxoacid dehydrogenase family of enzyme complexes, all of which play key roles in cell metabolism:<sup>9</sup>

- pyruvate dehydrogenase complex (PDC)
- branch-chain oxoacid dehydrogenase complex
- oxoglutarate dehydrogenase complex

The principal autoantibody response (present in 95% of patients) is directed at the enzyme 2, dihydrolipoamide acetyl transferase (E2), and enzyme 3 building protein (E3BP) components of PDC. In each case the antibody response is directed at a protein domain binding a lipoic acid cofactor of fundamental importance for electron transfer, and hence for the metabolic function of the complex. The lipoic acid cofactor constitutes a key component of the dominant B cell auto-epitope. The similarity in structural characteristics of the dominant epitopes in PDC-E2 and PDC-E3BP is reflected in the fact that anti-PDC-E2 and anti-E3BP autoantibodies are fully cross-reactive by immunoblotting. Recent hypotheses regarding the mechanism of breakdown of immune tolerance to PDC have focused on the potential role played by the lipoic acid cofactor shared between the autoepitopes, and the apparent role it plays in shaping conformational auto-epitopes.

Seemingly identical antibody responses reactive with self-PDC, are also seen in some patients with infectious disease but in the apparent absence of liver pathology.<sup>10</sup> This observation suggests, first, that the serum AMA characteristic of PBC are (perhaps unsurprisingly) unlikely to play a direct role in the induction of target cell damage and, secondly, that the development of antibodies reactive with self-PDC is immunologically 'permissible'.

**Fig 1. A model for the pathophysiology of the symptoms of primary biliary cirrhosis.**



There is a fundamental difference between the breakdown of immune tolerance to PDC developing in the context of bacterial infection and that in the wholly different situation of PBC. In the former case, tolerance breakdown appears to be restricted to the B cell compartment, whereas in PBC, autoreactive B cell responses are accompanied by now well characterised autoreactive T cell responses directed at, in particular, PDC-E2.<sup>11</sup> In contrast to the more widely occurring antibody responses, major histocompatibility complex class I and II restricted T cell responses directed, in part at least, against epitopes derived from the inner lipoyl domain of PDC-E2, and spanning the lipoic acid binding residue, are apparently restricted to PBC patients.<sup>12,13</sup> These autoreactive T cells are enriched in the liver compared with the peripheral blood T cell pool,<sup>14,15</sup> and the precursor frequency is highest in early disease when active BEC damage is at its peak.<sup>15,16</sup>

Although direct cytotoxic activity against autologous BEC has yet to be demonstrated, these studies, together with the histological demonstration of BEC apoptosis in the context of a T cell-rich mononuclear cell infiltrate, provide strong circumstantial evidence that autoreactive T cell responses specific for self-PDC derived epitopes are directly implicated in the pathogenesis of PBC. The key question that has to be answered to understand the pathogenesis of PBC is, therefore, why do cytotoxic T cell responses specific for the ubiquitous self-antigen PBC cytotoxic mechanisms become targeted at apparently healthy BEC in PBC?

#### *Animal modelling of immune tolerance to pyruvate dehydrogenase complex*

Studies in patients have provided enormous insight into the pathological processes occurring in PBC (this autoimmune disease is arguably the one with the best characterised autoreactive immune response profile), but provide limited oppor-

#### Glossary

AMA	antimitochondrial antibody
APC	antigen-presenting cell
BEC	biliary epithelial cell
CFA	complete Freund's adjuvant
E2	enzyme 2 (dihydrolipoamide acetyltransferase)
E3BP	enzyme 3 building protein
PBC	primary biliary cirrhosis
PDC	pyruvate dehydrogenase complex
TLR	toll-like receptor

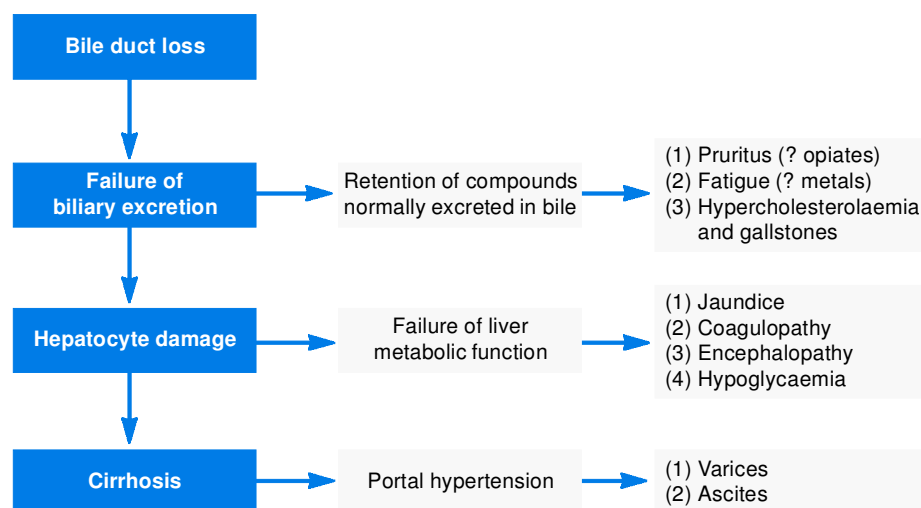
tunity to study the mechanism of tolerance breakdown, and even less opportunity to apply our knowledge to the development of specific forms of immunotherapy.<sup>17</sup> The inherent problem with studying mechanisms of tolerance breakdown in patients is that PBC appears to have a long clinical prodrome.<sup>18</sup> Thus, the key steps in the breakdown of immune tolerance will usually have occurred many years before their clinical presentation. A murine model has therefore been used to characterise mechanisms of breakdown of tolerance to self-PDC. These studies have yielded important new insights into the pathogenesis of PBC.<sup>19–22</sup>

Under normal conditions SJL/J mice are fully tolerant of self-PDC, mounting neither antibody nor T cell responses following sensitisation with the self-antigen.<sup>21</sup> In this sense, they resemble normal humans. By five weeks after sensitisation with non-self PDC (in these studies of bovine origin predicted to have 95% sequence identity with the murine homologues) SJL/J mice have high titre antibodies and splenic T cell proliferative and interferon- $\gamma$  secretory responses reactive with sensitising non-self-PDC. The anti-PDC antibody responses but not, strikingly, the T cell responses are fully cross-reactive with self-PDC.

These findings suggest that the initial T cell response following sensitisation with non-self-PDC is to non-conserved epitopes. The resulting B cell response is, however, directed at conserved conformational epitopes, reflecting the evolutionarily conserved, functionally driven conservation of the quaternary structure of PDC. This split in tolerance breakdown is analogous to that occurring in humans following infection by bacteria containing PDC.

SJL/J mice can be induced to break T cell tolerance by cosensitisation with self- and non-self-PDC<sup>21</sup>. One potential mechanism to explain this would be epitope spreading from non-conserved to conserved T cell epitopes within

**Fig 2. A classical early pulmonary biliary cirrhosis lesion demonstrating bile duct damage, portal tract granuloma formation and mononuclear cell infiltration.**



foreign-PDC driven by local inflammatory and immune factors. In the cosensitisation setting, we hypothesise that the presence of cross-reactive B cells drives epitope spreading. Two potential mechanisms lead to this effect:

- Self-PDC bound to cross-reactive surface immunoglobulin on the surface of B cells could be internalised, processed and presented by activated B cells capable of acting as professional antigen-presenting cells (APC).<sup>23</sup>
- Local formation of immune complexes consisting of self-PDC and cross-reactive anti-non-self could promote effective take-up processing and presentation of self-epitopes by other such APCs.

Breakdown of T cell tolerance in this model is simultaneously associated with the development of marked portal tract infiltrates. There are more infiltrates than seen in all animals receiving adjuvant including, as specific controls for this experiment, animals receiving self-PDC alone in adjuvant. The characteristic portal tract infiltrate (rich in CD3+ T cells, with a majority of CD4+ cells, and containing T cells reactive *in vitro* with self-PDC) is accompanied by inflammatory lesions of the intrahepatic bile ducts.<sup>19,21</sup>

Breakdown of T cell tolerance to self-PDC (accompanied by portal tract changes) can be induced, presumably through the process of epitope spreading, in a number of further settings. Over time (20–30 weeks), the phenomenon occurs in SJL/J mice sensitised with non-self-PDC alone. In this setting, breakdown of tolerance depends greatly on the nature of the adjuvant. Tolerance breakdown occurs in animals sensitised with non-self-PDC in H37Ra (mycobacterial) boosted complete Freund's adjuvant (CFA) but not in non-boosted (standard H37Ra concentration) CFA or incomplete Freund's adjuvant. The role played by the H37Ra in promoting tolerance breakdown appears to be in promoting APC activation through the toll-like receptors (TLRs), as use of specific TLR ligand in sensitisation can replace the need for boosted CFA. These highly conserved pattern recognition molecules, which are expressed on APCs, play critical roles in mediating the innate immune/inflammatory response through specificity for highly conserved, microbially derived structures such as lipopolysaccharide, the ligand for TLR4. Sensitisation with non-self-PDC in the context of oligonucleotides containing unmethylated CG motifs (a motif seen particularly frequently in bacterial DNA and which represents the ligand for TLR9) not only promotes breakdown of T cell tolerance but does so significantly more rapidly than sensitisation in boosted CFA (5–6 weeks vs 20–30 weeks).

An alternative source of altered self-PDC able to promote breakdown of T cell tolerance to self-PDC is self-PDC chemically modified to alter its structure. Preliminary studies from our group suggest that sensitisation of SJL/J mice with artificially modified PDC induces polyclonal antibody responses with reactivity against both modified and unmodified native self-antigen. The greatest T cell response is directed against modified antigen, but there is again breakdown of T cell tolerance to unmodified native antigen.

The final possible source of altered self-PDC, with the

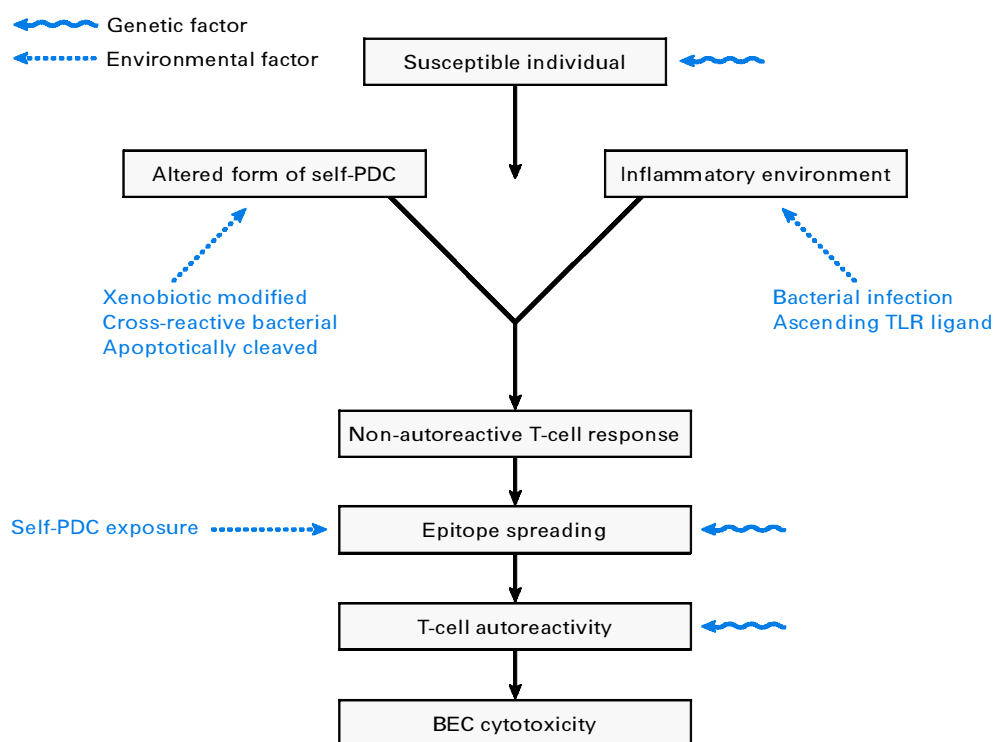
potential to contribute to breakdown of T cell tolerance to self, is self-PDC modified during the process of apoptosis. Apoptosis of BEC occurs from early in the disease process in PBC (accompanied by surface expression of modified PDC). Upregulation of autoantigens on the surface of cells undergoing apoptosis has been described,<sup>24</sup> and our current *in vitro* studies confirm that this is also the case for PDC. Moreover, cleavage of PDC-E2 by caspases activated as part of the apoptosis cascade has been demonstrated (*in vitro* at least). Together, these studies provide powerful, albeit circumstantial, evidence to suggest that the expression pattern, and possibly structure, of the normally mitochondrially restricted PDC are modified as cells undergo apoptosis, and that this process occurs early in disease pathogenesis. Studies are ongoing to examine how far self-PDC modified during apoptosis can promote breakdown in T cell tolerance to self and what is the nature of the inflammatory environment that promotes this tolerance breakdown.

### A model for the pathogenesis of primary biliary cirrhosis

Early human studies guided the design of the animal modelling studies by defining the questions regarding the mechanism of tolerance breakdown. From these studies we developed a model of disease pathogenesis applicable to human disease (Fig 3). It suggests that the presence of three independent factors is necessary and sufficient to extend the breakdown of T cell tolerance to self-PDC – which represents what seems to be the key step in the conversion of a harmless process to one associated with autoreactive tissue damage:

- *Immunogenetic susceptibility*: breakdown of T cell tolerance to self-PDC has so far been seen in only a single strain, the autoimmune susceptible SJL/J mouse.
- *Exposure to immunoreactive non-self-PDC with minimum and maximum degrees of homology to self*: sufficient sequence difference has to be present to allow the development of a T cell response to non-conserved T cell epitopes, whilst there has to be sufficient structural homology present to ensure the cross-reactivity of the anti-non-self antibody, with self seemingly necessary to drive epitope spreading.
- *Exposure to the necessary inflammatory environment*: the nature and extent of the inflammatory environment seems to determine the 'ease' with which epitope spreading will occur.

Based on these observations, we have developed the following model of PBC pathogenesis. As with the murine model, susceptibility to PBC is likely to be at least partially genetically determined. Prevalence of PBC is significantly increased in first-degree relatives of PBC patients, and there is an increase in the prevalence of other autoimmune diseases in both PBC patients and their relatives.<sup>25</sup> A recent (as yet unreplicated) twin study has suggested a concordance rate as high as 75% in monozygotic twins. The few data available with regard to the identity of the susceptibility loci are derived from relatively small case-control association studies with poor reproducibility.



**Fig 3. A model for the pathogenesis of primary biliary cirrhosis** (BEC = biliary epithelial cells; PDC = pyruvate dehydrogenase complex; TLR = toll-like receptor).

In susceptible individuals, the key occurrence appears to be exposure to immunogenic, cross-reactive non-self-PDC in a sufficiently inflammatory context to drive epitope spreading. One potential source of cross-reactive non-self-PDC would be bacterial derived antigen. In this case, the liver tropism of PBC could be envisaged as resulting from source PDC encountered in the context of mucosal bacterial infection – for example, bacterial cholangitis or the bacterial urinary tract infections previously demonstrated to occur at increased frequency in PBC patients – and inducing anti-PDC-E2 antibody responses even in non-patients.

A second potential source of altered self-PDC would be chemically modified self-antigen. In this case, the tropism of the disease would result from the unique role played by the liver in conjugating compounds, or perhaps biliary excretion of potential adducts able to modify BEC PDC. This xenobiotic model is of particular interest, given the recent observation that AMA from PBC patients have a higher affinity for peptides based on the lipoic acid binding domain of PDC-E2, in which lipoic acid has been modified to replicate potential halogenated xenobiotics, than to peptides with the native lipoic acid cofactor. This observation is compatible with chemically modified PDC being the sensitising antigen.<sup>26</sup>

A third, at least potential, source of modified self-PDC would be antigen cleaved during apoptosis. In this case, the tropism of the disease might result from the tropism of the agent(s) inducing apoptosis. However, it should be noted that the question of the immunogenicity of apoptotic BEC and/or caspase cleaved self-PDC has yet to be studied in vivo. In this regard, particular interest has focused on heavy metals which are concentrated in the bile during biliary excretion and which

might, intriguingly, be at higher environmental levels in some of the postindustrial areas which appear to have populations exhibiting high prevalences of PBC2.

In this model, the inflammatory environment could again be provided by bacterial infection. Alternatively, the high levels of bacterial products found in ascending portal blood as a result of leakage across the gut wall might fulfil this role, providing a further mechanism for the tissue tropism of the disease. In situations where insufficient drive for epitope spreading might be present, leakage of intact native PDC from necrotic cells might provide an additional drive to epitope spreading, as demonstrated in the murine modelling.

As yet this remains a model, but it has the advantage of reconciling a number of previous seemingly disparate observations in PBC. Should this model prove correct, one important implication is that, given the diverse forms of altered self-PDC able to initiate and potentiate the process, there is unlikely to be any single 'cause' of the disease.

## Coda

One of the great intellectual contributions made by Thomas Addison to medical science was the concept of pathophysiology. It is perhaps fitting that, over the years, numerous investigators (including those cited here and many others) have applied his philosophy of understanding physiology in order to explain pathology so effectively in the study of his 'other' disease.

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