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TOPIC HIGHLIGHT

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Etiopathogenesis of primary biliary cirrhosis

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Abstract

Primary biliary cirrhosis (PBC) is an autoimmune disease of the liver characterized by progressive bile duct destruction eventually leading to cirrhosis and liver failure. The serological hallmark of the disease is the presence of circulating antimitochondrial antibodies (AMA). These reflect the presence of autoreactive T and B cells to the culprit antigens, the E2 subunits of mitochondrial 2-oxo-acid dehydrogenase enzymes, chiefly pyruvate dehydrogenase (PDC-E2). The disease results from a combination of genetic and environmental risk factors. Genetic predisposition is indicated by the higher familial incidence of the disease particularly among siblings and the high concordance rate among monozygotic twins. Environmental triggering events appear crucial to disrupt a pre-existing unstable immune tolerance of genetic origin allowing, after a long latency, the emergence of clinical disease. Initiating mimotopes of the vulnerable epitope of the PDC-E2 autoantigen can be derived from microbes that utilize the PDC enzyme or, alternatively, environmental xenobiotics/chemical compounds that modify the structure of native proteins to make them immunogenic. A further alternative as a source of antigen is PDC-E2 derived from apoptotic cells. In the effector phase the biliary ductular cell, by reason of its proclivity to express the antigen PDC-E2 in the course of apoptosis, undergoes a multilineage immune attack comprised of $CD4^+$ and $CD8^+$ T cells and antibody. In this article, we critically review the available evidence on etiopathogenesis of PBC and present interpretations of complex data, new developments and theories, and nominate directions for future research.

Key words: Autoantibodies; Autoreactive T cells; 2-oxoacid dehydrogenase; Biliary epithelial cells; Primary biliary cirrhosis

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INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease of autoimmune origin characterized by a striking female predominance, high titer serum anti-mitochondrial autoantibodies (AMA), disease-specific antinuclear autoantibodies (ANAs), and an autoimmune-mediated destruction of the small and medium size intrahepatic bile ducts[1]. PBC is a peculiar, yet representative, organ-specific autoimmune disease. The presence of serum AMA and autoreactive T and B cells, in conjunction with the co-occurrence of other autoimmune diseases, all point to an autoimmune pathogenesis for PBC. Although most patients with PBC have AMA against the E2 subunit of the mitochondrial pyruvate dehydrogenase complex (PDC), there is no direct correlation between the titer of AMAs and disease severity. However, certain disease-specific antinuclear antibodies (ANAs) are present in about one third of patients and these carry a risk for more severe and progressive disease[2].

A multifactorial genetic background is suggested by a higher incidence of the disease among first-degree relatives[3], by the high concordance rate among monozygotic twins[4], and by an apparent role for X chromosome defects in PBC, based on the observation that women with PBC have preferential loss of one X chromosome

in peripheral white blood cells[5,6]. A vital question in the pathogenesis of PBC is why biliary epithelial cells (BEC) in particular are the primary target of pathology despite the ubiquitous presence of the PDC autoantigen in all tissue cells. Recent studies suggest that enhanced apoptosis in BEC is a critical step in ductular destruction in PBC[7,8], and some clues exist on mechanisms by which apoptosis in BECs cause the tissue-specific autoimmune reactivity characteristic of PBC.

GENETICS IN PBC

It is currently accepted that PBC pathogenesis is multifactorial, with genetic and environmental factors interplaying to determine disease onset and progression. Although the etiology of PBC remains enigmatic, there are several items of data indicating that genetic predisposition contributes strongly to the overall pathogenesis of PBC. The lines of evidence are these: (1) Data from monozygotic twins indicate that the concordance rate of PBC in monozygotic twins is 63%[4], among the highest reported for autoimmunity; (2) Approximately 6% of patients with PBC have a first-degree relative that also suffers from PBC[3]; (3) There is a high female:male disease incidence ratio (8:1), with suggestions of a significant role for X chromosome defects in PBC, based on the observation that women with PBC have a significantly enhanced monosomy X frequency in peripheral white blood cells compared to age-matched healthy women[5] and that the X chromosome loss is preferential[6]. Interestingly, similar genetic defects were also found in women with systemic sclerosis and autoimmune thyroid disease[9], but not with systemic lupus erythematosus[10]. Future studies should assess whether haploinsufficiency for specific X-linked genes may lead to loss of tolerance; (4) PBC is exceptional among autoimmune diseases in having controversially variable associations with alleles of the major histocompatibility complex (MHC, HLA); only a weak and regional association with HLA *DRB1*08* has been widely confirmed[11], although there is growing evidence on a protective association with HLA *DRB1*11* and **13*[12,13].

Several association studies have attempted to identify gene loci associated with PBC but no family study of genetic linkage has been performed. Associations are often not applicable to all populations but available evidence suggests that a "multi-hit" genetic model might apply to PBC, with different genetic variants conferring initial susceptibility, and others influencing subsequent disease progression. Genetic influences operative in PBC may reflect mutations transmitted through germline genes, or conceivably, somatic mutations in hemopoietic precursor cells[14].

In summary, a susceptible genetic background is considered to be necessary, but is not sufficient to explain either PBC onset or the strong female predominance[15]. Thus several environmental factors have been invoked as additional elements in tolerance breakdown.

ENVIRONMENTAL FACTORS

Bacterial infection in various settings has been repeatedly invoked in the etiopathogenesis of PBC. This etiology is usually linked to the concept of molecular (epitope) mimicry. The cross-reactivity of AMA with prokaryotic antigens has been reported for a number of microbes. This cross-reactivity is not particularly surprising given the conserved sequence of PDC-E2 across all species, from eubacteria to mammals.

We provided experimental evidence suggesting that *Novosphingobium aromaticivorans*, a ubiquitous xenobiotic-metabolizing Gram-negative bacterium, is the best microbial candidate yet for the induction of PBC[16,17]. Briefly, we can extrapolate theories on microbial molecular mimicry in PBC as follows. The microbial motif CpG enhances IgM production in peripheral blood mononuclear cell cultures, with CD27+ memory B cells in PBC patients being responsible for this IgM production through Toll-like receptor (TLR) 9 signaling. Also, CpG can stimulate AMA production and expression of TLR9, CD86, and one of the potassium channels, KCa3.1, in B cells of PBC patients. Moreover upregulated expression of TLR9 and CD86, and AMA secretion induced by CpG, can be suppressed by a specific blocker of the KCa3.1 channel, namely TRAM. These data indicate that B-cell immunity of PBC patients depends on an enhanced innate immune response and imply that TRAM-34 can influence B-cell autoimmunity in PBC[18].

Another source of antigenic mimicry is xenobiotics. These are foreign compounds that may either alter or complex to defined self or non-self proteins, inducing a change in the molecular structure of the native protein sufficient to induce an immune response[19]. Such immune responses may then result in the cross-recognition of the self molecule, which could in turn perpetuate the immune response, thus leading to chronic autoimmunity. Interestingly, most xenobiotics are metabolized in the liver, thereby increasing the potential for liver-specific alteration of proteins. Recent data demonstrate that certain chemical/xenobiotic compounds can induce AMA and are in fact recognized by PBC sera with higher affinity compared to the analogous self protein and that such compounds are found in products in common use as food flavorings and cosmetics[20-23]. This implicit involvement of cosmetics could contribute to the female predisposition to PBC.

ROLE OF BEC

PBC is characterized by destruction of the small and medium size intrahepatic bile ducts, lined by BECs (cholangiocytes). BECs express cell surface adhesion molecules which permit adhesion and recognition of lymphocytes. Moreover, several studies have demonstrated that BECs of both healthy and diseased liver have the capacity to increase the expression of adhesion molecules, ICAM-1 and others, MHC class Ⅰ and Ⅱ, TNF-alpha, interferon (IFN) -gamma

Table 1 Immunopathological characteristics of biliary epithelium in PBC[90]

	Normal	PBC
Expression level of PDC-E2	+	+ + +
Adhesion molecules		
- ICAM-1	+	+ +
- VCAM-1	-/+	+
- LFA-1	-/+	+
- E-selectin		+ +
Biliary intra-epithelial lymphocytes	Large bile ducts, few $CD4^+$	Small bile ducts, increased $CD4^+CD28^-$
Apoptosis-related molecules		
- Fas (CD95)	-	+
- granzyme B	-	-/+
- perforin	-	-/+
- bcl-2	+ +	-
BEC phagocytosis of apoptotic BECs	-	+ +
Cytokines		
- INF-γ	-	+ +
- IL-2	-	+ +
- IL-6	-	+ +
- IL-6 receptor	-	-/+
- TNF-α	-/+	+ +
- TNF receptor	-/+	+ +

and IL-1[24-26] upon stimulation with proinflammatory cytokines[27]. Up-regulation of VCAM-1 and LFA-1 can also be identified[28]. Adhesion molecules expressed on the BEC surface, and the up -regulation by proinflammatory cytokines, which are abundant in the course of inflammatory reactions, allow BECs to modulate the intensity and localization of inflammatory reactions. The other immune feature attributed to the BECs is a capacity to act as APCs. Several studies demonstrate that BECs express HLA class Ⅱ[27,29], and such expression is increased after injury and after stimulation with IFN-gamma and IL-1. BECs also express accessory molecules responsible for the second (co-stimulatory) signal to T cells, CD80, 86 (B7-1, B7-2)[30]. These interactions with T cells might also be responsible for bile duct loss, one of the fundamental characteristics of progression of disease.

Data obtained in recent years point towards apoptosis as a leading mechanism for ductopenia. Years ago, Harada and colleagues demonstrated susceptibility to apoptosis via the perforin/granzyme B pathway, and this was enhanced by interaction of CD95 (Fas) with CD178 (FasL) in BECs of patients with PBC[31]. The hypothesis was further confirmed when apoptotic BECs were shown to express CD40, and Fas and FasL, with transcriptional up regulation of the latter molecules after stimulation with CD154 (CD40L), culminating in apoptosis[32]. Odin and colleagues discovered that glutathiolation of the lysine-lipoic acid moiety of PDC-E2 was dramatically reduced by serum AMA[33]. Recently it has been demonstrated that apoptotic cells are phagocytosed by BECs and consequently could be an endogenous source of autoantigens from BECs[34-36]. Importantly, these findings support the concept that tissue specific damage in PBC is due to cell type-specific differences in apoptosis, and phagocytosis of apoptotic cells.

Antigenicity of BEC self-molecules, or highly homologous epitopes, could also be related to their role in mucosal immunity. Like other epithelial cells, BECs actively transfer IgAs, and in PBC these IgAs have specificity for PDC-E2. These specific IgA-type AMA can be detected in almost all body fluids of patients with PBC, including saliva, urine and bile[37,38]. Further, Fukushima and colleagues[39] detected deposits representing co-localization of such antibodies with PDC-E2 (or a highly homologous molecule) at the apical surface and in the cytoplasm of BECs, and also detected their presence in liver allografts in patients with recurrent PBC after receiving a liver transplant. To assess the direct pathogenicity of the IgA antibody class, Matsumura and colleagues exposed canine kidney cells transfected with the human polymeric Ig receptor to highly purified AMA-IgAs, thereby inducing caspase up-regulation, and thus providing evidence for direct toxic effects[40]. The immunogenic characteristics of BECs in PBC are summarized in Table 1. Finally, the still unknown role of autophagy in autoimmunity could in the future provide interesting data for the pathogenesis of PBC[41].

B CELLS AND AUTOANTIBODIES

As mentioned, the presence of serum AMA and autoreactive B cells strongly endorses the concept of an autoimmune pathogenesis of PBC[42-44].

AMA is highly specific for PBC and can be detected in nearly 100% of patients, when sensitive diagnostic methodologies based on recombinant antigens are used[45]. They are directed against members of the 2-oxoacid dehydrogenase complexes (2-OADC) existing in the inner membrane of mitochondria. Among them, the major autoantigen is the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2). The epitopes for this antibody to the E-2 subunit localize to three domains of PDC-E2 component: (1) the inner (and outer) lipoic acid (lipoyl) domains; (2) the E3 binding domain; and (3) the catalytic and E2-binding domain[46]. Reactivity at lower frequency is also found against other 2-oxoacid dehydrogenase complexes (2-OADC), the 2-oxo glutarate dehydrogenase (OGDC-E2) and the branched-chain 2-oxo acid dehydrogenase (BCOADC-E2), involved respectively in the citric acid cycle and in amino acid catabolism. The 2-OADC autoantigens in PBC are summarized in Table 2.

Although the E-2 subunits of the three E-2 subunits are structurally similar, immunochemical studies have shown that the reactivities are independent, and do not depend on cross-reactivities, at least at the antibody level[47]. Antibodies to PDC-E2 and to the other 2-OADC enzymes are capable of inhibiting PDC-E2 enzyme activity *in vitro*, but this has not been shown to occur *in vivo*. Targeting of enzymes is a common feature of autoantibodies detected in patients with autoimmune

Table 2 Mitochondrial and nuclear autoantigens in PBC

		Autoantigens
Mitochondrial antigens	E2 subunits of 2-OADC	PDC-E2
		OGDC-E2
		BCOADC-E2
	Pyruvate dehydrogenase complex	E3BP
		PDC E1α
Nuclear antigens	Nuclear pore complex	gp210
		nucleoporin 62
	Multiple nuclear dots	Sp100
		PML
	Anticentromere	

2-OADC: 2-oxo-acid dehydrogenase complex; PDC: Pyruvate dehydrogenase complex; OGDC: Oxoglutarate dehydrogenase complex; BCOADC: Branched chain 2-oxo-acid dehydrogenase complex; E3BP: Dihydrolipoamide dehydrogenase (E3)-binding protein.

diseases, as is the inhibition of their activity by these autoantibodies. A pathogenic role for AMA is uncertain, since no clinical correlations with levels of AMA can be found, and in certain experimental animal models there is occurrence of serum AMA but no overt PBC-like liver lesions[14]. The role of the lipoic acid co-factor attached to lysine$_{173}$ (K_{173}) in the composition of the epitope recognized by AMA is unclear. Both lipoylated and non-lipoylated PBC-E2 react with AMA and the question is to what degree does lipoic acid serve to enhance antigenicity.

In addition to AMA, PBC sera can present other disease-specific autoantibodies, particularly anti-nuclear (ANA) specificities[2]. PBC-specific ANA reactants include nuclear pore glycoproteins of the inner nuclear membrane, gp210[48] and p62[49], with a detection rate up to about 30% and with an apparently higher prevalence among AMA-negative PBC. This subtype of PBC-specific ANA has been shown to correlate with disease severity and progression[50,51]. Other PBC-specific nucleoprotein reactants include the Sp100-promyelocytic leukemia (PML) autoantigen antigen that gives the characteristic fine nuclear dot pattern by immunofluorescence[52]; both appear specific for PBC, but the prevalence differs, being from about 20% to 30%. Finally anti-centromere antibodies occur in PBC (-10% of cases) often in association with a limited scleroderma syndrome. Recently, it has been demonstrated that anti-centromere antibodies were a significant predictive factor in PBC for the development of portal hypertension[51,53]. Table 2 specifies the nuclear autoantigens in PBC.

There seems no way of incorporating the co-occurrence of AMA and ANA into a unifying theory of pathogenesis of PBC, other than specifying both reactivities as reflecting a systemic failure of maintenance of immune tolerance.

T CELLS

Autoreactive $CD4^+$ and $CD8^+$ T cells are demonstrably involved in the pathogenesis of PBC and, histologically, infiltration of presumably autoreactive T cells in the liver and periductular spaces is one of the major features of the disease[54,55]. Both $CD4^+$ and $CD8^+$ T lymphocytes can be purified from biopsy samples of PBC patients and both subsets recognize epitopes of PDC-E2[56]; moreover, using recombinant fragments of PDC-E2 it has been demonstrated that there is a sequence overlap in the PDC-E2 specific T and B cell epitopes[57]. The minimal T-cell epitope for $CD4^+$ T cells was identified as amino acid residues 163 to 176 (GDLLAEIETDKATI), within the inner lipoyl domain of PDC-E2[57]. Phenotypically, the PDC-E2$_{163\text{-}176}$ T-cell clones were positive for CD4, CD45RO, and T cell receptor (TCR) αβ. The MHC Class Ⅱ human leukocyte antigen (HLA) -restriction molecules for this epitope have been identified as HLA-DR53 (B4*0101)[58]. In addition, PDC-E2$_{163\text{-}176}$-specific $CD4^+$ T-cell clones recognize other functionally related mitochondrial autoantigens, including OGDC-E2 and BCOADC-E2, and also E3BP[59]. More specifically, these T-cell clones were cross-reactive with the amino acid residues 100 to 113 of OGDC-E2, residues 90 to 103 of BCOADC-E2, and residues 34 to 47 of E3BP, all located in the respective E2-lipoyl domain of these enzymes; thus the suggestion is that OGDC-E2$_{100\text{-}113}$, BCOADCE$_{290\text{-}103}$, and E3BP$_{34\text{-}47}$ all represent $CD4^+$ T-cell epitopes.

$CD8^+$ T cells (CTLs) from peripheral blood of patients with PBC have been studied in the context of MHC Class I HLA-A2.1 restriction, and have been found to identify amino-acid residues 159-167 and 165-174 of PDC-E2[57]. Specific MHC class I restricted CTLs can also be generated by *in vitro* stimulation with antigen pulsed dendritic cells[60] from blood of patients with PBC, but not from healthy controls, indicative of the presence in PBC of specific precursors of PDC-E2 -reactive T cell clones in peripheral blood. Interestingly, there was a greater increase in numbers of CTL precursors in blood in early *versus* advanced stages of PBC, and in the same study there was a 10 -fold increase in specific CTLs in the liver compared to the peripheral blood, supporting the role of these cells and their specific recruitment in the evolution of bile duct injury in PBC. Thus the two major subsets of T cells recognize the same or very close amino acid sequences within the same epitope regions in the lipoyl domain, thus supporting the hypothesis of a common etiological trigger mechanism, potentially molecular mimicry, associated with other particular immune modifications.

Coming now to $CD4^+CD25^{high}$ natural regulatory T cells (Tregs), a decreased reactivity appears to contribute to a number of human autoimmune diseases[61-65] including PBC. A relative reduction of Tregs compared with healthy controls was detected and, as well, the ratio of hepatic Tregs over hepatic $CD8^+$ cells in PBC patients was lower than that in patients with chronic hepatitis C or autoimmune hepatitis[66,67].

INNATE IMMUNITY IN PBC

Innate immunity is a first line of defense against

infections and neoplasms, but its importance for adaptive immunity has been appreciated only recently, and its role in the induction of autoimmunity is only partially known[68]. The cellular components of innate immunity, including dendritic cells (DC) and other professional APCs[69], and natural killer T cells (NKT), are known to have a regulatory function by modulating the quality and quantity of subsequent adaptive immune responses, including antigen-specific antibody and T cell responses. Innate immunity in PBC patients is characterized by an increased response to pathogen-associated stimuli, as indicated by higher levels of pro-inflammatory cytokines secreted *in vitro* by monocytes after exposure to micro-organisms[70].

NK/NKT cells have been linked to autoimmune diseases in murine models, including autoimmune diabetes in NOD mice and experimental autoimmune encephalomyelitis, a model of multiple sclerosis[71], and the role of such cells in autoimmunity in general is attracting increasing attention. In PBC, Chuang and colleagues recently demonstrated a marked increase in the frequency and absolute number in blood and liver of NK cells. Moreover, in the same study, the cytotoxic activity and perforin expression by isolated NK cells were significantly increased, associated with increased levels of plasma IL-8 and the expression of CD128a (IL-8 receptor) on such cells. In contrast, the levels of IFN-γ, IL-6 and IL-8 synthesized by NK cells were significantly decreased in PBC compared to controls[72].

Hyper-responsiveness of the innate immune system of itself would be insufficient to account for the breakdown of natural immune tolerance, but these alterations might come to influence the initiation and perpetuation of the subsequent adaptive autoimmune response.

CYTOKINES

In PBC, a Th1 cytokine predominance has been reported in serum and liver[73], and a high prevalence of INF-γ, a Th1 cytokine, has been detected as a transcriptional up-regulation[74]. Moreover, BECs of patients with PBC overexpress TNF-α and the corresponding receptor, thus favoring the idea of a paracrine activity of, and effect on these cells, leading to their proliferation and, potentially, to apoptosis[75]. Recent findings further suggest the involvement of cytokine-cytokine receptor interactions in the effector stages of the pathogenesis of PBC[72]. Whilst T cells and NKT cells are major sources of cytokines, B cells, endothelial cells, macrophages and other cell types also contribute to cytokine production. Furthermore, different types of APC, genetic background, availability of costimulator molecules, and types and amounts of antigenic stimuli may also influence the differentiation of Th0 cells into either the Th1 or Th2 cell pathways, each with their particular cytokine profiles. Of course, cytokines also come into play in the earlier inductive stages of PBC, in particular transforming growth factor-beta (TGF-β). Deficiency of TGF-β is prejudicial to immunoregulatory functions, as illustrated by recent mouse models of PBC (see below).

ANIMAL MODELS

The occurrence of a spontaneous animal model would be extremely helpful in elucidating causation and progression of PBC, but none has been identified, and there is some element of "artificiality" with induced models. However, recently, there have been developed three informative genetically manipulated mouse strains that simulate features of human PBC[76].

The first of these mouse models is a congenic variant of the non-obese diabetic (NOD) mouse designated NOD.c3c4 that presents as an autoimmune larger bile duct cholangiopathy and PBC-like serology, with AMA positivity of 50%-60% and ANA positivity of 80%-90%[77]. Histologically, there is lymphocytic infiltration within portal tracts with appearances of chronic nonsuppurative destructive cholangitis and epithelioid granuloma formation, although certain features of the bile duct lesions differ from those in human PBC, particularly the occurrence of cystic changes[77]. Detailed analysis of the introgressed genetic intervals that determine the autoimmune switch from pancreatic insulitis to cholangitis is awaited.

The second of the mouse models was derived by transgenic introduction of a dominant negative form of TGF-β receptor Ⅱ (dnTGF-βRⅡ)[78]. These mice have inflammatory cholangitis and show 100% AMA positivity against PDC-E2. TGF-β receptor Ⅱ is essential for signal transduction of TGF-β, which regulates activation of lymphocytes. This model suggests a specific dysfunction of T cells with impaired TGF-β signaling which, in the presence or absence of B cells, is implicated in the pathogenesis of a PBC-like disease, at least in mice[78]. Interestingly, it has been demonstrated that CD1d-restricted NKT cells in these mice are a critical factor in liver injury[79].

The third of the mouse models depends on knockout of the gene for the IL-2 receptor (IL-2Rα knockout mouse)[54]. These mice have inflammatory cholangitis with lymphocyte infiltration around the portal tracts accompanied by cholangiocyte injury, and show 100% AMA positivity against PDC-E2 and 80% ANA positivity. The IL-2Ra is the CD25 molecule which, when highly expressed on $CD4^+$ T cells, is a marker for cells with immunoregulatory activity. This model further implicates deficiency of TGF-β-dependent regulatory pathways in the pathogenesis of PBC.

Another useful model has been developed by experimental immunization with xenobiotically modified molecular variants of the PDC-E2 epitope region. Such immunization appears promising in that AMA have thus been elicited in different animal species, rabbits, guinea pigs and mice, as recently reviewed[14].

PATHOGENIC MECHANISMS

Several theories have been proposed for the

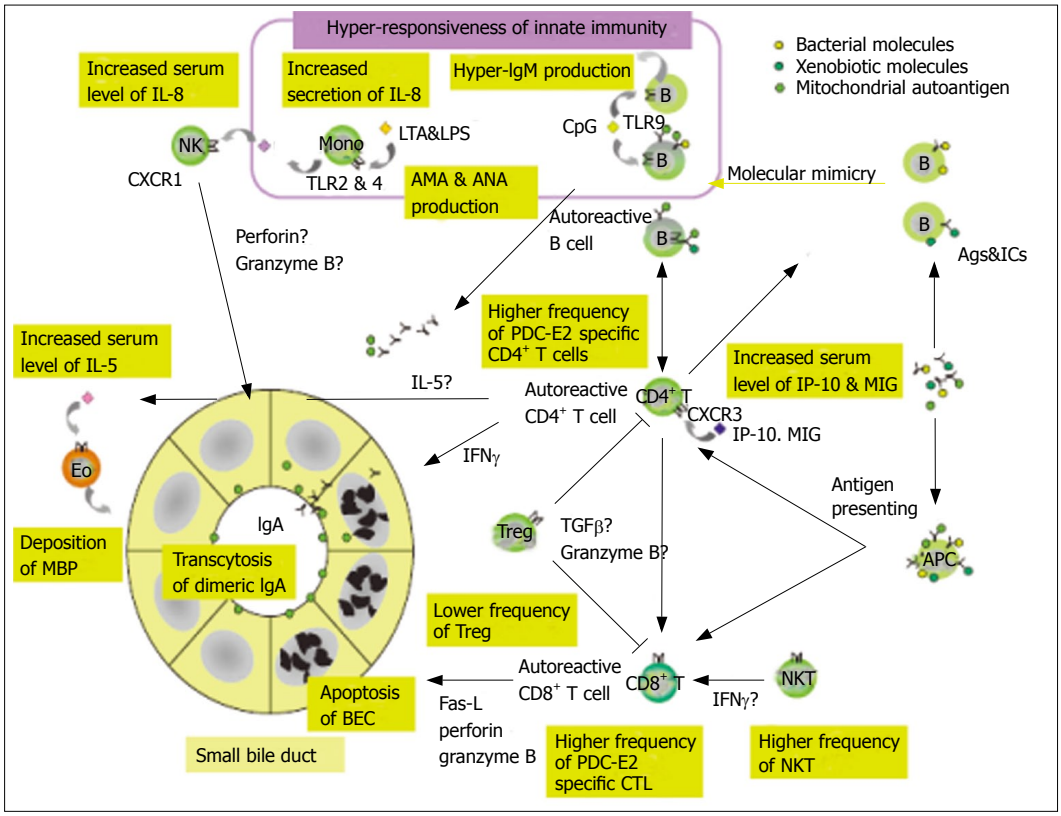

Figure 1 Model of pathogenic mechanisms in primary biliary cirrhosis (PBC). PBC is initiated by an autoantigenic stimulus (upper, right) provided either by a bacterial mimic of the autoepitope of PDC-E2, a xenobiotically modified PDC-E2, or "spillage" of native mitochondrial autoantigens derived perhaps from apoptotic cells. Hyper-responsiveness of innate immunity (top, centre) can facilitate autoantigenicity; bacterial Cpg enhances IgM production and cellular expression of TLR9. Genetic susceptibility is critical overall, and depends particularly on multiple inherited deficits in immune tolerance, mostly as yet undefined. APCs that become activated (lower, right) by stimulation through TLRs present immunogenic self peptides (or mimics) *via* MHC Class II molecules to autoreactive $CD4^+$ T lymphocytes (centre) which in turn activate $CD8^+$ cytotoxic T lymphocytes and B lymphocytes that produce AMA. Treg lymphocytes (lower, centre) that normally restrain activated autoreactive T cells are deficient in PBC, thus further impeding T cell tolerance. Effector mechanisms converge on the target cell in PBC, the BEC (lower left), which can be damaged by injurious cytokines (IFN-γ) from $CD4^+$ T cells, direct cytotoxicity (Fas-L, perforin, granzyme B) from $CD8^+$ T cells, or transcytosis of IgA-AMA. A toxic effect might even be supplied by activated eosinophils (centre, left) by release of eosinophil MBP. BECs thus undergo apoptosis and in doing so contribute immunogenic mitochondrial PDC-E2 autoantigen to sustain a self-perpetuating autoimmunization process and, by reason of a BEC-specific anomaly of apoptosis retain PBC-E2 intact in apoptotic blebs (see text), so conferring particular vulnerability on these cells. Ags: Antigens; AMA: Antimitochondrial antibodies; ANA: Antinuclear antibodies; APC: Antigen-presenting cell; BEC: Biliary epithelial cells; CTL: Cytotoxic T lymphocytes; ICs: Immune complexes; IL: Interleukin; IFN: Interferon; IP-10: Interferon-γ-inducible protein 10; LTA: Lipoteichoic acid; LPS: Lipopolysaccharide; MIG: Monokine induced by γ-interferon; MBP: Major basic protein (primary cytotoxic granule protein); NKT: Natural killer T cells; PDC-E2: Pyruvate dehydrogenase complex E2; TGF: Transforming growth factor; TLR: Toll-like receptor; Treg: Regulatory T cells.

etiopathogenesis of the immune-mediated tissue injury observed in PBC (Figure 1). Such theories are not necessarily independent, but rather each may be directed to different phases of etiopathogenesis. In other words, we need to consider processes particular to initiation; processes particular to perpetuation, notably deficiencies in immune tolerance; and processes particular to the selective destruction of BECs, with the assumption that these express the target of the disease in an accessible form, namely the AMA autoantigen PDC-E2.

Initiation

Initiation has been considered already and possibilities include microbial infection or chemical-xenobiotic modification of the PDC-E2 epitope sequence with tolerance-breaking effects due to molecular mimicry. Alternatively mere spillage of autoantigen after cellular injury and apoptosis could suffice, as shown recently in our laboratory[80].

Perpetuation

Perpetuation involves particular consideration of genetically-based tolerance deficits in PBC, and here more data are sorely needed. Consideration was given to this aspect in a recent review from this laboratory[14]. We can refer here to studies on the critical role of $CD4^+$ $CD25^{high}$ regulatory T cells (Tregs) in the prevention of autoimmune disease in murine models. It is postulated that Tregs are important for the prevention of autoimmunity and maintenance of self-tolerance, and studies have demonstrated that the transfer of T cells lacking the Treg subset into athymic nude mice results in the development of various T cell–mediated autoimmune diseases[61,81]. PBC patients display significantly lower frequencies of Tregs as percentages of total TCR-$\alpha\beta^+$/$CD4^+$ T cells, which may contribute to the failure in tolerance in PBC[66,82,83].

Destruction

Destruction involves a multilineage attack by $CD4^+$ and $CD8^+$ T cells, and B cells, on the vulnerable biliary

ductile, and here our hypothesis would state that the immunodominant AMA autoantigen PDC-E2, which is normally located in the mitochondrial inner membrane, is aberrantly expressed on the cell surface of the BEC and thus is immunologically recognized. Several possibilities have been visualized. First, although *in situ* hybridization studies of *PDC-E2* mRNA showed no significant difference in the amount of PDC-E2 transcript present in PBC liver compared with other liver diseases, PDC-E2 may be selectively overexpressed in small bile duct BECs perhaps as a result of aberrations of apoptosis[14]. Second, variants of PDC-E2 may cause an abnormal turnover of the molecule, leading to the accumulation of PDC-E2 in these subpopulations of cells. It is possible that toxic substances disposed of by the liver may accumulate in the biliary epithelium and potentially modify the PDC-E2 molecule locally, leading to the production of such variants. Third, altered *PDC-E2* mRNA could be produced by the abnormal transcription of PDC-E2. For example, it is possible that abnormal splicing during synthesis of *PDC-E2* mRNA would substitute an endoplasmic reticulum targeting signal instead of a mitochondrial targeting signal, thereby enabling PDC-E2 to be delivered into the endoplasmic reticulum and Golgi apparatus via a secretory route to be expressed on the cell surface of biliary ducts, instead of into mitochondria. Although direct evidence supporting these mechanisms is currently lacking, it remains possible that the molecules that are expressed and identified on the ductular surface of BECs, and recognized by anti-PDC-E2 antibodies, may not be PDC-E2 itself, but are PDC-E2 mimics that cross-react with human PDC-E2. Some experimental data seem to support this hypothesis.

Another hypothesis that might explain the selective targeting of bile ducts in PBC is that the autoantigen-specific immunoglobulin A (IgA) antibody plays a role. IgA is the principal isotype of immunoglobulin in epithelial surfaces, including biliary epithelium. If AMA-IgA autoantibodies are responsible for the specific destruction of BECs in PBC, it is possible that this occurs by disrupting cell metabolism of the cells i.e. the AMA-IgA bound to the mitochondrial antigen induces cellular dysfunction and so accounts for the tissue specificity. Interestingly, IgA from PBC patients colocalized with PDC-E2 inside the cells and on the apical membrane of BECs[84]. These data support the idea that both the aberrant polar expression of PDC-E2 and the trafficking of IgA in BEC are possible mechanisms for selective damage of BECs. Thus, the apical staining of BECs revealed by anti–PDC-E2 monoclonal antibodies could also be accounted for by the presence of an immune complex formed from secreted IgA and mitochondrial enzyme autoantigens.

CONCLUSIONS AND FUTURE PERSPECTIVES

There have been many substantial advances in the understanding of PBC since the molecular identification in 1988 of PDC-E2 as the major reactant for characteristic AMA response. Possible initiators of PBC have emerged as environmental chemical xenobiotics, or microorganisms that utilize the shared culprit autoantigen PDC-E2. Strong genetic predisposition is certain from case study data, noting here the female predisposition and family clustering, but formal genome wide studies are not yet available. It is highly likely that multiple deficits in immune tolerance will prove important, and that these will be genetically based; recent mouse models are certainly pointing in this direction. Other unexplained features of PBC include susceptibility to infections, likely associated with the aberrant humoral and cellular reactivities[85,86]. It is crucial to ascertain whether there is a pathogenic role of AMA in the bile duct damage of PBC and, if so, how this is mediated. Further investigation of innate immune mechanisms in PBC is called for, as is the role of the BEC itself in stimulation and perpetuation of the peribiliary inflammatory process.

It is almost a truism that only knowledge of the etiopathogenetic mechanisms will open the door to effective therapies for diseases such as PBC[87], yet the major advance in the therapy of PBC with UDCA[88] has come without insight into primary causes of the disease, just as successful biotherapies of rheumatoid arthritis have emerged without knowledge of the primary cause of that disease. Currently, we should press on with development and study of animal models, encourage the application of predictably informative genomic studies, and seize on the clues we have to environmental provocations.

At the practical level, clinicians should be alert to the need for early diagnosis, during the long latent period of the disease, in the susceptible middle-aged female population to ensure that such subjects do gain benefit from disease-retarding therapy, at whatever stage their disease may be[88]. To a degree, this is already happening if we compare what PBC was like 20 years ago[89] to what it is today[90].

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