The pathogenesis of primary biliary cirrhosis

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ABSTRACT

Primary biliary cirrhosis (PBC) would develop when the immune system comes across a *microorganism* with proteins similar to those in the pyruvate dehydrogenase complex E2 (PDC-E2), or a neoantigen resulting from a xenobiotic-modified autoantigen. This would lead to an *innate immune* response where TLRs would play a pivotal mediating role, which would give rise to a local microenvironment favoring an *adaptive immune* response. Such response would be particularly strong in individuals with selected genetic characteristics. The genetic characteristics underlying this predisposition remain unknown, but they likely entail small numbers of scarcely-active regulatory T cells. The AE2 anion exchanger, which is deficient in patients with PBC, may reduce the number and activity of regulatory T cells. NK cells are also pivotal in the preparation of an adaptive response, as they release a number of cytokines and chemokines that favor and recruit antigen-presenting cells to activate B and T cells – CD4+ Th1 and CD8+. An activation of the former would increase the production of IgM and anti-mitochondrial IgG and IgA antibodies against PDC-E2. An activation of CD8+ cells, also sensitive to PDC-2 as aberrantly expressed on the surface of BECs and SECs, would result in apoptosis for these epithelial cells, and in small bile-duct destruction. Immune response is likely inadequately suppressed because of the small numbers of scarcely-active regulatory T cells, the latter resulting from low genetic expression and activity of the AE2 transporter.

Key words: Primary biliary cirrhosis. Pathogenesis. Autoimmunity. Xenobiotics. Genetics.

INTRODUCTION

Primary biliary cirrhosis (PBC) is an inflammatory disease of unknown etiology that affects almost exclusively women; lesions selectively compromise small bile ducts, hence the disease results in chronic cholestasis. While PBC is the most widely accepted designation for this disease, it is inappropriate since the condition has several stages with no liver cirrhosis but only chronic, non-suppurative, destructive cholangitis.

Elementary lesions in PBC concentrate in small septal and interlobular bile ducts, where biliary epithelial cells (BECs) show various degrees of degeneration. The walls of these injured ducts commonly exhibit interepithelial CD8+ and CD4+ Th1 T cells surrounded by dense infiltrates of CD4+ and CD8+ B cells, among other cell types. Over time degenerative changes in BECs become more pronounced, and these cells and their associated bile ducts eventually disappear. In this stage epithelioid-cell aggregates or non-caseating granulomas are usually found in the vicinity of or around involved bile ducts. These lesions, which are not pathognomonic of PBC, have been designated florid duct lesions or chronic non-suppurative destructive cholangitis (1,2).

PATHOGENESIS OF PBC

Small bile duct lesions

The mechanism through which typical PBC lesions arise is unknown; however, enough data have been collected of late to endorse that the destruction of small bile ducts is the result of an immune aggression that leads to apoptosis in BECs (3-5).

While the deep mechanisms determining apoptosis in these cells remain unknown, factors such as Fas/FasL, TRAIL (TNF-related apoptosis-inducing ligand), and...
Immune response in PBC

While molecular mechanisms determining BEC apoptosis are not clearly understood, it is accepted that autoimmune response is pivotal in the pathogenesis of lesions found in PBC. A study of immunity in patients with PBC reveals changes both in humoral and cell-mediated immune responses, some of them most likely related to small bile duct damage. Major changes include the presence of anti-mitochondrial antibodies (AMA) in the blood, an aberrant expression of HLA-II molecules and 

Humoral immunity

A most typical change in PBC is high AMA titers in more than 95% of patients (22), and both anti-nuclear (30%) and anti-centromere (<10%) antibodies may also be found. Anti-nuclear antibodies are found particularly in the absence of AMA, and anti-centromere antibodies are detected when PBC is associated with self-limited scleroderm.a. AMA may appear before any other manifestation occurs, and this fact predicts that the disease will eventually manifest clinically (23-25). Only a minority of patients (5%) have undetectable AMA (26); however, these patients have clinical, histological, and cell-mediated immune changes that are identical to those of the remaining 95% (27). AMA are antibodies that recognize the o xo-acid dehydrogenase complex (OADC), including the piruvate dehydrogenase complex (PDC), 2-oxoglutarate dehydrogenase complex (OGDC), branched-chain o xo-acid dehydrogenase complex (BCOADC), and sub-units E1a, Elb, and E3 of PDC binding protein or protein X (28-30). All these complexes are located in the inner mitochondrial membrane and play a role in keto-acid oxidative decarboxylation and amino-acid catabolism. The main epitope in all these enzymatic complexes, be it for AMA, CD4+ cells and CD8+ cells, resides within their lipoil domains (31,32).

AMA likely have no pathogenetic role. A number of facts could hardly be explained should AMA be responsible for this condition. For example: a) these antibodies persist after liver transplant when no lesions are present in grafts; b) there is no relationship between AMA titers and liver damage severity; c) some patients with PBC have no AMA and exhibit lesions identical to those seen in the presence of AMA; d) AMA formation may be induced in animal models by injecting PDC-E2, and this is associated with no biochemical changes or PBC-related liver lesions (33); and e) while 2-OADC is ubiquitous and may be seen throughout the body, inflammatory response is confined to BECs and salivary epithelial cells (SECs) (27,34).

The fact that the autoimmune response specifically targets BECs and SECs suggests that these cells must have some peculiarities. One of these may be that these cells, in contrast to others, aberrantly express PDC-E2 on its apical surface (35-37). This is an early phenomenon during PBC, one that develops much earlier than class-II HLA molecules (38). The reason why PDC-E2 is expressed on cell surfaces is not clearly understood. This occurs despite these cells lacking PDC-E2 messenger RNA (mRNA) (39). Hence these proteins must come from the extracellular environment, including the bile (40), or other phagocytized cells (41,42). Others have mentioned that PDC-E2 is released by mitochondria into the cytoplasm during apoptosis, but self-reactive epitopes are present on the surface of still intact cells (43). BECs have been seen to phagocitize apoptotic cells (44). Their presence as a consequence of apoptotic cell phagocytosis is of particular interest since in the course of this process cell molecules, including PDC-E2 components (45), experience changes leading to neoantigenic peptide formation (46-49). Despite the changes that PDC-E2 may undergo during apoptosis, epitopes that are recognized by AMA and self-reactive T and B cells remain in apoptotic BECs (44,50). This is because PDC-E2 experiences no lipoil domain glutathiolation in these cells, thus escapes degradation, and can then be expressed on the cell’s surface. In cells other than BECs and SECs these proteins are catabolized in a different manner –PDC-E2 undergoes glutathiolation, experiences degradation by apoptosis-related enzymes, and disappears as an autoantigen.

Apical staining, as seen in BECs from patients with PBC, results from complex formation between IgA AMA and PDC-E2, which suggests that IgA may play a role in
the destruction of small bile ducts. Regarding this, a PDC-E2-specific monoclonal IgA has been seen to enter BECs via the polymeric immunoglobulin receptor (pIgR); there it binds PDC-E2’s catalytic component (51), forms complexes with it, and inactivates it (52-55). In this way AMA may induce mitochondrial dysfunction and apoptosis (56). The fact that BEC incubation with serum from patients with PBC results in caspase activation and cell apoptosis advocates for this notion (57). BEC-bound AMA probably have a dual origin. On the one hand they may originate in the bile; on the other, they may have been synthesized by B cells within portal spaces. In fact, the bile of patients with PBC contains IgG and IgA with anti-PDC-E2 activity (58), and 10% of B cells present in portal spaces release PDC-reactive antibodies (59).

Against the pathogenetic role of PDC-E2 in the BECs of patients with PBC stands the fact that transgenic mice expressing PDC-E2 on the surface of BECs have no liver or biliary lesions (60). This suggests that developing lesions require, in addition to an aberrant expression of PDC-E2 on the cell’s surface, some molecular change in this complex, whether caused by xenobiotics, microorganisms (61,62), or apoptosis. During the latter some autoantigens, usually tolerated by the body, experience small molecular changes that render them intolerable to the immune system, which responds with aggression against cells harboring them.

Cell-mediated immunity

While the role of humoral immunity in the pathogenesis of PBC lesions remains unclear, evidence suggests that T cells play a key role in BEC and SEC death, and in bile and salivary duct destruction (3,10,20,63). Since early during the disease portal spaces of patients with PBC are infiltrated with B, T and plasma cells (20,64), and involved bile duct walls are infiltrated with intraepithelial CD8+ CD4+, particularly CD4+CD28- (65,66), lymphocytes reactive to PDC-E2 (3,19,20,31,64,67) and native human antigen (68,69). While activated CD4+ cells (CD45RO) specifically recognize peptide PDC-E2 (20,70,71), CD8+ cells in these patients recognize the PDC-E2 (51,167) epitope (3,63). As can be seen both cell types are reactive to the same amino-acid sequences or to nearby sequences in the lipoi domain.

CD4+CD28+ T cells are believed to potentially play a role in the pathogenesis of autoimmune diseases such as PBC (65). These cells are highly increased in these conditions, express high amounts of IFNγ (72,73), are self-reactive and cytolytic, and survive many years because of their excessively expressing Bcl-2 and resistance to apoptosis (74).

Both the adaptive and innate autoimmune responses are regulated by two distinct CD4+ T cell types – Th1 and Th2. Th1 cells produce proinflammatory cytokines (IL2, IFNγ, TNFα), help cytotoxic CD8+ cells, regulate cell-mediated immune response, and activate NK cells. Th2 cells produce anti-inflammatory cytokines (IL4, IL5, IL6, IL10 e IL13), influence humoral response, and impact the differentiation and activation of B cells into plasma cells. In PBC immune response is Th1 in type (75-78). Indeed, monociliated cells near small bile ducts express IFNγ mRNA, which correlates to portal inflammatory activity (40). In addition, peripheral blood mononuclear cells (PBMCs) in patients with PBC preferentially synthesize Th1 cytokines (79,80).

Inflammatory cell subpopulations infiltrating tissues mostly depend on chemokines released by altered cells, since these cytokines attract lymphocytes fitted with their receptors. The plasma and portal spaces of patients with PBC have revealed high protein 10 (CXCL10), monokine MIG (CXCL9), and fractalkine (CX3CL1) levels. While the former two are synthesized by macrophages exposed to IFNγ, the latter is produced by BECs in response to IFNγ and TNFα (81,82). The CX3CL1 receptor may be found in CD4+ and CD8+ lymphocytes, CXCR3 and CCR5 receptors are preferentially expressed by Th1 cells (83,84), and CCR3 and CCR4 receptors by Th2 cells (85). Early during PBC mononuclear cells in portal infiltrates and around bile ducts have CXCR3 and CX3CL1 receptors (85), which accounts for the Th1 CD4+ cells present in this disease. Also osteopontin, a molecule that contributes to mononuclear cell recruitment and granuloma formation, abounds in portal spaces of patients with PBC (86).

Portalspaces and spaces between epithelial cells also contain CD8+ lymphocytes that play a role in the degeneration and death of BECs with aberrant expression of PDC-E2 and class-I and -II HLA molecules (3,87-91). Other cells also found in the biliary epithelium of interlobular ducts include CD20+ (B) cells, which advocates for a role of humoral immunity in the pathogenesis of PBC (92). This should not be surprising when consideration is given to the inter-relation between B and T cells, regarding the fact that the latter favor the former’s proliferation (93).

CD4+CD25 regulatory T cells (Treg) play a key role in the prevention of T-cell-mediated autoimmune conditions (94,95), and their numbers and function decrease following the loss of immune tolerance and immune aggression. The molecule Foxp3 (Forhead P3) is a Treg function marker (95-98). Its detection in biopsies from patients with PBC shows, in contrast to other liver conditions, that they are very scarce in portal spaces (99). Experimental evidence suggests that this reduction of Treg cells plays a role in the pathogenesis of PBC. Function of these cells may be delayed in mice by blocking TGFβRII receptors, which are specific of Treg lymphocytes. Mice with this blockade develop portal infiltration with CD4+ and CD8+ cells, bile duct destruction, high AMA titers, and elevated IFNγ and TNFα levels (100-102). This laboratory model also demonstrates the importance of the...
TGFβ pathway for the prevention of autoimmune response. This growth factor conditions the suppressive capacity of T<sub>reg</sub> cells as it is required for Foxp3 synthesis in these cells (103). The cause of this T<sub>reg</sub> decrease is unknown but may be genetic in origin, as it is also found in first-degree female relatives. Patients with PBC have been seen to exhibit a low expression of the AE2 anionic exchanger gene (104,105) – AE2 plays a role in intracellular pH regulation (106) through bicarbonate secretion. When this exchanger’s activity is low cells – including lymphocytes – become alkaline, and their activity decreases (107). AE2-deficient mice develop lesions similar to those of primary biliary cirrhosis – PDC-E2-specific AMA appear, the CD8<sup>+</sup> population expands, and T<sub>reg</sub> numbers decline (108). A genetic defect involving AE2 may modify the immune response and result in the development of PBC-related lesions.

**Innate immunity**

Innate immunity represents a first-line defensive system that becomes immediately activated against microorganisms. Its cellular component – macrophages, dendritic cells (DCs), antigen-presenting cells (APCs), NK cells – determines adaptive immune response quantity and quality, including T- and B-cell response and antibody production (109-111). These cells play a dual role – on the one hand they stimulate B- and T-cell response against infection, on the other hand they establish this cell tolerance to autoantigens, and hence prevent autoimmunity (112). In patients with PBC, this type of immunity is activated, reflected by the fact that exposure of these patients’ PBMCs to CpG results in the production of AMA and various cytokines (IL-1β, IL6, IL8, TNFα) (113). TLRs (Toll-like receptors) play a role in innate immunity activation (114). These receptors are activated by some bacterial products, most particularly lipopolysaccharides (LPSs) and non-methylated oligonucleotides (CpG). TLR4 receptors, which are amply distributed in all tissues (115), start an innate immune response against gram-negative bacteria (116). In contrast to other liver conditions, in PBC, these receptors are vastly increased in the biliary epithelium and periportal hepatocytes, and, in them, expression extent correlates to disease stage (117). B-cell activation occurs via these receptors (118), hence increased IgM levels commonly found in patients with PBC are likely a memory B-cell response to their stimulus by bacterial substances. Exposure to these products may itself determine an increase in these receptors in PBC (119). Indeed, BEC stimulation with LPS increases TNFα production (120), which together with other proinflammatory cytokines (IFNγ) increases TLR4 expression on the cell surface (121,122). Other TLRs that may play a role in the pathogenesis of PBC include TLR9. These receptors have been thought to be activated in PBC as also occurs in various rheumatologic conditions (123,124).

PBMC (125) or memory CD27<sup>+</sup> B-cell (126,127) stimulation with CpG – a TLR9 ligand – in patients with PBC increases TLR8 and TLR9 expression in these cells, CD86 expression (monocytes, activated B cells), and IgM and AMA production.

**ENVIRONMENTAL FACTORS**

PBC risk factors include urinary tract infection, smoking, use of reproductive hormones, nail enamel (128), and living near a garbage dump (129). These environmental factors may initiate the autoimmune mechanisms that destroy small bile ducts (Fig. 1).

Numerous data suggest that some organisms or their products may play a role in the pathogenesis of PBC (130,131). Indeed, bacterial infection is common in these patients (132), and bacterial products have been consistently found in the liver tissue of patients with PBC (133,134). The mandatory passage of portal venous blood through the liver renders this organ a preferential target for bacterial products entering the body via this
route. On passing through the liver micro-organisms or their products (135,136) would bind TLRs, activate innate immunity, and then activate adaptive immunity after the exposure of immune cells to bacterial peptides related to those in mitochondrial complexes (137).

Micro-organisms may play a role in the pathogenesis of PBC through several mechanisms. We saw that their entry into the body starts innate immunity via TLRs. In addition, similarities between some bacterial and mitochondrial antigens (molecular mimetism) may determine loss of tolerance to one’s own mitochondrial antigens (137-142). Peptide sequences in bacterial antigens that are similar to peptide sequences in mitochondrial products may be recognized by T-cell receptors and induce the latter’s response to self antigens (143). It is possible that microbial infection disappears without a trace other than the presence of memory T cells capable of recognizing microbial antigens and mitochondrial self antigens.

Micro-organisms involved in the pathogenesis of PBC include *Escherichia coli*, some mycobacteria, *Novosphingobium aromaticivorans*, *Chlamydia pneumonia*, and *Lactobacillus delbruecki*, among others — *Propioibacterium acnes* (134), *Lactobacillus delbrueckii* (144), *Azoto bacter vinelandii*, *Pseudomonas putida*, *Helicobacter pylori*, *Streptococcus intermedius* (145), and retroviruses —.

The involvement of *Escherichia coli* is based on many suspect observations. For instance, this bacterium has proteins similar to PDC-E2 (146); the serum and AMA of patients with PBC react with *E. coli* sequences that closely resemble epitope PDC-E2 212-226 (141,146); feces from these patients contain R forms of *E. coli* that specifically react with AMA (146). Regarding the potential pathogenic role of mycobacteria, AMA frequently develop during tuberculosis (147); lesions similar to those of PBC can be induced in mice through immunization with mycoplasma organisms (148), and cross reactivity has been found between protein HSP65 of *Mycobacterium gordonae* and epitope E2 212-226 in PDC (149,150). Despite such observations the pathogenetic role of mycobacteria cannot be accepted yet because some of these observations could not be universally confirmed, and no such bacteria have been cultured in liver tissue thus far. The same goes for *Chlamydia pneumoniae* despite the fact that some investigators have reported the presence of this organism’s messenger RNA (mRNA) and antigens in liver tissue (151). Others confirmed the presence of antibodies against *C. pneumoniae* in the serum of patients with PBC but failed to identify this organism’s mRNA or antigens (152). While some consider cross reactivity to exist between the serum of patients with PBC and this bacterium, such reactivity is much lower than that seen with *E. coli* or *N. aromaticivorans*, and other authors never revealed it (153). *Novosphingobium aromaticivorans* has aroused special interest. This gram-negative bacterium is widely distributed in the soil and water. It has four lipoic acid domains highly homologous with human autoantigens. It has been seen that 100% of patients with PBC have antibody titers against *N. aromaticivorans* lipoic domains that are up to 1,000 times higher than those found against *E. coli* lipoic domains (154,155), and similar to those seen against PDC-E2 in these patients. This reactivity can be detected in asymptomatic PBC and in early-stage disease. Because of all this, this bacterium may possibly be directly involved in the pathogenesis of PBC. The retroviral etiology of PBC is based on the identification of viral particles and retroviral sequences within BECs in 75% of patients with PBC (156). In addition PDC-E2 is expressed by cholangiocytes following retroviral infection (157). Lastly, some have managed to improve this condition with lamivudine and zidovudine (158). Despite all this, the role of retroviral infection has not been demonstrated (159) as some authors have found no evidence for viral infection (160).

Xenobiotics, including xenobiotic drugs, may play a part in the pathogenesis of PBC given their capacity to modify mitochondrial proteins and induce an autoimmune response against them. B cells and T cells specific for these neoantigens may cross react with original mitochondrial proteins. The fact that AMA from patients with PBC react with new PDC-E2 epitopes generated by replacing lipoic acid in peptide PDC-E2 212-226 with a number of similar synthetic structures does suggest so (161,162). *Octinoic acid*, usually found in perfumes, lipsticks, and food additives, is recognized by the serum from patients with PBC but not by the serum of control subjects or patients with other autoimmune diseases (161), and rabbits immunized with *bromohexanoate* ester-modified bovine serum albumin develop very high AMA titers that can inhibit PDC-E2; despite this, these animals do not develop PBC lesions (163,164). However, autoimmune cholangitis was induced when this was performed in Guinea pigs (165). All these findings support the idea that the exposure of predisposed subjects to selected xenobiotics may modify the immunogenic characteristics of PDC-E2 and induce PBC lesions.

**Genetic factors**

While the ultimate cause of PBC remains unknown, evidence suggests that genetic factors must play a role (166-168). This is pointed out by a 5% frequency of PBC among first-degree relatives of patients (169), which is 50 to 100 times higher than among the general population (170); relative risk among the offspring is 10.5 to 31.0, and it is 59 among daughters, and 10 among siblings (171). Concordance is 63% among homozygous twins (172). Furthermore, of all relatives of patients with PBC 2-13% are AMA carriers (171-175). Last, the disease is particularly common in selected geographical regions (176).

Given its association with other autoimmune conditions whether the HLA system can be related to PBC has been investigated. However, the role of class-I HLA anti-
gens is uncertain (177,178). There is seemingly a closer relationship to class-II HLA molecules since an association of PBC with the DRB1*08 (DRB1*0801) allele has been commonly described among Caucasians (179,180), with DRB1*8 and DRB1*11 among Italians (181), and with DRB1*0803 among the Japanese (182,183). More recently an association was found for the DQA1*0401 allele and DRB1*0803 haplotype with disease progression (184). However, subsequent studies could not demonstrate this (178). On the other hand, DRB1*11 and DRB1*13 seem to have a protective role (180,185). Demonstrating this (178). On the other hand, DRB1*08 (179,180), with an association of PBC with the DRB1*08 (DRB1*0801) allele has only applied to a minority of patients, hence the seeming secondary role. Regarding class-III HLA antigens results are also inconclusive. This region contains the gene coding for TNFα, but its relation to PBC is uncertain (186).

Other genetic factors that were studied to account for the high familial risk of having PBC include polymorphic varieties of immunomodulating molecules — chemokines, IL-1 (187), IL10 (188), vitamin D receptor (189), CTLA-4 (190,191), a T-cell activation inhibitor (192), vasoactive compound-producing enzymes, proteins involved in bile acid transportation and secretion (193), CYP2E1 (194), and IL-2α receptor (195). With the exception of vitamin D receptor polymorphisms, no clear association has been found between PBC and a specific gene to this day (166,178,196).

The latter does not by itself suffice to this end. This demands the presence of environmental factors to establish a loss of immune tolerance to autoantigens.

A genetic characteristic of PBC that cannot be ignored is that this disease affects almost exclusively women. The study of chromosome X has revealed that monosomy X is present in 5% of women with this disease, which is significantly higher than the frequency of this anomaly among age-matched women, namely 2% (197). A lost chromosome X may explain the preference of PBC for women (198). It has been suggested that this chromosome may contain a gene essential for immune regulation. In fact, autoimmune diseases, including PBC, are common in Turner’s syndrome (XO) (199,200). Lastly, microchimerism, that is, fetal cells persisting in the maternal circulation after childbirth, may also account for the predominance of this disease in women. Its role is supported by the finding of fetal DNA in the liver of 42% of women with PBC many years after pregnancy (201). However, this hypothesis has not been corroborated by all (177), and is not believed to play a role in the pathogenesis of PBC (202,203).

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