REVIEW

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Primary biliary cholangitis: pathogenic mechanisms

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Purpose of review

Primary biliary cholangitis (PBC) is characterized by autoimmune damage of intrahepatic bile ducts associated with a loss of tolerance to mitochondrial antigens. PBC etiopathogenesis is intriguing because of different perplexing features, namely: a) although mitochondria are present in all cell types and tissues, the damage is mainly restricted to biliary epithelial cells (BECs); b) despite being an autoimmune disorder, it does not respond to immunosuppressive drugs but rather to ursodeoxycholic acid, a bile salt that induces HCO_3^- rich choleresis; c) the overwhelming female preponderance of the disease remains unexplained. Here we present an etiopathogenic view of PBC which sheds light on these puzzling facts of the disease.

Recent findings

PBC develops in patients with genetic predisposition to autoimmunity in whom epigenetic mechanisms silence the Cl^-/HCO_3^- exchanger AE2 in both cholangiocytes and lymphoid cells. Defective AE2 function can produce BECs damage as a result of decreased biliary HCO_3^- secretion with disruption of the protective alkaline umbrella that normally prevents the penetration of toxic apolar bile salts into cholangiocytes. AE2 dysfunction also causes increased intracellular pH (pHi) in cholangiocytes, leading to the activation of soluble adenylyl cyclase, which sensitizes BECs to bile salt-induced apoptosis. Recently, mitophagy was found to be inhibited by cytosolic alkalization and stimulated by acidification. Accordingly, we propose that AE2 deficiency may disturb mitophagy in BECs, thus, promoting the accumulation of defective mitochondria, oxidative stress and presentation of mitochondrial antigens to the immune cells. As women possess a more acidic endolysosomal milieu than men, mitophagy might be more affected in women in an AE2-defective background. Apart from affecting BECs function, AE2 downregulation in lymphocytes may also contribute to alter immunoregulation facilitating autoreactive T-cell responses.

Summary

PBC can be considered as a disorder of Cl^-/HCO_3^- exchange in individuals with genetic predisposition to autoimmunity.

Keywords

Ae2 KO mice, antimitochondrial antibodies, biliary bicarbonate umbrella, epigenetic mechanisms, female predominance, miR-506, mitophagy, Na^+–independent Cl^-/HCO_3^- anion exchanger 2, pHi disturbance, promoter hypermethylation, soluble adenylyl cyclase

INTRODUCTION

Primary biliary cholangitis (PBC) is an autoimmune disease that affects interlobular bile ducts causing ductopenia and progressive cholestasis. It is characterized by the presence (in 95% of the patients) of antimitochondrial antibodies (AMAs), overwhelming female preponderance (10 : 1) and frequent association with sicca syndrome and other autoimmune diseases, such as Hashimoto's thyroiditis, reumathoid arthritis and scleroderma [1–7]. PBC-specific AMAs are directed against components of the 2-oxo dehydrogenase complexes, principally the inner lipoyl domain in the E2 component of the pyruvate dehydrogenase complex (PDC-E2), but also the branched chain 2-oxo-acid dehydrogenase (BCOADC-E2) and 2-oxoglutarate dehydrogenase complex (OGDC-E2) [8,9]. Additionally, and regardless of AMA status, 30% of patients may

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KEY POINTS

- PBC arises as a result of epigenetic downregulation of AE2 in both cholangiocytes and lymphoid cells in patients with genetic predisposition to autoimmunity.
- AE2 deficiency in cholangiocytes has two main consequences: it reduces biliary bicarbonate secretion, which disrupts the protective alkaline umbrella with subsequent penetration of protonated bile salts into biliary epithelium and it induces an increase of pHi, which favors cell apoptosis via sAC activation.
- It is proposed that pHi elevation in cholangiocytes would impair mitophagy leading to oxidative stress, accumulation of defective mitochondria, enrichment in PDC-E2 and presentation of mitochondrial antigens to the immune system provoking loss of immune tolerance to mitochondrial proteins and production of AMA.

develop different PBC-specific antinuclear autoantibodies presenting multiple nuclear-dot or nuclear-membrane staining patterns [10]. These autoantibodies include the highly specific anti-gp210 and anti-p62 (with 95% specificity for PBC each) as well as anti-sp100, anti-PML and anti-sp140 [5,10].

PBC has variable presentation. Although there are patients who evolve rapidly to advanced forms of liver damage, in most cases, the progression is slow with long lasting asymptomatic evolution in some individuals [1]. However, if the disease is left untreated, the persisting damage of bile ducts leads to increasing cholestasis with jaundice, pruritus and hypercholesterinemia, ending up in biliary cirrhosis requiring liver transplantation [11,12].

Nowadays, growing clinical awareness and widespread use of AMA test in the community allow diagnosis of PBC at an early stage when daily administration of ursodeoxycholic acid (UDCA) is particularly efficient at improving biochemical markers, attenuating bile duct injury and halting the evolution of the disease to advanced cholestasis and liver failure [13–15]. Indeed, PBC patients who respond favorably to UDCA (more than 60%) have a life expectancy similar to that of age and sex-matched normal population [16–18]. With ongoing development of novel therapies, combination regimes are expected to achieve adequate control of the disease in those patients who do not respond to UDCA alone [11,19,20]. In fact, sufficiently powered phase III clinical trials have shown that two drugs, the FXR agonist obeticholic acid and bezafibrate (a PPAR-α agonist that reduces bile salt synthesis and increases biliary phospholipid secretion), provide benefit in combination with UDCA to patients with incomplete response to UDCA monotherapy [11,19].

PATHOLOGIC FINDINGS AND IMMUNOLOGICAL MECHANISMS

The distinctive pathological finding in PBC is the presence of dense portal and periportal inflammatory infiltrates surrounding the small and medium-sized intrahepatic bile ducts. Inflammatory cells permeate the biliary epithelium, which appears disrupted, with irregularities of the biliary lumen. The inflammatory infiltrate is composed mainly of $CD4^+$ and $CD8^+$ T cells, macrophages, B lymphocytes, plasma cells, NK and NKT cells, and variable presence of eosinophils [21]. Infiltrating $CD8^+$ T cells exert a direct cytotoxic attack on bile duct epithelium, whereas $CD4^+$ cells are helper T (Th) cells that act by producing inflammatory cytokines, which stimulate autoreactive cytotoxic $CD8^+$ T cells and autoantibody production by B cells [22,23▪▪]. As a reflection of the loss of tolerance to PDC-E2, $CD4^+$ and $CD8^+$ T cells reacting against this mitochondrial protein are abundantly found in the liver of patients with PBC and, interestingly, both B and T-cell epitopes include the lipoyl domain of E2 [24]. Regulatory T cells (Treg) are reduced in the liver and peripheral blood of patients with PBC [25], a defect which likely facilitates autoimmune responses.

In PBC, B-cell follicles are frequently observed in the portal tract in the vicinity of biliary ducts. These lymphocytic aggregates – which are termed tertiary lymphoid structures (TLSs) when present outside secondary lymphoid organs – can be found in the target tissues of other autoimmune diseases. They are characterized by the presence of high endothelial cell venules and an organized disposition of B and T cells in connection with follicular dendritic cells. TLSs provide a cytokine and chemokine-rich milieu, where B cells, follicular dendritic cells and T cells interact enabling affinity maturation of effector immune cells fostering humoral and cellular autoimmunity [26]. It should be mentioned that early PBC is characterized by IL-12-driven Th1 type of immune response, whereas IL-23-mediated Th17 type of response prevails in later stages promoting fibrosis in advanced disease [27].

Although PBC-specific AMA are not directly pathogenic, immunocomplexes formed by AMA and the cognate antigen can activate local and regional dendritic cells boosting immunity against E2 subunit. In fact, it has been shown that macrophages from PBC patients, but not from normal individuals, produce high amounts of proinflammatory cytokines, including IL-6, TNFα and IL-12, when incubated with apoptotic bodies from BECs and AMA, likely reflecting M1 polarization of the phagocytic cells [23▪▪,28].

Notably, PDC-E2 is upregulated in injured BECs and, because of its lack of glutathiolation, it may remain intact in apoptotic bodies from these cells [29,30,31▪]. The apoptotic blebs of damaged BECs can be engulfed by antigen-presenting cells to stimulate adaptive immunity against this mitochondrial antigen particularly when the individual has a genetic background predisposing to autoimmunity.

The question arises as to what is the basic alteration of BECs that causes cell stress, PDC-E2 enrichment and apoptosis.

BILIARY EPITHELIAL CELL DYSFUNCTION IN PRIMARY BILIARY CHOLANGITIS: AN ESSENTIAL DETERMINANT OF BILIARY EPITHELIAL CELL STRESS LEADING TO IMMUNE-MEDIATED BILE DUCT INJURY

BECs are polarized cells, which play a central role in bile formation by secreting HCO_3^- and water to the ductular lumen, thus, fluidizing and alkalizing canalicular bile [32▪▪,33,34]. Although cholangiocytes represent about 3–5% of total liver cell population, they generate 25–40% of total bile flow in humans, indicating how active are these cells at transporting water and HCO_3^- to bile [32▪▪,33,35].

Ductular secretion of HCO_3^- takes place via the Cl^-/HCO_3^- exchanger AE2 (also known as SLC4A2) located at the apical membrane of BECs [34,36,37▪▪,38▪▪]. Notably, *AE2* mRNA levels are much higher in cholangiocytes than in hepatocyes [39]. During the postprandial period, the gastrointestinal hormone secretin binds the secretin receptor at the basolateral membrane of BECs resulting in increased intracellular cAMP levels with subsequent PKA activation and transfer of cytoplasmic vesicles containing AE2, the Cl^- channel cystic fibrosis transmembrane regulator (CFTR) and the water channel aquaporin 1 (AQP1) to the luminal membrane [40]. This event promotes CFTR-mediated exit of Cl^- to the lumen, generating a Cl^- gradient, which stimulates Cl^-/HCO_3^- exchange via AE2, leading to increased biliary HCO_3^- and water secretion [32▪▪,33,41]. CFTR also mediates ATP release to bile [42]. Luminal ATP stimulates apical P2Y receptors followed by activation of the apical, type III inositol 1,4,5-triphosphate receptor (InsP3R, a Ca^{2+} release channel present at the endoplasmic reticulum) with subsequent rise of intracellular Ca^{2+} levels. Increased intracellular Ca^{2+} enhances luminal Cl^- gradient by activating the apical Ca^{2+}-dependent Cl^- channel TMEM16A, thus, promoting AE2-mediated Cl^-/HCO_3^- exchange and biliary HCO_3^- secretion [42]. Acetylcholine can also stimulate biliary HCO_3^- secretion via activation of basolateral InsP3Rs (types I and II) and elevation of intracellular Ca^{2+} [42].

AE2 deficiency in primary biliary cholangitis

In the early 1990s, it was shown that PBC patients exhibit reduced *AE2* mRNA levels in the liver and in peripheral blood mononuclear cells [43▪▪]. Defective hepatic *AE2* expression in PBC was corroborated by immunohistochemistry studies demonstrating reduced AE2 staining at the apical membrane of BECs of interlobular bile ducts [38▪▪]. Notably, it was found that UDCA therapy improved hepatic AE2 expression in parallel with amelioration of cholestasis [38▪▪,43▪▪]. Consistent with the concept that PBC is associated with deficient HCO_3^- transport to bile, PET imaging revealed that secretin-induced biliary HCO_3^- secretion was impaired in PBC patients and that UDCA therapy corrected this defect [44▪▪]. In-vitro studies using isolated cholangiocytes further confirmed diminished AE2 activity in specimens from individuals with PBC [45].

The notion that AE2 deficiency is central in the pathogenesis of PBC was supported by findings in *Ae2a,b* knockout mice. These animals recapitulate most features observed in PBC patients, including development of AMA, increased IgM levels, disturbed immunoregulation, progressive cholestasis and nonsuppurative cholangitis [46–48]. In fact, all aged *Ae2a,b* knockout mice (15–25 months) exhibit portal mononuclear infiltration with abundant cytotoxic lymphocytes, bile duct damage and ductopenia [47]. Moreover, *Ae2a,b*-deficient mice manifest an altered cholangiocyte gene expression profile consistent with the existence of oxidative stress and enhanced antigen presentation [46].

Upregulation of miR-506 in primary biliary cholangitis cholangiocytes

In the last years, it was found that miR-506 (has-miR-506–3p/5p), a micro RNA that targets both *AE2* and *InsP3R3*, is upregulated in BECs from PBC patients compared with either patients with primary sclerosing cholangitis or healthy controls [49,50]. Noteworthy, transfection of PBC cultured cholangiocytes with antimiR-506 oligonucleotides increases AE2 activity [49], whereas experimental overexpression of miR-506 in cholangiocytes diminishes AE2 expression and activity, causes PDC-E2 overexpression, impairs mitochondrial energy metabolism and promotes oxidative stress, endoplasmic reticulum stress and bile acid induced-apoptosis [31▪]. Moreover, lymphocytes from PBC patients are activated and proliferate when cocultured with miR-506-overexpressing cholangiocytes

[31▪]. Accordingly, in PBC patients, the upregulation of miR-506 in cholangiocytes might impair biliary HCO_3^- secretion while promoting a proinflammatory and immunogenic phenotype in BECs. Of interest, the *MiR-506* gene is located in the X chromosome, a fact that might be related to the female predominance observed in PBC [49]. Of note, proinflammatory cytokines stimulate *MiR-506* promoter activity [31▪], suggesting that inflammatory processes generated by environmental factors might contribute to AE2 downregulation in the biliary epithelium of PBC patients via increased miR-506 expression.

AE2 deficiency increases pHi, enhances the sensitivity to proapoptotic signaling and may potentially affect mitophagy in BECs

In addition to be a crucial mediator of biliary HCO_3^- secretion, AE2 is an acid loader involved in regulating intracellular pH (pHi) [41]. Accordingly, AE2 dysfunction in BECs results in increased pHi [41,48,49]. Notably, it was found that intracellular alkalization activates the HCO_3^- sensor soluble adenylyl cyclase (sAC), which sensitizes BECs to bile salt-induced apoptosis [51,52▪▪].

In addition, deficient AE2 activity might likely impinge on a variety of cell functions, including autophagy and mitophagy, which are processes critically dependent on pHi [53▪▪]. In particular, mitophagy, the specific autophagic elimination of mitochondria, is induced by cytosolic acidification, whereas intracellular alkalization impairs this process, ultimately leading to the accumulation of defective mitochondria, production of reactive oxygen species and activation of cell death pathways [53▪▪,54]. When dysfunctional mitochondria are not properly eliminated, the activation of intracellular innate immunity sensors, such as the NLRP3 inflammasome, stimulates the production of proinflammatory cytokines, such as IL-1β and IL-18 [55▪,56]. Thus, mitophagy represents a critical cell-intrinsic, quality-control and anti-inflammatory mechanism, which is likely disturbed in PBC BECs. Defective mitophagy would make these cells to overexpress mitochondrial antigens and to acquire a proinflammatory and immunogenic phenotype. Importantly, Matheoud *et al.* [57▪▪] showed that the blockade of mitophagy was accompanied by presentation of high levels of mitochondrial antigens on major histocompatibility complex class I, an observation which is clearly relevant to the pathogenesis of PBC.

The potential involvement of dysregulated mitophagy in the pathogenesis of PBC is attractive as it might explain the enrichment of BECs in mitochondrial proteins and their increased presentation to the immune system. Interestingly, it has been recently shown that there are sex differences in the pH of intraphagolysosomal milieu, which is more acidic in women than in men [58▪▪]. Accordingly, it would be expected that defects in acidifiers like AE2 might affect mitophagy to a greater extent in women. Moreover, mitochondrial dynamics have been found to be markedly influenced by sex hormones [59]. PBC is characterized by an overwhelming female preponderance and although autoimmune diseases are in general more common in women than in men, it is tempting to hypothesize that a preferential disturbance of mitophagy in women in a context of defective AE2 function might contribute to explain the strong female bias of the disease.

Defective 'biliary bicarbonate umbrella' in primary biliary cholangitis

As indicated above, in addition to disturbing the intracellular milieu of cholangiocytes, impaired AE2 expression results in reduced alkalization of bile. Ten years ago, Beuers *et al.* [60] proposed that the bile duct epithelium is protected against the toxic effects of bile salts by the presence of a HCO_3^-–rich glycocalyx covering the luminal pole of cholangiocytes (a protective barrier designated as the 'biliary bicarbonate umbrella'). This alkaline protective barrier is generated by the active AE2-mediated secretion of HCO_3^- to bile. Thanks to this alkaline shield, glycine-conjugated bile salts with a pK_a of 4, like glycochenodeoxycholic acid, are maintained in a polar membrane-impermeant form and therefore are not able to enter BECs [60,61▪▪]. When AE2 activity is impaired, glycine-conjugated bile salts remain in an apolar state and penetrate into cholangiocytes promoting oxidative stress, cell senescence and apoptosis [51,62,63▪].

The therapeutic effect of ursodeoxycholic acid

UDCA promotes biliary HCO_3^- secretion and therefore strengthens the defensive alkaline barrier, which protects BECs [61▪▪]. The fact that most PBC patients have a positive response to UDCA while immunosuppressive drugs (including newer biologics used to modify the immune system) do not provide a clear benefit [19,64–68] supports the notion that AE2 deficiency and a defective biliary HCO_3^- umbrella are key determinants of bile duct damage in PBC. The mechanisms by which UDCA increases biliary HCO_3^- secretion involve the activation of PI3K/Akt pathway, which stimulates the release of ATP leading to the extrusion of Cl^- to the

lumen through the Ca^{2+}-dependent Cl^- channel TMEM16A and subsequent Cl^-/HCO_3^- exchange [61▪▪]. In addition, UDCA upregulates *AE2* gene expression when acting in conjunction with glucocorticoids [69]. Moreover, UDCA induces nitric oxide synthase in liver tissue and promotes Mrp2-mediated canalicular secretion of nitric oxide in the form of S-nitrosoglutathione (GSNO), a compound that displays potent antiapoptotic effects on BECs [70]. Additionally, GSNO was shown to enhance UDCA-mediated ATP release by cholangiocytes further boosting biliary bicarbonate secretion [70].

GENETIC, EPIGENETIC AND ENVIRONMENTAL FACTORS

Genetic factors

Abundant evidence has been accumulated supporting the view that PBC occurs as a result of a dysfunction of the biliary epithelium in individuals with genetic predisposition to autoimmunity. Genome-wide association studies [71,72] and dense fine-mapping association analysis [73] have related susceptibility to PBC with variations in genes regulating innate and adaptive immunity, similarly to what occurs in autoimmune diseases responsive to immunosuppressive drugs [11,23▪▪]. However, studies in discordant monozygotic twins showing differences in gene expression profile and DNA methylation [74,75] indicate that besides genetics, epigenetic factors contribute to the development of the disease.

Concerning *AE2* gene variations, conventional genotyping of selected single nucleotide polymorphisms (rs2069443, rs2303933, rs2303937 and rs2303941) in Japanese PBC patients revealed associations with disease susceptibility and/or anticentromere antibody production [76]. In whites, however, no association of *AE2* single nucleotide polymorphisms with PBC susceptibility was found, though two variants were reported to influence AMA status [77], whereas the synonymous variation rs2303932 was associated with reduced PBC progression in French patients receiving UDCA [78].

Environmental and epigenetic factors

Environmental elements may affect the phenotype of patients, particularly via epigenetic changes (DNA methylation patterns, noncoding RNAs like microRNAs and noncoding long RNAs, histone modifications, such as acetylation, methylation, phosphorylation, ubiquitination and sumoylation) leading to disturbed gene expression. So far, a significant number of epigenetic changes have been reported in PBC, which include altered expression of microRNAs (thoroughly reviewed by Rodrigues *et al.* [4]) as well as methylation abnormalities of genes (either hyper or hypomethylation) on the X chromosome and autosomal chromosomes [4,23▪▪,74,79]. When affecting promoter regions, hypermethylation is consistently related to silencing of gene expression, whereas hypermethylation in intragenic regions is usually associated with enhanced expression [80].

Recently, we reported that there is a significant increase in CpG-cytosine methylation affecting selective promoter regions of the *AE2* gene both in liver and PBMCs from PBC individuals [81▪]. Moreover, mean methylation rates inversely correlate with mRNA levels [81▪]. Of note, selective CpG-hypermethylation clusters in PBC affected relevant nuclear factor sites/motifs [81▪,82–84].

Among environmental exposures affecting DNA methylation, smoking has been associated with high risk of PBC [85–87], being also related to increased DNA-methyltransferase expression [88]. Therefore, DNA methylation affecting *AE2* and additional genes may constitute a link between genetic and environmental risk factors in PBC pathogenesis.

THE ROLE OF AE2 DEFICIENCY IN THE DYSREGULATION OF IMMUNE RESPONSE

As mentioned, in PBC, *AE2* mRNA levels are decreased not only in the liver but also in peripheral lymphoid cells [43▪▪]. Thus, altered pHi because of defective AE2 activity may affect the function of immune cells in PBC patients [48,89]. Indeed, we found that *Ae2a,b*-deficient mice exhibit reduced Treg population, progressive expansion of $CD8^+$ T cells and increased production of IL-12 and IFNγ [46,47]. Notably, we observed that $CD4^+$ T cells express several transporters that mediate cytosolic acidification, including AE2, AE1 and Na^+–HCO_3^- cotransporter, whereas the last two are poorly expressed in $CD8^+$ T cells. Thus, CD8 T cells rely heavily on AE2 for intracellular acidification. Accordingly, *AE2*-deficient $CD8^+$ T cells have high pHi and, upon stimulation, become more alkaline, produce more IL-2 and exhibit increased proliferative response [48]. These findings suggest that AE2 deficiency in lymphoid cells might contribute to dysregulated T-cell responses in PBC patients.

PBC is characteristically a disease affecting middle-aged women. Data from *Ae2a,b*-knockout mice illuminate the effect of age in the development of the disease [47]. In young *Ae2a,b*-knockout mice, portal tracts were infiltrated with activated T cells expressing PD1 and producing high amounts of IFNγ (this cytokine induces PD-L1 expression in cholangiocytes

and in other cell types of the portal tract). In young knockout animals, the clonal expansion of autoreactive lymphocytes within the liver was inhibited by PD1/PD-L1 interaction. In contrast, in old *Ae2a,b*-knockout mice, *PD1* gene expression was epigenetically silenced by DNA methylation in $CD8^+$ T cells. This age-related epigenetic downregulation of PD1 allowed autoreactive $CD8^+$ T cells to escape PD1/PD-L1-induced apoptosis, favoring clonal expansion and enhanced lymphocytic infiltration of portal tracts with aggravation of bile duct lesion. Similar epigenetic changes in patients with PBC would explain why this disease is commonly manifested later in life, between 40 and 60 years of age.

CONCLUSION

PBC results from autoimmune attack to the epithelium of interlobular bile ducts. PBC develops in patients with a genetic background predisposing to autoimmunity. The target of the autoimmune reaction is the mitochondrial antigen E2 component of the 2-oxo dehydrogenase complexes, in particular PDC-E2, which is overexpressed by stressed BECs. The immune-mediated cholangiocellular injury appears to be secondary to the failure of BEC to secrete HCO_3^- to bile due to AE2 deficiency. This defect is likely because of epigenetic factors involving miR-506 upregulation and *AE2* promoter methylation. Impaired biliary HCO_3^- secretion disrupts the defensive bicarbonate umbrella allowing the penetration of hydrophobic bile acids into BECs resulting in senescence and apoptosis of these cells. In addition, AE2 deficiency alters cholangiolar pHi leading to sAC activation and increased sensitivity to bile salt-induced apoptosis. Increased pHi likely disturbs mitophagy, with resulting accumulation of faulty mitochondria, and this would trigger

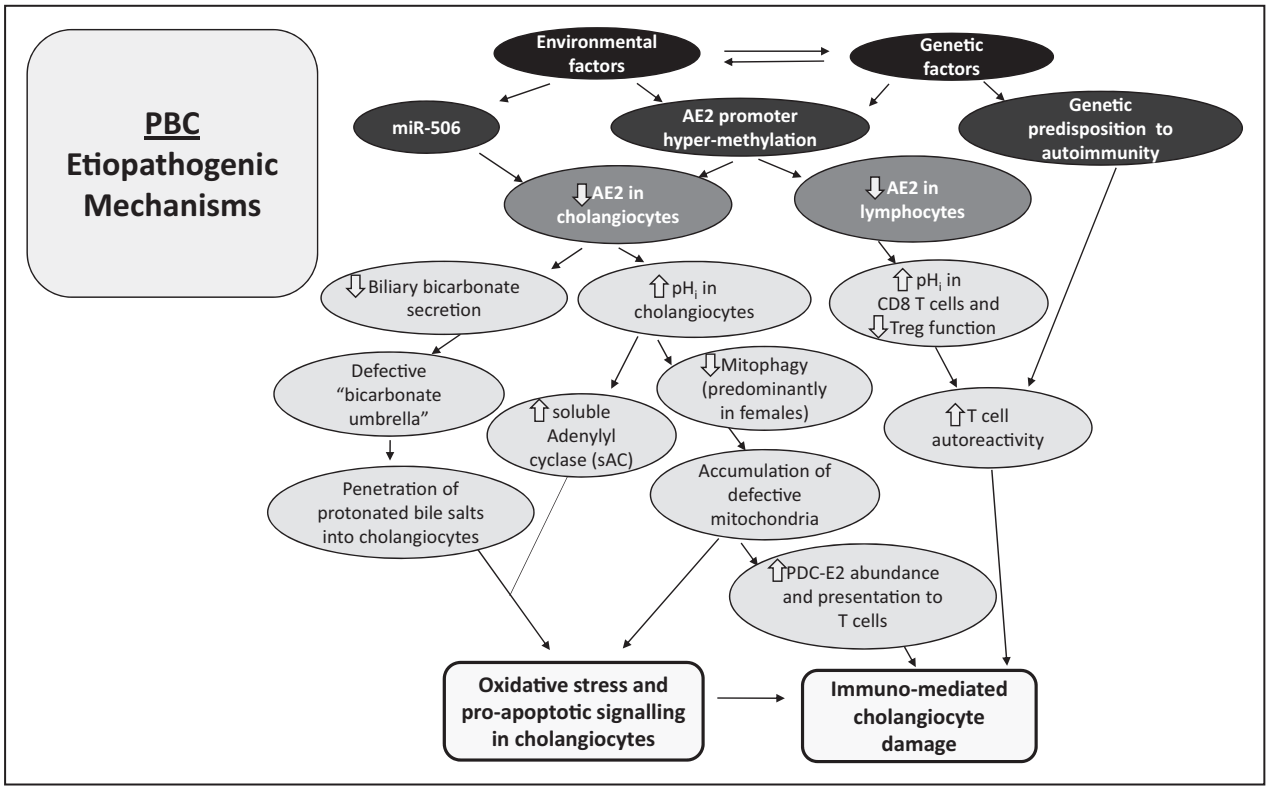

FIGURE 1. PBC: Etiopathogenic mechanisms. Epigenetic, genetic and environmental factors concur in causing PBC. Epigenetic mechanisms (including microRNAs and promoter hypermethylation) induce downregulation of AE2 in both cholangiocytes and lymphocytes. Defective AE2 function in cholangiocytes decreases biliary bicarbonate secretion while increasing intracellular pH (pHi). The disruption of the protective bicarbonate umbrella allows penetration of apolar bile salts into hepatocytes promoting cell apoptosis, which is favored by the activation of soluble adenylyl cyclase (sAC) because of the elevation of pHi. This alteration may also affect mitophagy, especially in women (in whom the endolysosomal milieu is more acidic than in men). Impaired mitophagy would lead to oxidative stress, accumulation of defective mitochondria, PDC-E2 overexpression and presentation of mitochondrial antigens to the immune system. These changes lead to immuno-mediated cell damage specially in individuals with genetic predisposition to autoimmunity. On the other hand, AE2 deficiency in lymphocytes, particularly in CD8 T cells, may contribute to enhance autoreactive T-cell responses.

oxidative stress while promoting the presentation of mitochondrial antigens to the immune system. It could be hypothesized that as women exhibit a more acidic intraendolysosomal milieu than men, AE2 deficiency may disturb mitophagy more intensely in women, thus, contributing to explain the marked predominance of the disease in women. In PBC, AE2 deficiency also affects lymphocytes likely cooperating to the immunological derangement present in this condition. In summary, PBC could be considered as a disease that occurs in patients with genetic predisposition to autoimmunity in which AE2 is epigenetically downregulated both in liver and lymphoid cells. These concepts are illustrated in Fig. 1.

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