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New in the pathogenesis of the primary biliary cholangitis asymptomatic stage

New insights into the pathogenesis of primary biliary cholanschgitis

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Abstract

Primary biliary cholangitis (PBC) is a chronic cholestatic progressive liver disease owned by to cholangiopathies. Damage to cholangiocytes triggers the development of intrahepatic cholestasis, progression of which leads to cirrhosis in the terminal stage of the disease. Accumulated scientific data indicate that damage to biliary epithelial cells (BEC, cholangiocytes) is most likely associated with intracellular accumulation of bile acids, which have a detergent, toxic effect on cell. Mechanisms leading to uncontrolled bile acids intake into BEC in PBC are associated with pH change in bile ducts lumen, which is determined by bicarbonate buffer system ("biliary HCO_3^- umbrella"). The impaired production and entry of bicarbonate (hydrocarbonate, HCO_3^-) from the BEC into the bile duct lumen is due to epigenetic changes in the expression of the X-linked *microRNA 506* (*miR-506*) gene. The growing body of knowledge on molecular mechanisms of cholangiocytes damage development in patients with PBC allows us to propose a hypothesis explaining the pathogenesis of the first morphologic (ductulopenia), immunologic (antimitochondrial autoantibodies, AMA) and clinical (weakness, malaise, rapid fatigue) signs of the disease in the asymptomatic stage. This review focuses on the consideration of these mechanisms.

Key Words: Primary biliary cholangitis (PBC); antimitochondrial autoantibodies (AMA); micro-RNA 506 (miR-506); inositol-1; 4; 5-trisphosphate receptor type 3 (InsP3R3; ITPR3); Cl-/HCO3- anion exchanger 2 (AE2); "biliary HCO3- umbrella"; dihydrolipoyltransacetylase (E2 subunit) pyruvate dehydrogenase (PDG) complex (E2 PDG)

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Core Tip: The review is devoted to the consideration of mechanisms contributing to the damage of E2 subunit of pyruvate dehydrogenase complex (E2 PDG), AMA formation and development of ductulopenia in primary biliary cholangitis already in the asymptomatic stage of the disease. A hypothesis explaining the pathogenesis of the first morphological (ductulopenia), immunologic (AMA) and clinical (weakness, malaise, rapid fatigue) signs of the disease in the asymptomatic stage is proposed.

INTRODUCTION

Introduction

Primary biliary cholangitis (PBC) is a chronic cholestatic progressive liver disease of probably autoimmune genesis, proceeding with destruction, necrosis and apoptosis of the epithelium of predominantly intralobular and septal bile ducts, in the terminal stage of which cirrhosis develops[1-3]. Primary biliary cholangitis is preceded by a long asymptomatic period[1,2]. During this time, there are no physical signs of the disease. Clinically, it may be manifested by fatigue, weakness and malaise. Detection of antimitochondrial autoantibodies (AMA) in serum at a titer of 1:40 or higher during this period serves as a pathognomonic marker for the development of PBC. The fact that AMA are detected many months and even years before the appearance of clinical signs of PBC indicates their primary immunopathogenetic role rather than a secondary phenomenon arising as a consequence of cholestasis[4,5]. At the same time, most

researchers emphasize the absence of correlation of AMA titer with disease activity and duration[4-7]. Disclosure of the causes and mechanisms of AMA formation may contribute to understanding the pathogenesis of the development of clinical, morphologic, biochemical, and immunologic signs of PBC.

Antimitochondrial antibodies are not strictly specific for PBC[4]. They are classified as class M immunoglobulins (IgM), which react with multiple antigens in mitochondria, designated as M1-M9[8]. The highly sensitive and most frequent (>95%) autoantibodies found in PBC are anti-M2 IgM[8]. In patients with the classical course of PBC, antigenic components of AMA have been shown to be related to the dihydrolipoyltransacetylase (E2 subunit) of the pyruvate dehydrogenase (PDG) complex (E2 PDG), which localizes to the inner mitochondrial membrane[8]. Experimental data on immunization of laboratory animals with E2 PDG as a recombinant polypeptide, leads to AMA formation but not to cholangiocyte damage[9]. This indicates that AMAs are not a factor triggering the destruction of biliary epithelial cells (BECs, cholangiocytes).

It is still unclear how the E2 antigen of PDG, being located on the inner membrane of mitochondria, can be a target of immune effector mechanisms. It is extremely important to understand why the E2 PDG antigen of biliary epithelial cells located in small and medium-sized bile ducts is involved in this process[4]. There is no clear answer to these questions up to now. The theory of antigenic mimicry was discussed earlier.

AMA and the theory of antigenic mimicry

The pyruvate dehydrogenase complex in prokaryotes has structural similarity to that of eukaryotes[10]. Antibodies derived from the serum of patients with PBC have been shown to react with yeast and bacterial proteins[11,12]. Therefore, it has been suggested that AMAs in PBC arise due to cross-reactivity to exogenous bacterial antigens (antigenic mimicry)[13,14] and that the disease may have a bacterial origin[15]. But no one has been able to find clear evidence of any infectious agent[4]. In addition, in classical bacterial antigenic exposure, IgM are first produced and rise in the blood. After 3-4 wk, immunoglobulins G (IgG) take their place. IgM in this case can persist in the patient's body up to 3 mo, followed by their decline. But in PBC the level of IgM-related AMA

does not disappear and even does not decrease during the long-term development of the disease, which does not fully explain the disorders of the immune system and does not really fit into the bacterial nature of antigens triggering the production of AMA. Although, with prolonged exposure to thymus-independent antigens, IgM synthesis can become stable[16]. But this requires the continuous presence of thymus-independent antigen in the patient's body and decreased immune tolerance to it. The probability that bacterial antigen is constantly present in the organism of patients with PBC and triggers the production of AMA is small.[10].

It is more logical to assume that it is an antigen of own tissues of the human body, namely the epithelium of the biliary tract. But then for the production of AMA in patients with PBC it is necessary that E2 PDG should become an immunomodified antigen, leave the mitochondria, cholangiocyte and meet with immunocompetent cells, which will start the production of autoantibodies. To date, the triggers and mechanisms that initiate these processes in cholangiocytes remain unknown.

In the last decade scientific data on the importance of bicarbonate (hydrocarbonate, HCO_3^-) as a "protective umbrella" for cholangiocytes from the toxic effect of bile acids have appeared. It was shown that in PBC the production of hydrocarbonate decreases, which leads to increased bile acids intake into cholangiocyte (the theory of defective "biliary HCO_3^- umbrella"). These data allowed us to suggest that the gradual accumulation of bile acids in BECs may serve as a trigger mechanism for AMA formation, development of ductulopenia and one of the early clinical signs - weakness in the asymptomatic stage of PBC.

Aggression and defense factors of cholangiocytes.

It is well known that bile is an aggressive medium for cholangiocytes lining intra- and extrahepatic bile ducts. The presence of bile acids in bile, which have powerful detergent properties, can cause damage to the cell membranes of cholangiocytes. Hydrophobic bile acids are known to exhibit cytotoxicity to many cell types[17]. However, biliary epithelial cells under physiological conditions are exposed to very high (millimolar) concentrations of hydrophobic bile acids without signs of

cytotoxicity[18]. This resistance implies the presence of mechanisms protecting cholangiocytes from the toxic effect of bile acids.

Conjugation of bile acids and formation of mixed micelles with cholesterol and phospholipids are considered as defense mechanisms already at the level of hepatocytes, bile capillaries and Hering's canals[18]. Known defense factors that enter the bile during its passage through the bile ducts include the production and secretion of mucin and bicarbonate[19]. Under physiological conditions, the main function of cholangiocytes is biliary secretion of bicarbonate[20]. It is well known that HCO_3^- is produced by cholangiocytes all throughout the biliary tree. Mucin glycoprotein production is carried out by peribiliary glands (PBGs, bile duct glands)[21]. PBGs are located in the wall of large intra- and extrahepatic bile ducts and are directly connected with their lumen. Experimental data indicate that the glycocalyx covering the apical surface of large cholangiocyte membranes with glycosylated mucins and other glycan-containing membrane glycoproteins stabilizes the "biliary HCO_3^- umbrella", thus helping to protect human large cholangiocytes from bile acid toxicity[22]. The mucin produced by PBGs, protects cholangiocytes of only large bile ducts[19]. On this basis, cholangiocytes of large intra- and extrahepatic bile ducts have dual protection: mucin produced by PBGs and bicarbonate. Intralobular, interlobular, and septal bile ducts do not contain peribiliary glands, which is accompanied by the absence of mucin in them[21]. As a result, only bicarbonate serves as a factor of BECs defense at the level of intra-, interlobular and septal ducts.

Under physiological conditions, there is a balance between the factors of aggression (bile acids) and defense (bicarbonate and/or mucin secretion).

Cholangiocyte defense mechanisms

Cholangiocytes are polarized epithelial cells that line the intra- and extrahepatic bile ducts and are responsible for regulating bile volume, modifying bile, and maintaining bile pH (alkalinity)[23,24]. Cholangiocytes play an important role in modifying the composition of primary bile by secreting water, chlorine (Cl^-), and HCO_3^-[25], and by absorbing bile acid salts, amino acids, and glucose. Small and large cholangiocytes are

distinguished depending on their size and location in small and large bile ducts[26]. They are differently involved in the processes of secretion and absorption[27]. The secretion of HCO_3^- with human bile accounts for 25%-40% of the total volume of secreted bile and maintains physiologic pH in the lumen of bile ducts[17,28,29].

In the process of bile formation, predominantly conjugated bile acids and a minimal amount of unconjugated bile acids enter the bile capillary. Under physiological conditions, both conjugated and unconjugated bile acids are secreted into bile by hepatocytes in anionic (deprotonated, ionized, having a negative charge) form[30]. Bicarbonate, secreted by cholangiocytes into the lumen of the bile duct, due to its buffering properties creates a slightly alkaline pH of hepatic bile. This keeps bile acids in a deprotonated state. The ionized form of bile acids does not allow them to penetrate into BECs, due to the presence of negatively charged molecules of hydrocarbonate on the apical surface of the cytoplasmic membrane of cholangiocytes[30]. Thus, the secretion of hydrocarbonate ions protects cholangiocytes from uncontrolled transmembrane bile acid enter, which has been called "biliary HCO_3^- umbrella". The latter provides preservation of cholangiocytes and normal bile flow along the biliary tree.

The main regulators of bicarbonate production and secretion by cholangiocytes.

The pH fluctuation in bile ducts depends on the rate of bicarbonate production by cholangiocytes. The signaling pathways regulating HCO_3^- secretion differ in large and small cholangiocytes[31]. In small cholangiocytes, activation of bicarbonate secretion is due to biliary ATP secreted from the overlying hepatocytes of Hering's canals (Figure 1). Cholangiocytes express apical membrane proteins of the purinergic receptor (P2YR) family, which are stimulated by adenosine triphosphate (ATP)[32]. Luminal ATP binds to P2YR, stimulating intracellular Ca^{2+} ion release *via* inositol-1,4,5-trisphosphate receptor type 3 ($InsP_3R3$, ITPR3)[33]. In cholangiocytes, $InsP_3R3$ is the major receptor isoform that localizes in the apical region[31,34] and is involved in $InsP_3R3$-mediated cell signaling and Ca^{2+} secretion[35]. $InsP_3R3$ are the only receptors that promote the opening of intracellular calcium channels and the release of calcium ions[34]. Calcium is one of the messengers in the cholangiocyte that modulates and regulates diverse cellular

functions such as ion channel activation, secretion, cell proliferation, apoptosis, *etc.*[34,36]. Ca^{2+} release from subapical stores in the endoplasmic reticulum triggers and locally activates transmembrane 16A chloride (Cl^-) channels (TMEM16A) on the apical membrane of cholangiocytes (Figure 1)[36-38]. The appearing Cl^- concentration gradient on the apical membrane activates chloride/carbonate (Cl^-/HCO_3^-) anion exchanger 2 (AE2, also Slc4A2), which leads to the secretion of HCO_3^- into the lumen of the bile duct. In large cholangiocytes, except to the Ca^{2+}-dependent pathway of bicarbonate secretion, there is an additional mechanism functioning with the participation of the hormones secretin and somatostatin[39] (Figure 1). Secretin is produced by S-cells of the duodenal mucosa and stimulates the production of bicarbonate not only by the intestinal mucosa itself, but also by cholangiocytes and epithelial cells of pancreatic ducts[39]. Secretin regulates secretion of HCO_3^- and Cl^- into bile by large cholangiocytes through interaction with secretin receptors (SRs) located on the basolateral membrane of BECs[39-42] (Figure 1).

Figure 1. Schematic of bicarbonate secretion by small and large cholangiocytes[31].
Note: AE2 – chloride/carbonate (Cl^-/HCO_3^-) anion exchanger 2 (also Slc4A2); TMEM16A – transmembrane 16A chloride (Cl^-) channels; ATP – adenosine triphosphate; P2YR – purinergic receptor family; $InsP_3$ – inositol-1,4,5-trisphosphate; $InsP_3R3$ – inositol-1,4,5-trisphosphate receptor type 3; ER - endoplasmic reticulum; SR – secretin receptor; cAMP – cyclic AMP; Ach – acetylcholine; CFTR – cystic fibrosis transmembrane conductance regulator; M3R – muscarinic acetylcholine M3 *receptor*
As a result of this interaction, the formation of cyclic AMP (cAMP) is stimulated *via* G-protein. cAMP through adenylate cyclase (AC) activates the cystic fibrosis transmembrane conductance regulator (CFTR), causing the secretion of Cl^- ions into the bile duct[28]. The appearing Cl^- concentration gradient at the apical membrane of the cholangiocyte activates Cl^-/HCO_3^- anion exchanger 2, which leads to the secretion of HCO_3^- into the bile duct lumen, in return for the intracellular entry of Cl^- ions into the cholangiocyte[20,24,26,43]. In parallel, ATP from the cholangiocyte enters the bile duct

lumen by exocytosis, which stimulates the secretion of HCO_3^- by a Ca^{2+}-dependent mechanism[44].

Receptors to secretin and CFTR are not found in small cholangiocytes. Therefore, secretin is unable to stimulate in small cholangiocytes the secretion and entry of HCO_3^- and Cl^- into bile[24]. However, a Ca^{2+}-dependent mechanism of bicarbonate secretion is present in both small and large cholangiocyte types (Figure 1)[20,24].

Somatostatin, binding to the somatostatin receptor (SSTR), counteracts the stimulating effect of secretin, inhibits fluid secretion, and retard the production and entry of HCO_3^- from cholangiocytes into the lumen of the bile duct[45].

Mechanisms of bile acids protonation-deprotonation and their entry into cholangiocytes.

Uncontrolled, carrier-independent, passive diffusion of unconjugated primary bile acids into BECs is determined by their polarity and degree of protonation[18,46,47]. Protonation of bile acids is an exponential function of pH. When the pH of hepatic bile acidifies, bile acids may undergo protonation. The degree of protonation of bile acids depends on both their dissociation constant (pKa) and the pH of the bile. The pKa values for unconjugated primary bile acids are 5-6[48-50]. Conjugation of primary bile acids with amino acids reduces pKa values to 4-5 for conjugates with glycine and 1-2 for conjugates with taurine, which improves their solubility in water and reduces their lipophilicity[48-50]. The low pKa values of taurine conjugates of primary bile acids indicate that they are stronger acids than glycine conjugates. Therefore, taurine-conjugated bile acids will be in a dissociated (deprotonated) form even at acidic bile pH values. While glycine conjugates, with higher pKa values, are weak acids and at the slightest acidification of bile will quickly change to the protonated state [50].

Ionized (deprotonated, having a negative charge) bile acids are unable to overcome the "biliary HCO_3^- umbrella" on the outer hemileaflet of the apical cytoplasmic membrane of cholangiocytes[18,47]. In normal, a small amount of unconjugated protonated primary bile acids, enters the cholangiocyte. The neutral intracellular pH promotes the transport of unconjugated protonated primary bile acids further into the peribiliary vascular

plexus with subsequent return to hepatocytes and re-release into biliary capillaries[51]. Such a biliary-hepatic shunt, aims to prevent the accumulation of toxic bile acids with strong detergent properties in cholangiocytes[18,47].

Conjugated bile acids can be transported through apical and basolateral membranes of cholangiocytes with the help of specific transporters[26,52-56].

Conjugates of bile acids with glycine in human hepatic bile account for ¾ of all conjugated bile acids and have a pKa close to 4[57]. At physiological pH 7.4, glycine conjugates of primary bile acids, being relatively weak acids, will be partially protonated (become nonpolar), which promotes their penetration in micromolar amounts into cholangiocytes. Small shifts of local pH to acidic region in biliary ducts will lead to increase of protonated glycine conjugates of primary bile acids. A significant increase in the ratio of protonated:deprotonated glycine conjugated bile acids will lead to increased entry their into cholangiocytes.

Bile acid conjugates with taurine in hepatic bile account for ¼ of all conjugated bile acids. They are stronger acids and have a pKa of 1-2[57,58]. Therefore, changes in biliary pH will have little effect on their protonation. Most of the taurine conjugates of bile acids will be in an anionic form and will not be able to enter cholangiocytes. Because of this, taurine conjugates of primary bile acids have less toxicity to cholangiocytes.

Active functioning of bile acid transporters in the basolateral membrane of the cholangiocyte, as a rule, leads to rapid removal of hydrophobic bile acids from the intracellular space and their back delivery into hepatocytes[59]. Therefore, the accumulation of toxic bile acids with detergent properties in cholangiocyte does not occur in norm.

The theory of defective "biliary HCO_3^- umbrella" in PBC.

Studies of the last decade show that in PBC there is a decrease in the protective role of bicarbonate for cholangiocytes. The theory of defective "biliary HCO_3^- umbrella" is actively discussed[1,17,22]. This theory is based on a number of clinical and experimental works showing insufficient HCO_3^- supply to the bile ducts in PBC, which leads to a shift of pH of intraductal (hepatic) bile to the slightly acidic region and an increase of pH

inside the cholangiocyte to the slightly alkaline region. The reasons for the insufficient production of HCO_3^- by cholangiocytes remain unknown to date. The involvement of $InsP_3R3$ and AE2 in this process is discussed. It has been shown that in liver biopsy specimens and blood mononuclear cells of patients with PBC, expression of *InsP3R3* and *AE2* gene is reduced, indicating their dysfunction and involvement in the pathogenesis of this disease[60,61]. Decreased expression and activity of InsP3R3 and AE2, is associated with increased content of micro-RNA 506 (miR-506) in cholangiocytes[62]. Micro RNAs are small noncoding RNAs 22-23 nucleotides long that inhibit gene expression by full or partial pairing with initial sequences located in the 3'-untranslated regions (3'-UTR) of mRNA[62].

The 3'-UTR region of mRNA of InsP3R-3[62] and the 3'UTR region of mRNA of AE2[63] contain binding sites for miRNA-506. miRNA-506 binding to the 3'-UTR regions of mRNA of InsP3R-3 and AE2 prevents translation of these proteins. By doing so, miR-506 is a regulator of InsP3R3 and AE2 expression (Figure 2). The expression of miR-506 is probably expose to epigenetic regulation and can vary in different individuals as a result of polymorphisms in the NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) signaling pathway[30].

An increase in the amount and activity of miR-506 has been reported in cholangiocytes of patients with PBC[63]. The discovery of increased miR-506 activity in cholangiocytes of patients with PBC, which is an X-linked microRNA, leads to decreased expression and activity of $InsP_3R3$ and AE2, as well as potentially explains the prevalence of this disease in women[30] (Figure 2).

Decreased expression and activity of InsP3R3 in cholangiocytes in PBC[64] impairs intracellular $Ca^{2+}i$ ion secretion and its use as a messenger in signaling to the transmembrane Cl- channel TMEM16A[44]. The impaired Ca^{2+} signaling in cholangiocytes in PBC is evidenced by the absence of ATP stimulation of purinergic receptors (P2YR) on the apical membrane[36]. Decreased calcium-dependent activity of TMEM16A on the apical membrane of cholangiocytes leads to decreased secretion of Cl^- ions into the lumen of bile ducts, which is an accompanied by decreased activity of

chlorine/bicarbonate anion exchanger and impaired secretion of HCO_3^- BECs. In models of cholangiocytes expressing miRNA-506, InsP3R3-mediated reduction in intracellular Ca^{2+} release and decreased fluid and HCO_3^- secretion into bile ducts has been shown[36,44]. Binding of miRNA-506 to the 3'UTR of AE2 mRNA also contributes to decreased chlorine/bicarbonate anion exchanger activity and decreased hydrocarbonate secretion by cholangiocytes (Figure 2). Human cholangiocytes isolated from biopsy specimens of patients with PBC show decreased AE2 activity[65]. Due to this, the homeostasis of intracellular pH (pH*i*) in cholangiocytes and bile duct pH in patients with PBC may undergo changes[30].

Figure 2: Mechanism of *ITPR3* (*InsP$_3$R3*) and AE2 gene expression reduction due to the increase in the amount of miR-506 and its activity.

Note: $InsP_3R3$ (ITPR3) - inositol-1,4,5-trisphosphate receptor type 3; AE2 – Cl^-/HCO_3^- anion exchanger 2; miR-506 – micro-RNA 506; mRNA - matrix RNA;

Changes in intra- and extracellular pH in PBC associated with the loss of $InsP_3R3$ and decreased activity of AE2 promote protonation of bile acids, their entry into cholangiocytes and the development of damage to the latter[44].

Destruction of biliary epithelium of small intrahepatic bile ducts already at the early asymptomatic stage of PBC is most likely related to the imbalance between aggression factors (bile acids) and defense factors ("biliary HCO_3^- umbrella") of cholangiocytes. Since intralobular, interlobular and septal bile ducts, which are damaged in PBC, do not contain peribiliary glands producing mucin glycoproteins (mucin supraepithelial layer)[21], this defense mechanism for small cholangiocytes most likely does not play a pathogenetic role in the development of PBC.

Mechanism of cholangiocyte damage at destabilization of "biliary HCO_3^- umbrella"

Decrease of HCO_3^- supply to bile ducts, due to decrease of $InsP_3R3$ and AE2 activity, will shift pH in bile duct lumen to acidic region [66]. Simultaneously, due to the retention and accumulation of HCO_3^- in the cytosol of cholangiocytes, there will be a gradual alkalinization of intracellular pH*i* in patients with PBC[30,65,67]. Complete AE2 deficiency

will lead to intracellular alkalosis of cholangiocytes[61]. But reduced (rather than absent) expression of InsP3R3 and AE2 genes has been observed in patients with PBC[61].

Shift of pH to slightly acidic region in the lumen of bile ducts will increase the amount of protonated unconjugated and glycine-conjugated primary bile acids. This will lead to increased entry of them into the small cholangiocytes (intra-lobular, inter-lobular, septal) of the bile ducts. Once in the slightly alkaline pH*i* within the cholangiocytes, the protonated bile acids will undergo deprotonation. Alkalinization of pH*i* and ionization of glycine-conjugated and unconjugated primary bile acids within cholangiocytes, reduces the process of their difundation from intracellular to peribiliary space. As a result, there is a delayed and gradual accumulation of glycine-conjugated and unconjugated primary bile acids in small cholangiocytes. The theory of defective "biliary HCO_3^- umbrella" helps to explain the intracellular uncontrolled increased entry and accumulation of bile acids in small biliary epithelial cells.

The presence of mucin-containing glycocalyx layer on the apical surface of large cholangiocytes protects them from penetration and damaging effect of protonated conjugated and unconjugated bile acids.

Intracellular accumulation of hydrophobic bile acids is a prerequisite for their cytotoxic effects[68]. Being strong detergents, they are able to solubilize phospholipids and cholesterol from membrane structures of cholangiocytes, which leads to damage and destruction of cytoplasmic membrane and membranes of cell organelles (Figure 3). Herewith, entry and accumulation of unconjugated bile acids with stronger detergent properties in small cholangiocytes is more toxic for the cell than accumulation of conjugated bile acids.

Figure 3: Solubilization of phospholipids and cholesterol from membrane structures by bile acids.

Chronic damaging effect of bile acids on membrane structures triggers accelerated senescence, necrosis and/or apoptosis of BECs[69]. Bile acids destroy membranes of cell organelles and nuclear membrane in cholangiocytes with release of apoptogenic factors. The barrier function of the biliary epithelium is impaired, resulting in concomitant

damage, inflammation and oxidative stress. Cytokines, chemokines and pro-inflammatory mediators released by the cholangiocyte probably stimulate apoptotic and proliferative responses as well as activate fibrogenesis[70]. Bile acids also mediate their toxic, apoptotic effects through specific signaling pathways at the intracellular level. The intrinsic apoptotic pathway is activated, including mitochondrial translocation of BAX (BCL2-associated X protein), release of cytochrome C from mitochondria, activation of caspase 3, cleavage of PARP (Poly (ADP-ribose) polymerase) and DNA fragmentation[71]. There is evidence that miRNA-506 activates the apoptosis pathway upon stimulation with toxic bile acids[66].

Proinflammatory cytokines additionally increase miR-506 expression[30]. A vicious circle develops that supports senescence, apoptosis and proliferation of cholangiocyte. Ultimately, ductulopenia develops[30]. All this reflects the direct effects of bile acids on cholangiocytes rather than the nonspecific effects resulting from periportal inflammation[31].

From the pathophysiologic point of view, common to all cholangiopathies is the coexistence of cholangiocyte death and proliferation, as well as various degrees of portal inflammation and fibrosis[70]. Cell death induces the activation of inflammatory and profibrogenic pathways that trigger the development and progression of fibrosis, which gradually leads to the small bile duct ductulopenia[72]. The conceptual mechanisms of these processes have been described in reviews[69,72].

Disruption of apoptosis is considered a trigger of PBC and already in the asymptomatic stage leads to the development of small bile duct ductulopenia, one of the early morphologic signs of the disease[73].

Apoptosis depends on mitochondrial permeabilization associated with excessive intracellular accumulation of bile acids[74-76]. In addition, bile acids and incomplete apoptosis of BECs diverted to necrosis can lead to pathogenic effects on intracellular components with subsequent generation of AMA[77].

Mechanism of mitochondrial permeabilization and AMA formation

Solubilization of phospholipids and cholesterol from the outer membrane of mitochondria by bile acids leads to their permeabilization[78]. There is an increase in the permeability of the mitochondrial outer membrane to ions and solutes[71,78]. There is leakage of the contents of the intermembrane space into the cytosol and loss of membrane potential. Mitochondria swell, rupture of their outer membrane and release of apoptogenic factors occurs[78]. The inner mitochondrial membrane, the main target for AMA formation, is opened. Further solubilization by bile acids of phospholipids and cholesterol from inner membrane and destruction of mitochondria can lead to the release and degradation of pyruvate dehydrogenase complex (PDG). The latter includes three enzymes: pyruvate dehydrogenase (E1 PDG), dihydrolipoyltransacetylase (E2 PDG), and dihydrolipoyl dehydrogenase (E3 PDG)[73]. Each of these enzymes, in addition to the protein part, has cofactors: E1 PDG contains thiamine pyrophosphate as a cofactor, E2 PDG contains lipoic acid and coenzyme A, E3 PDG contains FAD and NAD. E1 and E3 PDG are protein complexes that do not contain lipid components. Therefore, they are not likely to be affected by bile acids accumulated in the cholangiocyte since they have an effect on lipid components. Sera from PBC patients do not show serologically detectable reactivity against E1 and E3 components of PDG[79].

E2 PDG is a lipoprotein and has two lipoic acid binding sites[8]. E2 PDGs contain an essential lysine residue in the lipoyl domain to which lipoic acid is covalently attached[8]. The lipoic-lysine bond at position 173 is highly conserved across species and is essential for antigen recognition[80]. AMAs target immunodominant epitopes containing lipoic acid.

The importance of chemical xenobiotics capable of modifying lipoic acid in E2 PDG has been previously shown for the appearance of serologic reactivity of this complex[81,82]. Alteration of the conformational structure of the lipoyl domain of E2 PDG, due to chemical modification of lipoic acid may contribute to the loss of immune tolerance[83,84]. Most likely, such chemical modifiers in PBC are bile acids accumulating in cholangiocytes at loss of protective properties of the "biliary HCO_3^- umbrella". Bile acids, having powerful detergent properties, can interact with lipoic acid of the antigen-

recognized site E2 PDG. The result of such interaction may be immunomodification of E2 PDG complex with acquisition of autoantigenic properties and loss of immune tolerance[66]. This assumption is supported by a number of studies performed at the end of the last century. In these works, it was shown that the main immunogenic region on E2 PDG recognized by sera from patients with PBC is localized in the lipoyl-containing domain[85-87]. The lipoic acid content of E2 PDG, meanwhile, is thought to play a role as a potent adjuvant[88]. The presentation of immunomodified E2 PDG complex to lymphocytes can lead to stimulation of T-cell subpopulation and specific production of AMA[66,88,89].

A defective "biliary HCO_3^- umbrella" triggers a continuous and endless process of accumulation and detergent action of bile acids on small cholangiocytes with the formation of AMA. Since the disruption of bicarbonate entry into the lumen of the bile duct is constant, the production of AMA will be continuous. As a result, an elevated level of IgM (M2) will be constantly maintained in the plasma of PBC patients.

The appearance of AMA in serum is another early immunologic pathognomonic sign of PBC, which appears already in the asymptomatic stage of the disease.

Dysfunction of PDG complex and the first clinical signs of asymptomatic stage of PBC

AMA detection in the asymptomatic stage of the disease is accompanied by the appearance of the first subjective clinical signs: weakness, malaise, fatigue, and decreased performance[90]. Fatigue is the most common symptom of PBC in the asymptomatic and early stage of the disease[91-93]. It has been reported that about 40-80% of patients experience fatigue as a symptom of PBC[94,95]. However, there is no correlation between fatigue and the severity or duration of the disease[95-98].

The mechanism of fatigue development is closely related to gradually progressive energy deficiency[99]. The latter is most likely related to the involvement of the pyruvate dehydrogenase complex in the pathologic process of PBC development. The pyruvate dehydrogenase complex is a very important metabolic enzyme. PDG functions in every cell and is required for the conversion of pyruvate to acetyl-CoA, which is incorporated

into the Krebs cycle and is essential for the body to obtain energy in the form of ATP[73]. As mitochondria in cholangiocytes permeabilize and PDG becomes involved in AMA production, there is a gradual decrease in ATP synthesis. This will lead to the development of local energy deficiency, which in turn will enhance the senescence and apoptosis processes of small BECs initiated by bile acids. A vicious cycle occurs, contributing to the progression of ductulopenia and AMA formation. Autoantibodies in this case are able to react with polypeptides which concerned to E2 PDG in mitochondria of almost any cells. It was shown that antibodies to PBC cross-react with polypeptides in mitochondria of beef heart, presumably related to E2 PDG[100]. ATP production decreases and energy deficiency develops throughout the organism.

The development of energy deficiency is accompanied by an increase in glycogenolysis and a decrease in glycogenogenesis. The data presented by Green *et al* indicate that already at the initial stages of PBC in the liver there is a gradual decrease in glycogen stores associated with increased glycogenolysis and decreased glycogenogenesis[101]. The authors have convincingly demonstrated that glucokinase activity fall significantly (down to zero) in patients with PBC, which indicates a decrease in glycogen formation in the liver[101]. At the same time, hexokinase (performs phosphorylation of hexoses), which is responsible for glycogen synthesis mainly in muscles, significantly increases in patients with PBC during this period compared to healthy individuals[101].

As a result of the developing energy deficiency in the asymptomatic stage of the disease, the first clinical signs of expressed weakness, rapid fatigue, decreased performance, functional status, and quality of life appear in patients with PBC[90,102-104].

CONCLUSION

The growing body of knowledge on the molecular mechanisms of cholangiocyte damage development in patients with PBC allows us to propose a hypothesis explaining the pathogenesis of the first morphologic (ductulopenia), immunologic (AMA) and clinical (weakness, malaise, rapid fatigue) signs of the disease in the asymptomatic stage (Figure 4).

Evidence suggests that in susceptible individuals, the unknown initial trigger causes an X-linked epigenetic change that leads to gene reactivation and increased expression of miR-506 [30]. Triggering increased synthesis and activation of miR-506 Leads to inhibition of InsP3R3 and AE2 translation [105]. As a result, bicarbonate entry into the bile duct lumen is reduced and HCO_3^- accumulation in the cytosol of cholangiocytes occurs [30]. Changes in extra- and intracellular pH alter the protonation (in the lumen of the bile duct) and deprotonation (intracholangiocyte) of bile acids. The uncontrolled entry and accumulation of unconjugated and glycine-conjugated bile acids into the BECs is increased.

The detergent properties of bile acids trigger cell membrane disruption, senescence and apoptosis of cholangiocytes, mitochondrial permeabilization, destruction and immunomodification of E2 PDG followed by AMA formation. Senescence, apoptosis and proliferation of cholangiocytes leads to the gradual development of ductulopenia. Involvement of PDG in the pathological process contributes to insufficient ATP synthesis, development of energy deficiency, and appearance of a nonspecific clinical sign - fatigue. The development of ductulopenia is accompanied by the development of intrahepatic cholestasis.

Figure 4. Mechanism of anti-mitochondrial antibody formation, development of duktulopenia, weakness, fatigue and malaise in the asymptomatic stage of primary biliary cholangitis: hypothesis

Cholangiocytes are the main target at the initial stage of PBC. But as soon as cholestasis develops, hepatocytes are also involved in the pathological process, which leads to their damage[30].

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