Primary Biliary Cholangitis

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Primary biliary cholangitis (PBC) is a chronic inflammatory autoimmune liver disease characterized by inflammation and damage of small bile ducts that frequently progress to liver cirrhosis and predominantly affects females. The key moment in the pathophysiology of the disease is loss of tolerance to PDC-E2, pyruvate subunit of the complex of dehydrogenase enzyme, located in the mitochondrial membrane. Combined genetic, epigenetic and environmental factors trigger the initial damage of the biliary epithelium in PBC, followed by the multilineage immune/inflammatory response to damaged cholangiocytes resulting in development of chronic biliary inflammatory disease.

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1. Introduction

Primary biliary cholangitis (PBC) is a chronic inflammatory autoimmune liver disease characterized by destructive lymphocytic inflammation of intrahepatic small bile ducts, increased serum levels of anti-mitochondrial antibodies (AMA) specific for mitochondrial autoantigens and higher incidence in the female population [1,2]. The key serological finding in PBC is the presence of disease-specific AMA antibodies that can be detected in more than 95% of patients [3]. These autoantibodies are specific to the antigenic determinant E2, located within different subunits of the complex of dehydrogenase enzyme, located in the mitochondrial membrane. These subunits are: 2-oxo-acid (2OADC-E2), pyruvate (PDC-E2), branched-chain 2-oxo-acid (BCOADC-E2) and 2-oxo-glutarate (OGDC-E2) [1]. The immunodominant autoantigen in PBC is PDC-E2. Loss of tolerance to PDC-E2 is accompanied by the development of cholangiocytes damage [1]. Until 2015, the name for this disease in the official nomenclature wasimary biliary cirrhosis (PBC), but the abbreviation remained the same [4].

The mechanisms of the disease onset and the pathogenesis are very complex and imply a loss of tolerance to autoantigens present in cholangiocytes, leading to the inflammation development with consequent damage of bile ducts, the development of cholestasis and liver fibrosis. There are several factors that play a role in PBC development: exposure to certain substances present in the environment, immunogenic predisposition, epigenetic control of the biliary epithelium, congenital and acquired immune response and disorder of bile acid production. Only recently, the combined role of immune mechanisms, disorders of bile salt production, biliary transport function and cholangiocyte apoptosis has been considered in the pathogenesis of PBC [5].

Now, it is considered that, in PBC, combined genetic, epigenetic and environmental factors are necessary for the initial damage of the biliary epithelium, followed by the immune/inflammatory response to damaged cholangiocytes that is responsible for the chronicity of the disease [6].

Although the pathogenesis and etiology of PBC is still unclear, the association of liver fibrosis in PBC and cancerogenesis is quite clear. PBC has been described as the risk factor for hepatocellular carcinoma [7,8], but a case of cholangiocellular carcinoma developed in the PBC patient has not been described and it is in accordance with the previous report that the liver microenvironment in biliary tract cancers is immunosuppressive [9]. Some cases of combined hepatocellular and cholangio cellular carcinoma have been reported [10,11].

2. Genetic and Environmental Factors in PBC

The results of numerous studies indicate the role of genetic factors as a risk factor in PBC development, showing a concordance of 63% in monozygotic twins and a higher incidence rate in certain families with a relative risk of 9.13–10.5 in the relatives in the first line of the relationship to 1.66 in the fifth line of the relationship [12,13]. The association of certain gene variants of HLA, but also non-HLA genes, and a higher risk for PBC development has been described. Products of the genes associated with increased PBC risk play the role in the modulation of PBC pathogenesis, but are also influenced by environmental factors [14]. The strongest association between PBC and *non*-HLA genes has been indicated for: *IL12RB2, STAT4, STAT1, CD80, IL12A, NFKB1, IL7R, TNFSF15, CXCR5, DDX6* and *RF8* genes whose products control the several immune reactions, e.g., antigen presentation, lymphocyte differentiation and immune response to microbes [15,16,17]. It seems that among these genes the most important role in the PBC pathogenesis play the genes whose products control IL-12 signaling and thus activation and differentiation of naive T lymphocytes toward inflammatory Th1 cells, but also by stimulation of IFN-γ production inhibit the Th17 cells development, thus playing bidirectional roles in PBC development [18]. Immunohistochemical studies of the livers obtained by PBC patients indicate the significance of the IL-12 and IL-23 signaling in PBC [19]. Some alleles of *CCL20* are associated with lower PBC risk [12], since CCL20:CCR6 interaction plays the role in differentiation and function of the mucosal lymph tissue, Th17 cells homing, biliary epithelium damage and function of effector CD8+ T cells in portal tracts [20,21,22].

It is considered that several environmental factors could be the triggers for the loss of tolerance to mitochondrial antigens, unleashing thus the key initial step in PBC development. Recurrent urinary tract infections caused by *Escherichia coli* induce the production of specific anti-PDC-E2 antibodies and thus increase the risk for PBC development [23]. It is proposed that similar mechanisms explain the increased risk of PBC developing after infections with other microorganisms such as: *Novosphingobium aromaticivorans*, *Helicobacter pylori*; *Chlamydia pneumoniae*; *Mycobacterium gordonae*; *Epstein-Barr* virus; *Cytomegalovirus* and *Toxoplasma gondii* [24,25]. In animal models of the disease it has been shown that xenobiotics, such as 2-octinoic acid, play a role in the pathogenesis of PBC [26].

3. Immune Dysregulation in PBC

PBC is characterized by multilineage immune dysregulation and a loss of auto-tolerance, resulting in targeted cholangiocyte damage [27,28]. Disease-specific anti-mitochondrial antibodies bind to immunodominant epitopes of PDC-E2 located in the inner mitochondrial membrane. PDC-E2 contains a lipoic acid-lysine bond necessary for this recognition and activation of the immune system [29,30]. Regardless of the fact that this autoantigen is ubiquitous, the targeted damage of cholangiocytes is probably a consequence of aberrant modification of mitochondrial PDC-E2, keeping intact the immunodominant epitope, within the apoptotic bodies of biliary epithelial cells. This immunogenic complex is recognized by circulating antibodies resulting in the formation of antigen–antibody complexes [31,32]. Increased levels of AMA in the serum and infiltration of the liver and portal spaces of PBC patients with CD4+ T and CD8+ T lymphocytes indicate the role of a specific immune response in PBC pathogenesis [33,34]. The population of effector memory CD8+ T lymphocytes, localized around the portal tracts in the livers of PBC patients recognizes antigenic sequences within the PDC-E2 domain that contain lipoic acid and contributes to targeted damage to the biliary tract [35,36]. Th17, Th1 and follicular helper T cells contribute to the development of the disease [37,38]. The stage of advanced fibrosis is associated with a shift of the immune response to the Th17 phenotype, with dominant production of IL-17, IL-6 and TGF-β, which has been confirmed in the infiltrates of liver sections obtained from PBC patients [39,40]. Follicular T helper cells, also found in greater number in the livers of patients with PBC, provide the necessary help to B lymphocytes to differentiate to cells capable of the production of an altered isotype of the specific antibodies [36]. A decreased number of Treg cells has been found in the livers of patients with PBC [41,42].

The importance of innate immunity in the development of PBC is indicated by the presence of granulomas and polyclonal IgM, but the mechanisms of innate immunity alone, without contribution of the acquired immunity, are not sufficient to cause a break in autotolerance [43]. Cholangiocytes express Toll like receptors (TLRs), which activated by various ligands including products of microorganisms, produce the proinflammatory mediators such as NF-κB, CX3CL1 and IL-8 that contribute to biliary epithelial cell damage and recruitment of immune effector cells into the portal tracts [44,45]. Increased expression of CX3CL1 in damaged cholangiocytes attracts CD4+ T and CD8+ T lymphocytes, which are found to be more abundant in the liver of patients with PBC [44]. A recent study indicated an increase in the number of suppressor cells of myeloid origin in the liver of patients with primary biliary cholangitis and correlation of their number with the biochemical parameters of the disease: the concentration of ALP and serum bilirubin [46]. In the presence of circulating AMA and apoptotic cholangiocytes, the expression of the proinflammatory cytokine IL-12 is increased in the macrophages, which indicates the link between apoptosis of the cholangiocyte and the innate immune system response [47]. A higher prevalence of NKT cells has been shown in the liver of PBC patients compared to healthy controls, as well as NK cells, that also contribute to cholangiocyte damage, autoantigen release and activation of autoreactive T lymphocytes [48,49]. Mucosal associated invariant T cells (MAIT cells), invariant mucosal T cells, are a relatively novel population of innate immune cells that produce inflammatory cytokines IFN-γ, TNF-α and IL-17, or independently, or by stimulation with microorganisms, present in a smaller percentage in the liver and blood of patients with PBC compared to controls [1,49,50].

All metabolites, nutrients and bacterial products as well as cells of the immune system found in the intestine through the portal circulation firstly go to the liver [51,52]. In addition, through the enterohepatic circulation of primary and secondary bile acids and immunoglobulins, the liver directly affects homeostatic processes and

absorption in the intestine [53,54,55]. Intestinal microbiome dysbiosis is either the result, or response to the development of certain diseases and may affect nutrient degradation, damage to epithelial tight junctions and thus increase the intestinal permeability [56]. Altered function of the intestinal epithelial barrier can result in increased diffusion of pathogen-associated molecular patterns of microorganisms (PAMP), damage-associated molecular patterns (DAMPs), free fatty acids and endotoxin into portal circulation all the way to the hepatic sinusoids and thus can be a trigger for liver damage and dysregulation of immune reactions in the liver, and if there are other factors that can contribute to the maintenance of the damage, chronic liver disease develops [57]. Kupffer cells are the first line of defense against pathogens that have reached the liver from the intestine [58]. If Kupffer cells activated by bacteria, lipopolysaccharide or toxins that have reached the liver obtain M1 phenotype, they produce proinflammatory cytokines IL-6, TNF and IL-1β, which activate profibrotic stellate cells in the liver, while M2 phenotype of Kupffer cells has the tolerogenic function, since these cells produce IL-10 and TGF-β, which increases the activity of immunosuppressive Treg cells [59]. Thus, microorganisms that reach the liver may be responsible for the development of PBC, because they play a role of innate immune responses triggers, which result in dysregulation of the immune system in the liver.

Intrahepatic MAIT cells are T cells with invariant TCRs that play the key role in the immune response to antigens raised by vitamin B metabolism mediated by intestinal bacteria. These antigens are presented to MAIT cells in the liver within MHC I molecules by macrophages, cholangiocytes, and B cells [59,60]. MAIT cells, also called the biliary epithelial defense system, are located mainly in the portal tracts where they can be activated by presented antigens and initiate a localized immune response that aims to control pathogenic microorganisms that reach the liver, including the recruitment of effector lymphocytes in the liver [61]. The number of MAIT cells in the liver of patients with PBC is significantly reduced compared to healthy controls, in contrast to conventional CD4+ T and CD8+ T lymphocytes, whose presence in the liver of PBC patients is significantly increased [60]. During the therapeutic treatment with ursodeoxycholic acid, the number of MAIT cells in the liver does not normalize, even if disease improvement is registered, which could be one of the mechanisms that explain progressive liver damage regardless of the therapeutic response [62].

Secretory IgA produced by plasma cells present in the portal system and secreted into the intestinal lumen together with bile acids may also play a role in protecting the biliary epithelium from microorganisms. Within the intestinal tract, secretory IgA binds directly to bacteria and thus traps them inside the mucus, allowing them to be expelled from the intestine by feces. In addition, these antibodies neutralize bacterial toxins and interfere with the binding of bacteria to the apical surfaces of enterocytes [63]. Lower concentrations of IgA on the intestinal surface of enterocytes of the duodenum in patients with PBC compared to healthy controls were observed, and it contributes to the disruption of the epithelial barrier [54,64].

Dysbiosis results in a change in immune activity in the intestine, increases the polarization of CD4+ T lymphocytes to the Th17 phenotype and increases the production of proinflammatory cytokines crucial for host defense against pathogens. Infection of mice with the bacterium *Citrobacter rodentium* results in apoptosis of enterocytes that release autoantigens and stimulate the differentiation and proliferation of autoreactive T lymphocytes [60]. Altered fecal microflora is present in patients with PBC [65,66]. These observations indicate the important role of

microorganisms in the initiation of pathological processes in PBC. Experimental models of PBC induced by infection with different microorganisms also indicate an important role of stimulation of innate immunity in triggering the pathological process. Infection of NOD.B6-Idd10/Idd18 mice with *Escherichia coli* results in an increase of anti-AMA titers and development of a severe form of cholangitis [23,24]. Additionally, NOD, C57BL/6 and SJL mice infected with bacterium *Novosphingobium aromaticivorans* have increased levels of specific antibodies in the sera and activated T cells that induce bile ducts damage [67]. *Novosphingobium aromaticivorans* contains molecules homologous to PDC-E2 autoantigen, which in infected mice initiate the production of PDC-E2 specific IgG and liver damage almost identical to those lesions developed during PBC in humans [68], with an increased number of NKT cells in the liver, with increased expression of the CD1d molecule [67]. This disease can be transferred by CD4+ and CD8+ T lymphocytes from a diseased mouse to healthy mice.